Chapter

Physical Inactivation of SARS-CoV-2 and Other Coronaviruses: A Review

Raymond W. Nims and Mark Plavsic

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for the ongoing pandemic of Coronavirus Disease 2019 (COVID-19). Other members of the enveloped RNA virus family *Coronaviridae* have been responsible for a variety of human diseases and economically important animal diseases. Disinfection of air, environmental surfaces, and solutions is part of infection prevention and control (IPAC) for such viruses and their associated diseases. This article reviews the literature on physical inactivation (disinfection) approaches for SARS-CoV-2 and other coronaviruses. Data for thermal (heat) inactivation, gamma irradiation, and ultraviolet light in the C range (UVC) irradiation have been reviewed. As expected, the susceptibilities of different members of the *Coronaviridae* to these physical inactivation approaches are similar. This implies that knowledge gained for SARS-CoV-2 should be applicable also to its emerging mutational variants and to other future emerging coronaviruses. The information is applicable to a variety of disinfection applications, including IPAC, inactivation of live virus for vaccine or laboratory analytical use, and waste stream disinfection.

Keywords: coronaviruses, *D* value, gamma irradiation, SARS-CoV-2, thermal (heat) inactivation, UVC inactivation

1. Introduction

The ongoing pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated disease, Coronavirus Disease 2019 (COVID-19), have resulted in the generation of a tremendous amount of literature on various aspects of the disease and the virus. Of importance to this chapter is the literature on physical disinfection strategies for the virus, and infection prevention and control (IPAC) strategies for reducing potential transmission of the virus. In addition, physical inactivation approaches are used for rendering patient samples safe for handling in laboratories conducting diagnostic assays. Certain physical inactivation approaches also are used as barrier technologies for rendering human and animal raw materials safe for use in biologics manufacture. The literature specific to SARS-CoV-2 that has been published in the past 18 months is supplemented by previous literature on other relevant human and animal coronaviruses. These include human coronavirus 229E (HCoV-229E), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), porcine

epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine hemagglutinating encephalomyelitis virus (PHEV), and canine, feline, and bovine coronaviruses (this list is not all-inclusive). For the present review chapter, the authors searched the literature for gamma irradiation, electron beam, high pressure, UVC, and heat (thermal) inactivation of coronaviruses in general, and in particular, the specific coronaviruses listed above. No limits were placed on date of publication, although, for obvious reasons, the data on SARS-CoV-2 were obtained from papers published since 2019.

Strategies for IPAC of SARS-CoV-2 include an impressive arsenal of pharmaceutical (vaccines, palliative therapies) and non-pharmaceutical interventions (face mask usage, social distancing, testing, contact tracing and quarantine), as well as chemical and physical approaches for liquid, surface, and air disinfection and for personal hygiene. In this chapter, we have attempted to review the physical inactivation efficacy data for SARS-CoV-2 and other coronaviruses. Our primary emphasis in this review is on IPAC, but other applications of physical inactivation approaches, such as rendering laboratory samples safe for handling within a biosafety I or II facility, and barrier treatments for inactivating potential contaminants in biologics animal-derived materials, are discussed.

2. Overview of physical viral inactivation approaches

The most commonly employed physical approaches for inactivating viruses are thermal (heat) inactivation (applied either to viruses in solutions or dried on surfaces); irradiation (applied to viruses in solutions, in solids, or dried on surfaces); and high pressure (most often employed for disinfection of food items). The irradiation approaches include gamma irradiation, X-irradiation, electron beam irradiation, and 254 nm ultraviolet light (UVC) irradiation. Irradiation with ultraviolet light in the A range and with visible light typically requires the addition of a photoactive chemical and, therefore, these are not truly physical approaches, but rather mixed physical/chemical approaches. The latter will not be dealt with in this chapter. Electron beam irradiation and high-pressure treatment are most commonly used for food preservation and the efficacy data to be found in the literature necessarily involve viruses of food concern (e.g., caliciviruses, astroviruses, reoviruses, picornaviruses, and adenoviruses) [1, 2]. Coronaviruses are not considered viruses of food concern [3] and, therefore, there are little or no data for inactivation of coronaviruses by electron beam irradiation and high-pressure treatment. As a result, there will be little discussion of these approaches in this chapter.

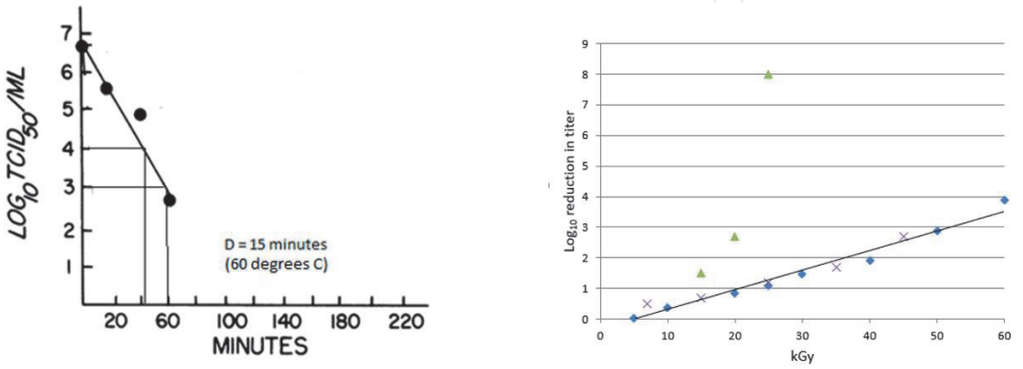
Physical inactivation approaches display efficacy for a broad range of viruses, including both lipid-enveloped and non-enveloped viruses. The factors determining virucidal efficacy for one virus type over another differ among the physical approaches. For instance, particle size appears to be the major determinant for inactivation efficacy of gamma, X-ray, and electron beam irradiation [4], while genomic structure (single vs. double strand, circularity, and relative content of pyrimidine dinucleotides) appears to be more important for determining UVC inactivation efficacy [5]. Thermal inactivation appears to be effective for both lipid-enveloped and non-enveloped viruses, and particle size does not appear to correlate with efficacy [6]. Having said this, the most highly resistant of viruses to heat inactivation are the non-enveloped parvoviruses, circoviruses, and polyomaviruses [6]. The orthogonality of mechanism of inactivation displayed by these physical approaches is convenient. If one approach is not practical for a given virus family, another approach may be applied. A good example is the parvovirus family of small non-enveloped viruses. These typically are highly resistant to thermal inactivation

and to gamma, X-ray, and electron beam irradiation but are quite susceptible to UVC irradiation [7].

Physical inactivation approaches also differ with respect to the types of sample matrices that may be treated. Thermal inactivation has the broadest range of matrix types, including liquids and surfaces. Of course, temperatures high enough to inactivate viruses may have adverse impacts on the sample matrices being irradiated. Gamma radiation has high penetrability, and can be used for liquids and solids, though the matrix to be irradiated must be brought in close contact with a gamma source, and such sources are available only at specialized irradiation facilities. In order to minimize potential side effects of gamma irradiation (free radicals, heat) and to maintain the integrity of the sample matrix (such as bovine serum), the typical gamma irradiation process requires keeping the sample to be irradiated at very cold temperature (typically, such samples are kept on dry ice during irradiation) [8]. Electron beam radiation has low penetrability, so is typically used for thin items such as food items [1]. Due to its low penetrability, ultraviolet light irradiation is effective only if the radiation reaches all portions of the matrix being irradiated [9]. It is a line-of-sight approach. It is used for inactivating viruses on non-porous surfaces and liquids which have low UVC-absorbance characteristics [9].

An advantage of physical inactivation approaches is the first-order behavior typically displayed for inactivation of viruses (see **Box 1**). This enables one to make informed predictions of inactivation efficacy at temperatures, times, fluences that have not specifically been tested empirically.

First-order viral inactivation by physical approaches. One commonality among the physical inactivation approaches is that, as a generality, the $\log_{10}$ reduction in virus titer observed following treatment is first-order (linear) with respect to time in the case of heat inactivation, or with applied dose (fluence) in the case of irradiation. Of course, there are exceptions, which are sometimes attributed to mixed virus populations with differential susceptibility to the inactivation approach. It is likely that the biphasic or non-linear behavior attributed to such mixed populations is due to experimental artifact, including the inclusion of data points which approach the limit of detection of the titration assays used, or simply the fact that most of the available virus has already been inactivated. The typical first-order behavior of the physical inactivation approaches enables the calculation of decimal reduction values (*D*) for a given virus, corresponding to the thermal treatment time or irradiation fluence associated with a 1-$\log_{10}$ reduction in virus titer. Knowing such a *D* value allows one to adjust the thermal contact time or the irradiation fluence such that a desired $\log_{10}$ reduction value may be achieved for a given virus. For instance, in the tables to follow in this chapter, thermal inactivation *D* values are plotted against temperature to allow estimation of log reduction at any given time and temperature. Similarly, gamma irradiation and UVC irradiation efficacy are expressed in terms of $\log_{10}$ reduction per kGy (gamma irradiation) or $\log_{10}$ reduction per mJ/cm^2 fluence (UVC). This enables one to estimate the effectiveness of the irradiation approach for inactivating a given virus under conditions not tested empirically.



Box 1.
Left panel: calculation of a D value for heat inactivation of a parvovirus at 60 °C (from [10]); right panel, first-order behavior for two data sets (× and ◊) and one data set displaying non-linear behavior (△) for inactivation of a parvovirus by gamma irradiation (from [4]).

3. Inactivation of coronaviruses by gamma irradiation

Gamma irradiation is commonly used for sterilization of plasticware (especially tissue culture flasks, bottles, pipette tips, and pipettes). For such applications, a high fluence (hundreds of kGy) may be used to kill any prokaryotic microbes and viruses [11]. When it comes to disinfecting surfaces, again, sufficiently high fluences may be employed to kill any microbes and viruses. For disinfection of frozen or liquid solutions, care must be taken to balance the need for adequate sterilization with maintenance of the expected performance of the solutions being irradiated [4]. Gamma radiation interacts with solutions in different manners, depending on a number of factors, including the temperature of the solution and the presence of radiation-scavenging compounds. At very low temperatures (< −60°C, for instance, and in the presence of radiation scavengers, such as concentrated proteins), the radiation impacts on the solution itself are limited, and the impacts on suspended microbes are more selectively targeted to vital macromolecules such as genomic material. These effects are termed "direct" radiation effects. At temperatures above freezing and in the absence of scavenging compounds, effects termed "indirect" are imparted to the solution. These are characterized as radiolysis products attributed to the interaction of photons with water, forming oxygen radicals that can damage not only suspended microbes but also any biological materials in solutions. As a result of the above, inactivation of viruses in solutions, such as animal serum or culture medium containing serum, is typically accomplished by irradiating the sample matrices frozen on dry ice [4, 8].

As mentioned already, gamma radiation is highly penetrating, therefore is ideal for pathogen reduction in deeply frozen containers of animal serum and other biological samples. The data pertaining to efficacy of gamma irradiation for inactivating coronaviruses [12–16] are displayed in **Table 1**. These data were collected using deeply frozen tissue culture medium containing small amounts of bovine serum (i.e., the harvest medium containing the virus that comprised the viral stocks tested). In each case, the sample temperature during irradiation was maintained through use of dry ice, so that primarily the direct effects of the radiation on the viral macromolecules were to be expected. As expected, based on the known mechanism of action of gamma radiation on the viruses, and the relatively large particle size (60–136 nm) of the coronaviruses, the inactivating efficacies of gamma irradiation on SARS-CoV, MERS-CoV, and SARS-CoV-2 were similar in the reported studies [12–16]. The consensus data indicate an efficacy of 0.5–0.9 $\log_{10}$ inactivation per kGy of gamma radiation. At the typical range of fluences administered to frozen animal serum (25–45 kGy), as an example, one would therefore expect >12 $\log_{10}$ inactivation of coronaviruses (i.e., 0.5 $\log_{10}$ inactivation per kGy × 25 kGy). It may be predicted that

Virus[a]	Temperature (°C)	Inactivation matrix	*D* value (kGy)	Efficacy ($\log_{10}$/kGy)	Efficacy at 25 kGy ($\log_{10}$)	Ref.
SARS-CoV-2	−80 (dry ice)	Culture medium	1.6	0.63	16	[12]
SARS-CoV-2	−80 (dry ice)	Culture medium	1.1	0.92	23	[13]
SARS-CoV	−80 (dry ice)	Culture medium	≤1.7	≤0.60	≤15	[14]
SARS-CoV	−80 (dry ice)	Culture medium	>0.15	ND[b]	ND	[15]
MERS-CoV	−80 (dry ice)	Culture medium	2.0	0.50	12	[16]

[a]*MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus.*
[b]*ND, not determined. The highest fluence tested (0.15 kGy) failed to cause ≥ one $\log_{10}$ inactivation.*

Table 1.
Efficacy of gamma irradiation for inactivating coronaviruses.

members of the coronavirus family, including future emerging species, should each be highly susceptible to inactivation by gamma radiation.

Greater efficacy for inactivating coronaviruses may be expected when irradiating solutions at higher temperatures (especially above freezing) and in the absence of radiation scavengers. This is due to the additional contribution of the indirect effects of gamma radiation. Of course, under these conditions, the matrix being irradiated may be degraded to the point where it no longer is useful for the intended application.

As indicated in the above, gamma irradiation should be considered a very effective physical approach for inactivating coronaviruses, such as SARS-CoV-2. Reports have suggested, for instance, the suitability of gamma irradiation for inactivation of SARS-CoV-2, while preventing loss of antigenic content, for use in preparing vaccines [17]. In a practical sense, however, the requirement for a gamma radiation source such as cobalt^{60} limit the general availability of this approach for routine use. Items or solutions to be gamma irradiated must be shipped to an irradiation facility to accomplish this.

4. Inactivation by UVC irradiation

As mentioned above, photons of light from various regions of the electromagnetic spectrum (i.e., visible, UVA, UVB, and UVC) have been used for inactivation of viruses. Available scientific literature indicates that light in the UVC range has the greatest efficacy for inactivating viruses, through a purely physical mechanism of action that does not depend on chemical radiation-sensitizing compounds. While visible light (405 nm) in the absence of photosensitizing agents has been shown to have efficacy for inactivating SARS-CoV-2, this activity is relatively weak, compared to that of UVC. For instance, a fluence of 288 mJ/cm^2 was required to cause a 2.58 $\log_{10}$ inactivation [18], equating to about 0.0090 $\log_{10}$/(mJ/cm^2), an order of magnitude greater than the UVC fluence required (see below). The reason for the unique efficacy of UVC light in the absence of sensitizing agents is thought to be the correspondence of the UVC light wavelength, typically 254 nm light from mercury vapor lamps, with the absorbance peaks of the target nucleic acids (~265 nm) [9, 19].

Only the efficacy of UVC light is discussed in the tables below. Unlike gamma irradiation, which can penetrate solids, UVC irradiation is a line-of-sight approach, which depends on exposure of target organisms to the radiation. The impacts to the target organism depend on the absorbed dose. As with gamma irradiation, the dose of UVC light applied can be expressed in a single fluence term that takes into account both dose rate and time. A variety of units have been used in the literature, which can lead to confusion when attempting to compare results between labs. We use the units mJ/cm^2 in this chapter, since most of the virus inactivation results to be found in the literature have been expressed in these units. Conversion of other fluence units, such as J/m^2 to mJ/cm^2 is straightforward, while exposures expressed in units of mW/cm^2 must be multiplied by the exposure time (in seconds) to convert to mJ/cm^2.

The mechanism of inactivation of viruses by UVC radiation is thought to involve interaction of the energetic photons with nucleic acids comprising the viral genome. Pyrimidine nucleotides (uracil, thymine, cytosine) are especially susceptible to the formation of covalent dimers following exposure to UVC. A more thorough discussion of mechanisms and pyrimidine dimer formation, and relevance for predicting efficacy for viruses of different genomic structure, is beyond the scope of this chapter. Readers are referred to excellent source papers [5, 20, 21].

There is some literature on coronavirus inactivation in liquid matrices by UVC radiation, and rather scanty information on irradiation of these viruses on solid surfaces or in aerosols. A summary of the evaluation of UVC efficacy for inactivating SARS-CoV-2 and other coronaviruses in liquid matrices is displayed in

Table 2. No attempt to cherry-pick the efficacy data has been made in assembling this table, although it will be readily apparent on review of this table that discrepant results in terms of *D* value and $\log_{10}$ inactivation per mJ/cm^2 have been reported. For an informed analysis of possible factors underlying these discrepant values, relating primarily to optical density of the liquid matrices and dosimetry difficulties, the reader is referred to Boegel et al. [19].

Neglecting the clearly discrepant values in this table, certain of which unfortunately have caused some confusion on the sensitivity of coronaviruses to UVC radiation [33], a consensus *D* value in the range of 0.5–2 mJ/cm^2 may be inferred. This *D* range corresponds to a consensus efficacy of 0.5–2 $\log_{10}$/mJ/cm^2 (**Table 2**). To put these *D* values into perspective, the most UVC-resistant viruses (adenoviruses and polyomaviruses), have UVC *D* values >50 mJ/cm^2 [6].

A summary of the evaluation of the inactivation of coronaviruses by UVC radiation on solid surfaces and in aerosols is provided in **Table 3**. As mentioned above, there are fewer reports for this topic within the literature. On a theoretical basis, UVC radiation accessibility to viruses dried on surfaces or present in aerosols should be optimal, therefore such considerations as impact of stirring or impact of matrix absorption of the radiation should not confound the efficacy results to the extent that these do in liquid matrix studies. Although the dataset in **Table 3** is limited, the agreement between observed *D* values between reports and between coronaviruses is fairly close, perhaps in keeping with the lessened impact of confounding factors mentioned above. The *D* values shown in **Table 3** also are in good agreement with the consensus *D* values (0.5–2 mJ/cm^2) from the liquid matrix studies.

Virus[a]	Wavelength (nm)	Inactivation matrix	*D* value (mJ/cm^2)	Efficacy ($\log_{10}$/mJ/cm^2)	Reference
SARS-CoV-2	254	Culture medium	1.7	0.59	[19]
SARS-CoV-2	254	Culture medium	6.7	0.15	[22]
SARS-CoV-2	254	Culture medium	1.8	0.56	[23]
SARS-CoV-2	254	Culture medium	0.5–7.5	0.13–2.0	[24]
SARS-CoV-2	282	Culture medium	12.5	0.080	[25]
SARS-CoV-2	254	Culture medium	98	0.010	[26]
SARS-CoV-2	265	Culture medium	0.6	1.7	[27]
SARS-CoV-2	254	Culture medium	0.016	Not calculated[b]	[28]
SARS-CoV	254	Culture medium	22	0.044	[29]
SARS-CoV	254	Culture medium	300	0.20	[15]
SARS-CoV	260	Culture medium	300	0.20	[30]
HCoV 229E	254	Culture medium	1.8	0.56	[19]
HCoV 229E	254	Culture medium	1.7	0.59	[31]
HCoV OC43	254	Culture medium	1.7	0.59	[19]
HCoV OC43	267	PBS	2	0.5	[32]
MHV	254	Culture medium	1.2	0.82	[19]
MHV	254	Culture medium	1.1	0.91	[31]

[a]*HCoV, human coronavirus; MHV, mouse hepatitis virus; PBS, phosphate buffered saline. SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.*
[b]*The reported inactivation kinetics were not first-order.*

Table 2.
Efficacy of Ultraviolet C (UVC) irradiation for inactivating coronaviruses in liquid matrices.

Virus[a]	Wavelength (nm)	Surface/Aerosol	*D* value (mJ/cm^2)	Efficacy ($log_{10}/mJ/cm^2$)	Reference
SARS-CoV-2	254	Plastic	1.4	0.71	[23]
SARS-CoV-2	222	Plastic	1.2	0.83	[34]
SARS-CoV-2	260–285	Stainless steel	1.6	0.63	[35]
SARS-CoV-2	260–285	N95 mask fabric	21	0.05	[35]
HCoV 229E	222	Aerosol	0.56	1.8	[36]
HCoV OC43	222	Aerosol	0.39	2.6	[36]
MHV	254	Aerosol	0.66	1.5	[37]
IBV	254	Aerosol	13.8	0.07	[38]

[a]*HCoV, human coronavirus; IBV, infectious bronchitis virus; MHV, mouse hepatitis virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2*

Table 3.
Efficacy of Ultraviolet C (UVC) irradiation for inactivating coronaviruses on surfaces or in aerosols.

For general reviews of UVC inactivation of coronaviruses in various matrices, the reader may also consult Boegel et al. [19], Hadi et al. [20], Chiappa et al. [39], and Helßling et al. [40]. Pendyala et al. [21] used efficacy modeling based on pyrimidine dinucleotide content to predict UVC efficacy for inactivating various alpha-, beta-, and gamma-coronaviruses. The conclusion of the modeling was that coronaviruses, as a family, are highly susceptible to UVC, and the *D* values obtained in the modeling for the various coronaviruses ranged from 18.0 to 28.1 J/m^2 (1.8–2.8 mJ/cm^2), aligning well with the consensus *D* values from **Tables 2** and **3**.

The data presented suggest that UVC irradiation is a very effective physical approach for inactivating coronaviruses, such as SARS-CoV-2. It is not surprising, therefore, that UVC has been proposed for a variety of applications, including indoor air sanitization [36, 41–44], inactivation of coronaviruses in water [33] or other solutions, inactivation of biological samples for downstream use in assays [22], and surface hygiene [34, 45], including sanitization of personal protective equipment [35, 46, 47].

5. Thermal (heat) inactivation

As is the case for gamma irradiation, heat can be highly penetrating, depending upon the inactivation matrix. For instance, heat transfer within liquids is typically efficient, so heat inactivation is a commonly employed method for inactivating adventitious agents (including viruses) in solutions. Heat inactivation is also commonly utilized for decontaminating non-porous surfaces. For some time, there has existed a dogma that heat inactivation of viruses is more effective when applied to solutions (liquid or wet inactivation) than to surfaces (carrier or dry inactivation). Exceptions to this have been noted recently [48, 49], and it is more correct to state that relative efficacy for wet vs. dry heating may depend upon the specific virus being inactivated.

The mechanisms underlying inactivation of viruses by heat are thought to be the same for both enveloped and non-enveloped viruses. The treatment is thought to result in leaky protein capsids, which allow penetration of the capsid by nucleases and loss of capsid contents to the environment. In either case, nucleases would be

expected to rapidly degrade the genomic material and render the viruses non-infectious [50]. If this mechanism is correct, heat inactivation efficacy should be similar for enveloped and non-enveloped viruses. Indeed, examination of wet heat inactivation data across virus families confirms this conclusion [6]. While certain viruses (e.g., animal parvoviruses and polyomaviruses) exhibit unusually high heat resistance, in general non-enveloped viruses do not appear to be significantly more resistant to heat than enveloped viruses [6].

The literature on heat inactivation of coronaviruses, including SARS-CoV-2, is extensive. The reports generally contain information on efficacy of one or more temperatures evaluated for one or more time periods. These studies [15, 29, 30, 51–65] generally do not report *D* values, only $\log_{10}$ reduction in titer obtained from heating at a given temperature for set time periods (e.g., 56°C for 30 min). Examples of this sort of heat inactivation data are given in **Table 4**. Note that in **Table 4**, data for temperatures greater than 45°C are displayed. Results at lower temperatures are associated with a great deal of variability. For readers interested in coronavirus stability at the lower temperatures (ambient to ~45°C), the following review papers may be consulted [65–70]. The data in **Table 4** indicate that inactivation of coronaviruses at temperatures between 48 and 54°C may be incomplete at exposure times up to 60 min. Temperatures ≥56°C are generally quite effective at exposure times of 10 min or greater, while temperatures ≥80°C are very effective within 1 or 2 min of exposure. Similar efficacies of heat inactivation for various members of the *Coronaviridae* are observed.

Relatively few reports of heat inactivation on carriers (dry heat) have been published for coronaviruses (**Table 5**). These studies [35, 71–73] have been concerned primarily with decontamination of personal protective equipment (gowns, N95 respirators) for reuse, although Fischer et al. [35] and Biryukov et al. [72] also evaluated inactivation of SARS-CoV-2 on stainless steel carriers. Estimates of *D* values for heat inactivation on surfaces (**Table 5**) range from ~7 min at 60°C (PEDV) to 11–35 min at 55–70°C (SARS-CoV-2).

The most useful heat inactivation results are expressed in terms of *D* values measured at three or more temperatures. The latter datasets enable the plotting of *D* vs. temperature curves, which, in turn, enable comparison of the efficacy of the heat inactivation results obtained in different laboratories, as well as estimation of *D* at non-measured temperatures. It should be noted that, while the kinetics of inactivation of viruses by heat at a given temperature are expected to be first-order with respect to time, the relationship between *D* and temperature is more complex [74]. In the past, the latter relationship has been plotted on semi-log scales ($\log_{10}$ *D* vs. time), resulting in linear plots from which *Z* values (°C per $\log_{10}$ change in *D*) could be calculated. These *Z* values could then be used to estimate *D* at non-measured temperatures. More recently, it has been discovered that the plot of *D* vs. temperature can be fit accurately with the power function. Examples of such plots for coronavirus heat inactivation are shown in **Figures 1** and **2**. The resulting line equation coefficients (**Table 6**) then may be used, in a more intuitive and straightforward manner, to estimate *D* at non-measured temperatures [74].

Some authors [66, 75] have taken the interesting and informative approach of combining the heat inactivation data from multiple individual reports to create summary plots of *D* vs. temperature. An example for heat inactivation of coronaviruses in liquids and on surfaces has been reported by Guillier et al. [66]. The portion of the dataset within the temperature range 40°C–70°C has been reproduced as **Figure 1** below. As can be appreciated from this figure, there is

Virus[a]	Temperature (°C)	Inactivation matrix	Inactivation efficacy	Reference
SARS-CoV-2	56	Culture medium	3.4 $\log_{10}$ in 15 min	[51]
	65	Culture medium	>6 $\log_{10}$ in 15 min	
SARS-CoV-2	56	Culture medium	4.3 $\log_{10}$ in 10 min	[52]
	70	Culture medium	>5.2 $\log_{10}$ in 10 min	
	90	Culture medium	>5.2 $\log_{10}$ in 10 min	
SARS-CoV-2	56	Culture medium	>3 $\log_{10}$ in 15 min	[53]
	95	Culture medium	>5 $\log_{10}$ in 1 min	
SARS-CoV-2	56	Culture medium	>4 $\log_{10}$ in 30 min	[54]
	65	Culture medium	>4 $\log_{10}$ in 15 min	
SARS-CoV-2	56	Culture medium	>5 $\log_{10}$ in 30 min	[55]
	92	Culture medium	>6 $\log_{10}$ in 2 min	
SARS-CoV-2	56	Culture medium	>5 $\log_{10}$ in 30 min	[56]
	98	Culture medium	>5 $\log_{10}$ in 2 min	
SARS-CoV	60	Phosphate buffered saline	>4 $\log_{10}$ in 15 min	[57]
SARS-CoV	56	Culture medium	>6 $\log_{10}$ in 90 min	[30]
	67	Culture medium	>6 $\log_{10}$ in 60 min	
	75	Culture medium	>6 $\log_{10}$ in 30 min	
SARS-CoV	56	Culture medium	>5 $\log_{10}$ in 30 min	[58]
	60	Culture medium	>5 $\log_{10}$ in 30 min	
SARS-CoV	56	Culture medium	>4 $\log_{10}$ in 10 min	[15]
	65	Culture medium	>4 $\log_{10}$ in 4 min	
SARS-CoV	58	Culture medium	4.9 $\log_{10}$ in 30 min	[59]
	68	Culture medium	≥4.3 $\log_{10}$ in 10 min	
SARS-CoV	56	Culture medium	>6 $\log_{10}$ in 30 min	[29]
MERS-CoV	56	Culture medium	4 $\log_{10}$ in 24 min	[60]
	65	Culture medium	4 $\log_{10}$ in 1 min	
PEDV	50	Culture medium	1.1 $\log_{10}$ in 60 min	[61]
	60	Culture medium	5 $\log_{10}$ in 30 min	
PEDV	48	Culture medium	1.7 $\log_{10}$ in 10 min	[62]
CaCoV	60	Culture medium	>4 $\log_{10}$ in 15 min	[63]
	80	Culture medium	>4 $\log_{10}$ in 1 min	
MHV	60	Culture medium	>4 $\log_{10}$ in 15 min	[63]
	80	Culture medium	>4 $\log_{10}$ in 1 min	
FIPV (Wt)	54	Culture medium	2 $\log_{10}$ in 15 min	[64]

[a]*SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; PEDV, porcine epidemic diarrhea virus; CaCoV, canine coronavirus; MHV, mouse hepatitis virus; FIPV (Wt), feline infectious peritonitis coronavirus (wild-type).*

Table 4.
Efficacy of heat inactivation for inactivating coronaviruses in liquid matrices.

Virus[a]	Temperature (°C)	Surface type	Inactivation efficacy	Reference
SARS-CoV-2	70	N95 mask fabric	3 $\log_{10}$ in 48 min	[40]
	70	stainless steel	3 $\log_{10}$ in 88 min	
SARS-CoV-2	70	N95 mask fabric	>5.5 $\log_{10}$ in 60 min	[71]
SARS-CoV-2	55	stainless steel	1 $\log_{10}$ in 35 min	[72]
PEDV	60	N95 mask fabric	≥3 $\log_{10}$ in 20 min	[73]

[a]*SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PEDV, porcine epidemic diarrhea virus.*

Table 5.
Efficacy of heat inactivation for inactivating coronaviruses on surfaces.

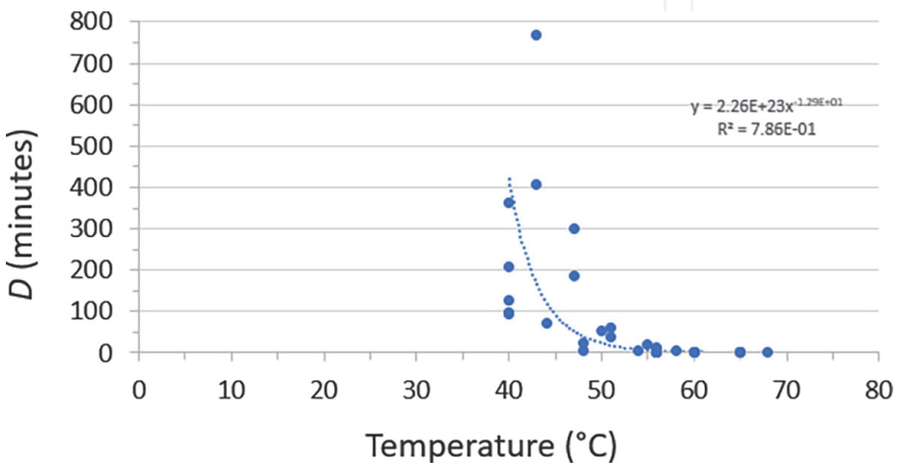


Figure 1.
*Relationship between decimal reduction value (*D*; time required for 1 $\log_{10}$ inactivation) and temperature for heating studies involving various coronaviruses. Data are from reference [66].*

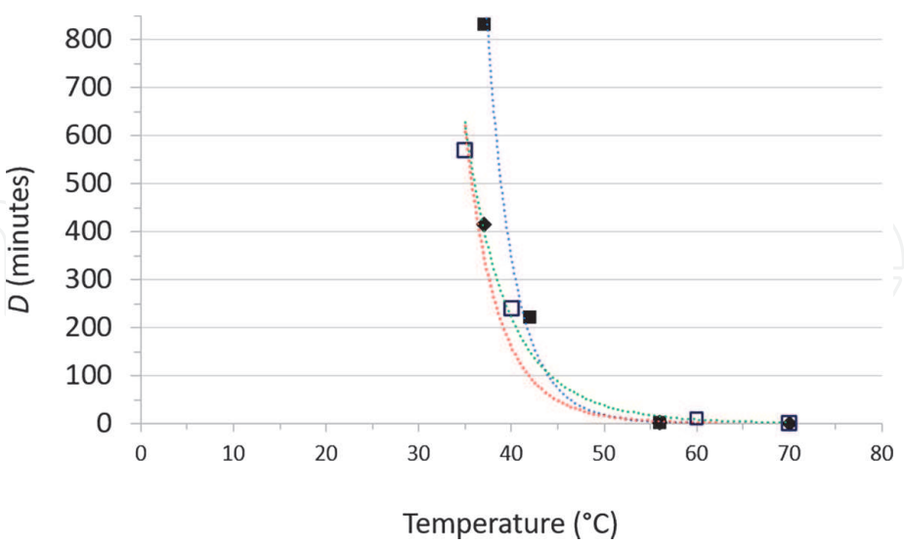


Figure 2.
*Relationship between decimal reduction value (*D*; time required for 1 $\log_{10}$ inactivation) and temperature for heating studies involving SARS-CoV-2. Data are from reference [75] (□), [76] (◆), and [77] ■).*

considerable variability in response at the lower temperatures, while greater concurrence is seen at temperatures of 50°C and above. The ability of the power function ($D = a \times \text{temperature}^{-b}$; where D is the decimal reduction value and a and b are calculated coefficients) to fit the combined coronavirus dataset is similar to

Coronavirus	*a*[a]	*b*	r^2	*D* at 56°C (min)	*Reference for D values*
Various *Coronaviridae*	2.26E+23	12.9	0.786	6.4	[66]
Alphacoronaviruses					
TGEV	4.38E+20	10.9	0.967	39	[78]
CaCoV	1.23E+09	4.92	0.856	3.1	[79]
PEDV	7.11E+15	8.57	0.953	7.4	[80]
Betacoronavirus					
SARS-CoV-2	2.70E+18	10.1	0.985	6.0	[76]
SARS-CoV-2	2.97E+23	13.1	0.996	3.7	[77]
SARS-CoV-2	9.52E+6	3.69	0.980	3.4	[51]
SARS-CoV-2 (modeled)	7.18E+14	7.81	0.998	16	[75]

[a]*Abbreviations used:* a *and* b*, coefficients for power function line equation* D = a × *Temperature;* D*, decimal reduction value; CaCoV, canine coronavirus; PEDV, porcine epidemic diarrhea virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGEV, transmissible gastroenteritis virus.*

Table 6.
Power function coefficients for D *vs. temperature curves for thermal inactivation of coronaviruses.*

the ability of this function to fit data for multiple temperatures for SARS-CoV-2 generated within a given laboratory (**Figure 2**).

In cases where a laboratory has generated *D* vs. temperature data for three or more temperatures, these data may be plotted as shown in **Figure 2**. This figure compiles line fit data from two empirical liquid inactivation studies for SARS-CoV-2 [76, 77]. The third line on this plot is the line fit obtained from modeling of SARS-CoV-2 inactivation at various temperature by Yap et al. [75]. The modeling by Yap and coworkers was performed on the basis of heat inactivation data generated by various labs, using as challenge viruses a variety of coronaviruses (SARS-CoV, SARS-CoV-2, MERS-CoV, MHV, PEDV, and TGEV) [75]. The agreement between the line fits for these three datasets in striking. It is apparent from the plots in **Figure 2** that it takes hours to achieve 1 $\log_{10}$ inactivation of SARS-CoV-2 at temperatures ≤40°C, while inactivation at temperatures greater than 50°C requires only min.

In **Table 6**, the power function line fit coefficients for heat inactivation studies evaluating various coronaviruses are displayed. The estimation of *D* at 56°C is shown as a means of demonstrating the utility of the power function line fitting approach for enabling comparison of datasets generated at different laboratories. Note that at 56°C, *D* values for the various coronaviruses range from 3 to 39 min, with the 39 min required for TGEV considered to be atypical.

Taken together, the data in **Figures 1** and **2** and **Tables 4–6** support the expectation that similar heat sensitivities are to be expected for various members of the *Coronaviridae* family. To put the *D* values shown in **Table 6** into perspective, more heat susceptible virus families include the *Rhabdoviridae* ($D_{56\ °C}$ ranging from 0.2 to 1.9 min) and *Retroviridae* ($D_{56\ °C}$ of 1.4 min), while less susceptible viruses include animal members of the *Parvoviridae* ($D_{56\ °C}$ > 10 hours) [6]. The heat susceptibilities displayed by the *Coronaviridae* are fairly typical of enveloped and non-enveloped viruses in general, except as noted above.

The literature that has been reviewed above indicate that heat inactivation is typically utilized for inactivation of coronaviruses in solutions, but this physical approach has also been used for decontamination of these viruses on surfaces, such as stainless steel and N95 respirator material. In addition, hot (≥63°C), humid (95%

relative humidity) air exposure for 1 hour has been described for decontaminating enveloped RNA virus (bacteriophage Phi6 used as a surrogate for SARS-CoV-2) dried on surfaces within aircraft [81].

6. Discussion

Physical pathogen inactivation approaches have a number of advantages. First among these is the fact that these approaches display efficacy for a broad range of pathogen types, up to and including bacterial and fungal spores. In the hierarchy of pathogen susceptibility to microbicides (sometimes referred to as the Spaulding scale [82]), only infectious proteins (prions) may remain resistant to these physical approaches as normally applied [83, 84]. Per the established hierarchy with regard to viral inactivation [85–88], non-enveloped viruses display much greater susceptibility to microbicides, while enveloped viruses are considered to be among the most susceptible of all pathogens to microbicides. For physical inactivation approaches, this hierarchy may be somewhat different. As mentioned in the introduction to this chapter, the orthogonal physical approaches may display complementary efficacies for different virus families, and efficacy is not solely determined by envelope status or particle size.

Secondly, to a certain extent, the physical approaches require additions of photons to the inactivation matrix, not molecules—as in the case of chemical inactivation. This means that the physical approaches can be used without the necessity of removing the inactivating agent from the inactivation matrix. For example, gamma irradiation can be applied to finished product in sealed containers, ultraviolet irradiation can be applied through glass or plastic tubing, and heat can be applied to containers of liquids. Each of the methods can be applied to surfaces without the need to subsequently remove an inactivating agent.

The first-order behavior of physical inactivation approaches, discussed previously in this chapter, is also a useful attribute. For instance, gamma irradiation and UVC inactivation efficacies are typically first-order with respect to applied fluence. Efficacy of heat inactivation is typically first-order with respect to time at any given temperature. This means that once a *log_{10} inactivation per fluence* value is obtained, efficacy at a different fluence (gamma irradiation or UVC) can be estimated. Similarly, once a *D* value is obtained at a given temperature for heat inactivation, the efficacy for a different contact time can be estimated with some confidence.

In this chapter, we have attempted to convert, where possible, inactivation results from different reports into the *log_{10} inactivation per fluence* values and the *D* values discussed above, so that the readers can make informed estimates of inactivation efficacy for these approaches under non-evaluated conditions. These estimates are quite straightforward in the case of gamma and UVC irradiation. For example, if 2 $\log_{10}$ inactivation per kGy gamma irradiation or per mJ/cm^2 UVC is measured in a study, then 4 $\log_{10}$ inactivation should be expected at 2 kGy or at 2 mJ/cm^2. For heat inactivation, if the *D* value at 65°C is 10 min, then 2 $\log_{10}$ inactivation should be expected after 20 min at the same temperature. The equations for the power function line fit of *D* vs. temperature plots [6] also allow one to estimate inactivation efficacy for non-measured temperatures. The plots shown in **Figures 1** and **2** can be thought of as depicting a 1 $\log_{10}$ inactivation *surface*. Any point on the line reflects the conditions necessary to achieve 1 $\log_{10}$ inactivation. Points to the right of this line will result in greater than 1 $\log_{10}$ inactivation, while points to the left of the line will result in less than 1 $\log_{10}$ inactivation.

As is apparent from this chapter, the three physical inactivation approaches discussed (gamma irradiation, UVC irradiation, and heat inactivation) each display

efficacy for all members of the *Coronaviridae* family and for SARS-CoV-2 in particular. The different approaches may be useful, in particular, for different applications. For instance, in case of IPAC, of the three approaches, UVC is most useful for decontaminating indoor air. For such an application, indoor air to be recirculated is passed through a unit which exposes the air to an appropriate UVC fluence. This can be done while the indoor spaces are being occupied. For surface inactivation, each of the three approaches may be useful, depending upon the surface to be decontaminated. For decontamination of liquid matrices, again, each of the three approaches could be useful. The disadvantages of the three approaches are:

- Gamma irradiation. Gamma irradiation must be performed at an irradiation facility. It is typically applied to inanimate surfaces, such as plasticware, at high fluences for sterilization. For decontaminating biological liquids, the irradiation is typically done at low temperature to avoid the damaging effects of indirect radiation effects.

- UVC irradiation. Ultraviolet light is a line-of sight-approach. If the inactivation matrix is shielded from the photons, or absorbs the photons, the efficacy for inactivation will be low. Establishing dosimetry under the actual inactivation conditions and assuring that all portions of the matrix receive photons is essential for efficacy. For IPAC, surface disinfection by UVC must be conducted while the indoor spaces are not occupied.

- Heat inactivation. Extent of inactivation depends on the temperature applied and the contact time, as well as on the specific virus being inactivated. Since coronaviruses appear to be very susceptible to heat inactivation, this approach is useful. Achieving and maintaining the desired inactivation temperature for the required contact time can be challenging. Heat and humidity have been used for IPAC, specifically for disinfecting aircraft cabins [81]. This approach also is commonly applied in the biologics industries.

For each of these physical approaches, a balance must be achieved between the desired $\log_{10}$ reduction in infectious virus level and the need to retain the desired attributes of the material being decontaminated. This includes inanimate surfaces, such as plasticware in the case of gamma irradiation [11]. To put this in another way, users are not always free to use extremely high fluences of gamma or UVC radiation, or extremely high temperatures as a means of assuring decontamination. Each of these physical approaches are capable of causing unintended damage to biological solutions and material surfaces. Treatment of indoor spaces with UVC radiation must be conducted when those spaces are unoccupied by humans.

7. Conclusions

This chapter represents a review of the literature on physical inactivation of SARS-CoV-2 and other members of the *Coronaviridae*. While physical approaches include X-irradiation, electron beam irradiation, and high pressure treatment, literature on those approaches for inactivation of coronaviruses were not identified during the search. Therefore, the chapter discusses only gamma irradiation, UVC irradiation, and heat inactivation. The *Coronaviridae* in general, and SARS-CoV-2 in particular, appear to be quite susceptible to each of these three physical inactivation approaches. The various approaches have utility for different applications. For instance, of the three approaches, UVC is most useful for indoor air

decontamination, each is useful for liquid or surface inactivation. Each approach has its advantages and disadvantages, which were discussed for the benefit of the reader.

Acknowledgements

No funding was obtained for conduct of this literature review. The authors thank Dr. M. Khalid Ijaz for constructive review of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Author details

Raymond W. Nims[1*] and Mark Plavsic[2]

1 RMC Pharmaceutical Solutions, Inc., Longmont, CO, USA

2 Fate Therapeutics, San Diego, CA, USA

*Address all correspondence to: rnims@rmcpharma.com

References

[1] Nims RW, Plavsic M. Efficacy of electron beam for viral inactivation. Journal of Microbial and Biochemical Technology. 2015;**7**:173-176

[2] Govaris A, Pexara A. Inactivation of foodborne viruses by high-pressure processing (HPP). Foods. 2021;**10**:215. DOI: 10.3390/foods10020215

[3] Anelich LECM, Lues R, Farber JM, Parriera VR. SARS-CoV-2 and risk to food safety. Frontiers in Nutrition. 2020;**7**:580551. DOI: 10.3389/fnut.2020.580551

[4] Nims RW, Gauvin G, Plavsic M. Gamma irradiation of animal sera for inactivation of viruses and mollicutes: A review. Biologicals. 2011;**39**:370-377

[5] Kowalski WJ, Bahnfleth WP, Hernandez MT. A genomic model for predicting the ultraviolet susceptibility of viruses. IUVA News. 2009;**11**:15-28

[6] Nims RW, Plavsic M. Intra-family and inter-family comparisons for viral susceptibility to heat inactivation. Journal of Microbial and Biochemical Technology. 2013;**5**:136-141

[7] Nims R, Plavsic M. Identification of worst-case model viruses for selected viral clearance steps. BioProcessing Journal. 2014;**13**:6-13

[8] Brown S, Croonenborghs B, Head K, Senescu M, Nims R, Plavsic M, et al. Gamma irradiation of animal serum: Maintaining the cold chain throughout the process. BioProcessing Journal. 2018;**17**. DOI: 10.12665/J17OA.Brown

[9] Meunier SM, Sasges MR, Aucoin MG. Evaluating ultraviolet sensitivity of adventitious agents in biopharmaceutical manufacturing. Journal of Industrial and Microbiological Biotechnology. 2017;**44**:893-909

[10] Harris RE, Coleman PH, Morahan PS. Stability of minute virus of mice to chemical and physical agents. Applied Microbiology. 1974;**28**:351-354

[11] Hemmerich K, Fitzgerald R, Hallett D, Nims R, Versteegen R. Gamma irradiation of animal serum: theoretical basis of impacts of gamma irradiation on biological and synthetic polymers. BioProcessing Journal. 2019;**18**

[12] Leung A, Tran K, Audet J, Lavineway S, Bastien N, Krishnan J. In vitro inactivation of SARS-CoV-2 using gamma irradiation. ABSA International. 2020;**25**(3):157-160. DOI: 10.1177/1535676020934242

[13] Jain R, Sarkale P, Mali D, Shete AM, Patil DY, Majumdar T, et al. Inactivation of SARS-CoV-2 by gamma irradiation. Indian Journal of Medical Research. 2021. DOI: 10.4103/ijmr.IJMR_2789_20

[14] Feldmann F, Shupert WL, Haddock E, Twardoski B, Feldmann H. Gamma irradiation as an effective method for inactivation of emerging viral pathogens. American Journal of Tropical Medicine and Hygiene. 2019; **100**(5):1275-1277. DOI: 10.4269/ajtmh.18-0937

[15] Darnell MER, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. Journal of Virological Methods. 2004; **121**:85-91. DOI: 10.1016/j.jviromet.2004.06.006

[16] Kumar M, Mazur S, Ork BL, Postnikova E, Hensley LE, Jahrling PB, et al. Inactivation and safety testing of Middle East respiratory syndrome coronavirus. Journal of Virological Methods. 2015;**223**:13-18. DOI: 10.1016/j.jviromet.2015.07.002

[17] Abolaban FA, Djouider FM. Gamma irradiation-mediated inactivation of enveloped viruses with conservation of genome integrity: potential application for SARS-CoV-2 inactivated vaccine development. Open Life Sciences. 2021; **16**:558-570

[18] Rathnasinghe R, Jangra S, Miorin L, Schotsaert M, Yahnke C, Garcia-Sastre A. The virucidal effects of 405 nm visible light on SARS-CoV-2 and influenza A virus. Scientific Reports. 2021;**11**:19470. DOI: 10.1038/s41598-021-97797-0

[19] Boegel SJ, Gabriel M, Sasges M, Petri B, D'Agostino MR, et al. Robust evaluation of ultraviolet-C sensitivity for SARS-CoV-2 and surrogate coronaviruses. Microbiology Spectrum. 2021;**9**(2):e00537-e00521. DOI: 10.1128/Spectrum.00537-21

[20] Hadi J, Dunowska M, Wu S, Brightwell G. Control measures for SARS-CoV-2: A review on light-based inactivation of single-stranded RNA viruses. Pathogens. 2020;**9**:737. DOI: 10.3390/pathogens9090737

[21] Pendyala B, Patras A, Pokharel B, D'Souza D. Genomic modeling as an approach to identify surrogates for use in experimental validation of SARS-CoV-2 and HuNoV inactivation by UV-C treatment. Frontiers in Microbiology. 2020;**11**:572331. DOI: 10.3389/fmicb.2020.572331

[22] Patterson EI, Prince T, Anderson ER, Casas-Sanchez A, Smith SL, Cansado-Utrilla C, et al. Methods of inactivation of SARS-CoV-2 for downstream biological assays. Journal of Infectious Diseases. 2020;**222**: 1462-1467

[23] Storm N, McKay LGA, Downs SN, Johnson RI, Birru D, de Samber M, et al. Rapid and complete inactivation of SARS-CoV-2 by ultraviolet-C irradiation. Scientific Reports. 2020;**10**: 22421. DOI: 10.1038/s41598-020-79600-8

[24] Biasin M, Bianco M, Pareschi G, Cavalleri A, Cavatorta C, Fenizia C, et al. UV-C irradiation is highly effective in inactivating SARS-CoV-2 replication. Scientific Reports. 2021;**11**:6260. DOI: 10.1038/s41598-021-85425-w

[25] Inagaki H, Saito A, Sugiyama H, Okabayashi T, Fujimoto S. Rapid inactivation of SARS-CoV-2 with deep-UV LED irradiation. Emerging Microbes & Infections. 2020;**9**. DOI: 10.1080/22221751.2020.1796529

[26] Heilingloh CS, Aufderhorst UW, Schipper L, Dittmer U, Witzke O, Yang D, et al. Susceptibility of SARS-CoV-2 to UV irradiation. American Journal of Infection Control. 2020;**48**:1273-1275

[27] Minamikawa T, Koma T, Suzuki A, Mizuno T, Nagamatsu K, Arimochi H, et al. Quantitative evaluation of SARS-CoV-2 inactivation using a deep ultraviolet light-emitting diode. Scientific Reports. 2021;**11**:5070. DOI: 10.1038/s41598-021-84592-0

[28] Sabino CP, Sellera FP, Sales-Medina DF, Machado RRG, Durigon EL, Freitas-Junior LH, et al. UV-C (254 nm) lethal doses for SARS-CoV-2. Photodiagnosis and Photodynamic Therapy. 2020;**32**: 101995. DOI: 10.1016/j.pdpdt.2020.101995

[29] Kariwa H, Fujii N, Takashima I. Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. Dermatology. 2006;**212**(suppl. 1): 119-123

[30] Duan S-M, Zhao X-S, Wen R-F, Huang J-J, Pi G-H, Zhang S-X, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomedical and

Environmental Sciences. 2003;**16**: 246-255

[31] Ma B, Linden YS, Gundy PM, Gerba CP, Sobsey MD, Linden KG. Inactivation of coronaviruses and phage Phi6 from irradiation across UVC wavelengths. Environmental Science & Technology Letters. 2021;**8**(5):425-430

[32] Gerchman Y, Mamane H, Friedman N, Mandelboim M. UV-LED disinfection of coronavirus: wavelength effect. Journal of Photochemistry and Photobiology. B. 2020;**212**:112044. DOI: 10.1016/j.jphotobiol.2020.112044

[33] Rozenberg Y, Lowentahl A. Can we use adenovirus validated ultraviolet systems for inactivation of SARS-CoV-2, the virus that causes COVID-19? Water ONLINE. 2020. Available from: https://www.wateronline.com/doc/can-we-use-adenovirus-validated-uv-systems-cov-the-virus-that-causes-covid-0001

[34] Kitagawa H, Nomura T, Nazmul T, Omori K, Shigemoto N, Sakaguchi T, et al. Effect5iveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. American Journal of Infection Control. 2021;**49**: 299-301

[35] Fischer RJ, Morris DH, van Doremalen N, Sarchette S, Matson MJ, Bushmaker T, et al. Effectiveness of N95 respirator decontamination and reuse against SARS-CoV-2 virus. Emerging Infectious Diseases. 2020;**26**(9): 2253-2255

[36] Buonanno M, Welch D, Shuryak I, Brenner DJ. Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. Scientific Reports. 2020;**10**:10285. DOI: 10.1038/s41598-020-67211-2

[37] Walker CM, Ko G. Effect of ultraviolet germicidal irradiation on viral aerosols. Environmental Science and Technology. 2007;**41**:5460-5465

[38] Deshmukh DR, Pomeroy BD. Ultraviolet inactivation and photoreactivation of avian viruses. Avian Diseases. 1969;**13**(**3**):596-602

[39] Chiappa F, Frascella B, Vigezzi GP, Moro M, Diamanti L, Gentile L, et al. The efficacy of ultraviolet light-emitting technology against coronaviruses: A systematic review. Journal of Hospital Infection. 2021;**114**:63-78

[40] Helßling M, Hönes K, Vatter P, Lingenfelder C. Ultraviolet irradiation doses for coronavirus inactivation: Review and analysis of coronavirus photoinactivation studies. GMS Hygiene and Infection Control. 2020;**15**

[41] Bedell K, Buchaklian A, Perlman S. Efficacy of an automated multi-emitter whole room UV-C disinfection system against coronaviruses MHV and MERS-CoV. Infection Control and Hospital Epidemiology. 2016;**37**(5):598-599

[42] Hitchman M. A new perspective of the chemistry and kinetics of inactivation of COVID-19 coronavirus aerosols. Future Virology. 2021;**15**(12): 823-835

[43] Buchan AG, Yang L, Atkinson KD. Predicting airborne coronavirus inactivation by far-UVC in populated rooms using a high-fidelity coupled radiation-CFD model. Scientific Reports. 2020;**10**:19659. DOI: 10.1038/s41598-020-76597-y

[44] de Abajo FJG, Hernández RJ, Kaminer I, Meyerhans A, Rosell-Llompart J, Sanchez-Elsner T. Back to normal: An old physics route to reduce SARS-CoV-2 transmission in indoor spaces. ACS Nano. 2020;**14**(7): 7704-7713

[45] Criscuolo E, Diotti RA, Ferrarese R, Alippi C, Viscardi G, Signorelli C, et al. Fast inactivation of SARS-CoV-2 by UV-C and ozone exposure on different materials. Emerging Microbes &

Infections. 2021;**10**. DOI: 10.1080/22221751.2021.1872354

[46] Rathnasinghe R, Karlicek RF Jr, Schotsaert M, Koffas M, Arduini BL, Jangra S, et al. Scalable, effective, and rapid decontamination of SARS-CoV-2 contaminated N95 respirators using germicidal ultraviolet C (UVC) irradiation device. Scientific Reports. 2021;**1**:19970. DOI: 10.1038/s41598-021-99431-5

[47] Derraik JGB, Anderson WA, Connelly EA, Anderson YC. Rapid review of SARS-CoV-1 and SARS-CoV-2 viability, susceptibility to treatment, and the disinfection and reuse of PPE, particularly filtering facepiece respirators. International Journal of Environmental Research and Public Health. 2020;**17**(17):6117. DOI: 10.3390/ijerph17176117

[48] Zhou SS, Wilde C, Chen Z, Kapes T, Purgill J, Nims R, et al. Carrier and liquid heat inactivation of poliovirus and adenovirus. In: Disinfection. London, UK: InTech; 2018. p. 76340

[49] Zhou SS, Wilde C, Chen Z, Kapes T, Purgill J, Nims, et al. Thermal inactivation of feline calicivirus and herpes simplex virus type 1: side-by-side suspension and carrier studies. Integrative Journal of Veterinary Biosciences 2020;**4**:1-6.

[50] Nims R, Zhou SS, Plavsic M. Identification of worst-case model viruses for low and high pH inactivation. BioProcessing Journal. 2017;**16**:7-14

[51] Batéjat C, Grassin Q, Manuguerra J-C, Leclercq I. Heat inactivation of the severe acute respiratory syndrome coronavirus 2. Journal of Biosafety and Biosecurity. 2021;**3**:1-3. DOI: 10.1016/j.jobb.2020.12.001

[52] 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. Scientific Reports. 2021;**11**:2418

[53] Burton J, Love H, Richards K, Burton C, Summers S, Pitman J, et al. The effect of heat-treatment on SARS-CoV-2 viability and detection. Journal of Virological Methods. 2021;**290**:114087. DOI: 10.1016/j.jviromet.2021.114087

[54] Kim Y-I, Casel MAB, Kim S-M, Kim S-G, Park S-J, Kim E-H, et al. Development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) thermal inactivation method with preservation of diagnostic sensitivity. Journal of Microbiology. 2020;**58**(10):886-891

[55] Pastorino B, Touret F, Gilles M, de Lamballerie X, Charrel RN. Heat inactivation of different types of SARS-CoV-2 samples: What protocols for biosafety, molecular detection and serological diagnostics? Viruses. 2020;**12**:735

[56] Auerswald H, Yann S, Dul S, In S, Dussart P, Martin NJ, et al. Assessment of inactivation procedures for SARS-CoV-2. Journal of General Virology. 2021;**102**:001539. DOI: 10.1099/jgv.0.001539

[57] Darnell MER, Taylor DR. Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products. Transfusion. 2006;**46**:1770-1777

[58] Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Medical and Microbiological Immunology. 2005;**194**:1-6

[59] Pagat A-M, Seux-Goepfert R, Lutsch C, Lecouturier V, Saluzzo J-F, Kusters IC. Evaluation of SARS-coronavirus decontamination procedures. Applied Biosafety. 2007;**12**(2):100-108

[60] Leclercq I, Batéjat C, Burguière AM, Manuguerra J-C. Heat inactivation of the Middle East respiratory syndrome coronavirus. Influenza and Other Respiratory Viruses. 2014;**8**(5):585-586

[61] Hofmann M, Wyler R. Quantitation, biological and physicochemical properties of cell culture-adapted porcine epidemic diarrhea coronavirus (PEDV). Veterinary Microbiology. 1989;**20**:131-142

[62] Hulst MM, Heres L, Hakze-van der Honing RW, Pelser M, Fox M. van der Poel WHM. Study on inactivation of porcine epidemic diarrhea virus, porcine sapelovirus 1 and adenovirus in the production and storage of laboratory spray-dried porcine plasma. Journal of Applied Microbiology. 2019;**126**: 1931-1943

[63] Saknimit M, Inatsuki I, Sugiyama Y, Yagami K-i. Virucidal efficacy of physico-chemical treatments against coronaviruses and parvoviruses of laboratory animals. Jikken Dobutsu. 1988;**37**(3):341-345

[64] Christianson KK, Ingersoll JD, Landon RM, Pfieffer NE, Gerber JD. Characterization of a temperature sensitive feline infectious peritonitis coronavirus. Archives of Virology. 1989; **109**:185-196

[65] Millar BC, Moore JE. Re-purposing of domestic steam disinfectors within the Hospital-at-Home setting: reconciliation of steam disinfector thermal performance against SARS-CoV-2 (COVID-19), norovirus and other viruses' thermal susceptibilities. Infection, Disease & Health. 2021;**26**: 156-159

[66] Guillier L, Martin-Latil S, Chaix E, Thébault A, Pavio N, Le Poder S, et al. Modeling the inactivation of viruses from the Coronaviridae family in response to temperature and relative humidity in suspensions or on surfaces. Applied and Environmental Microbiology. 2020;**86**(18):e01244-e01220. DOI: 10.1128/AEM.01244-20

[67] Kampf G, Voss A, Scheithauer S. Inactivation of coronaviruses by heat. Journal of Hospital Infection. 2020;**105**: 348-349

[68] Jiang Y, Zhang H, Wippold JA, Gupta J, Dai J, de Figueiredo P, et al. Sub-second heat inactivation of coronavirus using a betacoronavirus model. Biotechnology and Bioengineering. 2021;**118**(5):2067-2075

[69] Biryukov J, Boydston JA, Dunning RA, Yeager JJ, Wood S, Reese AL, et al. Increasing temperature and relative humidity accelerates inactivation of SARS-CoV-2 on surfaces. mSphere. 2020;**5**(4):e00441-e00420. DOI: 10.1128/mSphere.00441-20

[70] Morris DH, Yinda KC, Gamble A, Rossine FW, Huang Q, Bushmaker T, et al. Mechanistic theory predicts the effects of temperature and humidity on inactivation of SARS-CoV-2 and other enveloped viruses. eLife. 2021;**10**: e65902

[71] Singh G, Jorgenson J, Pringle T, Nelson T, Ramamoorthy S. Monitoring SARS-CoV-2 decontamination by dry heat and ultraviolet treatment with a swine coronavirus as a surrogate. Infection Prevention in Practice. 2021;**3**:100103. DOI: 10.1016/j.infpip.2020.100103

[72] Biryukov J, Boydston JA, Dunning RA, Yeager JJ, Wood S, Ferris A, et al. SARS-CoV-2 is rapidly inactivated at high temperature. Environmental Chemistry Letters. 2021;**19**:1773-1777

[73] Daeschler SC, Manson N, Joachim K, Chin AWH, Chan K, Chen PZ, et al. Effect of moist heat reprocessing of N95 respirators on SARS-CoV-2 inactivation and respirator function. CMAJ. 2020; **192**:E1189-E1197

[74] Nims R, Plavsic M. A proposed modeling approach for comparing the heat inactivation susceptibility of viruses. Bioprocessing Journal. 2013; **12**(2):25-35

[75] Yap TF, Liu Z, Shveda RA, Preston DJ. A predictive model of the temperature-dependent inactivation of coronaviruses. Applied Physics Letters. 2020;**117**:060601. DOI: doi.org/10.1063/5.0020782

[76] Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen H-L, Chan MCW, et al. Stability of SARS-CoV-2 in different environmental conditions. The Lancet Microbe. 2020;**1**(1):e10. DOI: 10.1016/S2666-5247(20)30003-3

[77] Wang TT, Lien CZ, Liu S, Selvaraj P. Effective heat inactivation of SARS-CoV-2. medRxiv (preprint). 2020. DOI: 10.1101/2020.04.29.20085498

[78] Laude H. Thermal inactivation studies of a coronavirus, transmissible gastroenteritis virus. Journal of General Virology. 1981;**56**:235-240

[79] Pratelli A. Canine coronavirus inactivation with physical and chemical agents. The Veterinary Journal. 2008; **177**:71-79

[80] Quist-Rybachuk GV, Nauwynck HJ, Kalmar ID. Sensitivity of porcine epidemic diarrhea virus (PEDV) to pH and heat treatment in the presence or absence of porcine plasma. Veterinary Microbiology. 2015; **181**(3–4):283-288

[81] Buhr TL, Young AA, Borgers-Klonkowski E, Kennihan NL, Barnette HK, Minter ZA, et al. Hot, humid air decontamination of aircraft confirmed that high temperature and high humidity are critical for inactivation of infectious enveloped ribonucleic acid (RNA) virus. Frontiers in Bioengineering and Biotechnology. 2020;**8**:592621. DOI: 10.3389/fbioe.2020.592621

[82] United States Environmental Protection Agency. Office of Pesticide Programs. Final Summary of the Disinfection Hierarchy Workshop. 2016. Available from: https://www.epa.gov/sites/production/files/2016-02/documents/dh_final_summary_of_workshop_-exploration_of_the_disinfection_hierarchy_meeting_summary_final_docx_2_4_16.pdf

[83] Taylor DM. Inactivation of prions by physical and chemical means. Journal of Hospital Infection. 1999;**43**(suppl 1): S69-S76

[84] Giles K, Woerman AL, Berry DB, Prusiner SB. Bioassays and inactivation of prions. Cold Spring Harbor Perspectives in Biology. 2017;**9**(8): a023499. DOI: 10.1101/cshperspect.a023499

[85] Klein M, Deforest A. Principles of viral inactivation. In: Block SS, editor. Disinfection, Sterilization, and Peservation. 3rd ed. Philadelphia: Lea and Febiger; 1983. pp. 422-434

[86] Sattar SA. Hierarchy of susceptibility of viruses to environmental surface disinfectants: A predictor of activity against new and emerging viral pathogens. Journal of the AOAC International. 2007;**90**(6): 1655-1658

[87] Ijaz MK, Rubino JR. Should test methods for disinfectants use vertebrate virus dried on carriers to advance virucidal claims? Infection Control and Hospital Epidemiology. 2008;**29**(2): 192-194

[88] Ijaz MK, Sattar SA, Rubino JR, Nims RW, Gerba CP. Combating SARS-CoV-2: leveraging microbicidal experiences with other emerging/re-emerging viruses. PeerJ. 2020;**8**:e9914. DOI: 10.7717/peerj.9914