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Carrier and Liquid Heat Inactivation of Poliovirus and Adenovirus

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Abstract

Viral inactivation is typically studied using virus suspended in liquid (liquid inactivation) or virus deposited on surfaces (carrier inactivation). Carrier inactivation more closely mimics disinfection of virus contaminating a surface, while liquid inactivation mimics virus inactivation in process solutions. The prevailing opinion has been that viruses are more susceptible to heat inactivation when suspended in liquid than when deposited on surfaces. In part, this reflects a paucity of comparative studies performed in a side-by-side manner. In the present study, we investigated the relative susceptibilities of the enteroviruses poliovirus-1 and adenovirus type 5 to heat inactivation in liquid versus carrier studies. The results of our side-by-side studies suggest that these two viruses are more readily inactivated when heat is applied to virus deposited on carriers. Decimal reduction values (i.e., the amount of time required to reduce the virus titer by one $\log_{10}$) measured at 46°C displayed the greatest difference between carrier and liquid inactivation approaches, with values ranging from 14.0 to 15.2 min (carrier) and from 47.4 to 64.1 min (liquid) for poliovirus. The corresponding values for adenovirus 5 were 18.2–29.2 min (carrier) and 20.8–38.3 min (liquid). At 65°C, the decimal reduction values were more similar (from 4 to 6 min) for the various inactivation approaches.
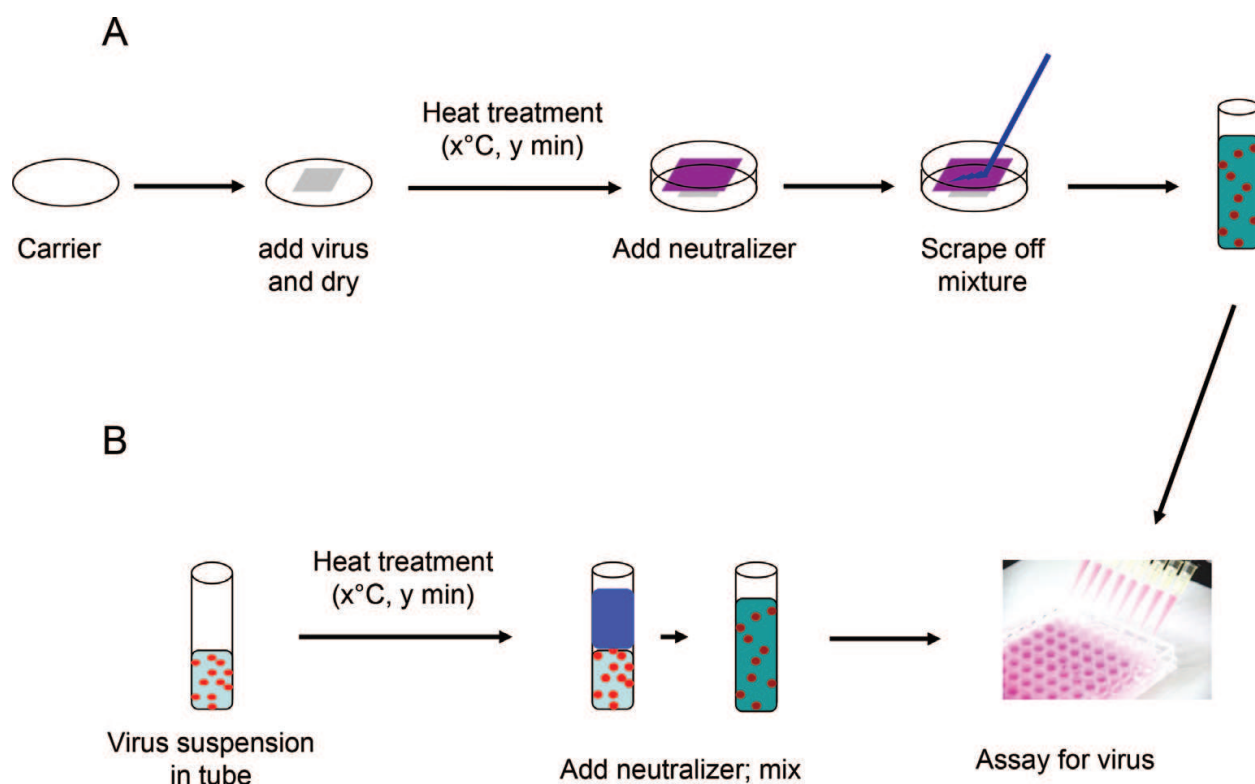
Keywords: adenovirus, carrier inactivation, enterovirus, liquid inactivation, poliovirus, thermal inactivation

1. Introduction

Heat (thermal) inactivation is one of several physical approaches that may be employed to inactivate viruses suspended in solutions or deposited on surfaces. Unlike chemical inactivation

approaches that often display greater efficacy for lipid-enveloped viruses than for nonenveloped viruses, heat inactivation has been found to display effectiveness for both enveloped and non-enveloped viruses [1]. Heating appears to open the viral capsid, exposing the genomic material to nucleases present in the immediate environment [2, 3]. Therefore, the capsid conformation appears to be the main determinant of heat inactivation susceptibility [3, 4], not the envelope status.

In the past, heat inactivation has more typically been evaluated in liquid inactivation studies. In these studies, a solution of known virus titer is heated at a given temperature for a given amount of time and the final titer is measured (**Scheme 1**). A decimal reduction value (D) in units of time required for one $\log_{10}$ decrease in titer is then calculated. Such studies are appropriate when evaluating the effectiveness of inactivation processes aimed at virus infectivity reduction in solutions (e.g., pasteurization). When the susceptibility of viruses deposited on a surface to heating is to be evaluated, such studies are most appropriately performed using carriers (**Scheme 1**) [5, 6]. A known amount of virus is applied to the carriers (small representative pieces of a given material type) and allowed to dry in the absence or presence of a matrix (such as blood, saline, or culture medium). After a given drying time, the carriers and virus deposited thereon are subjected to a given duration of heating at a given temperature. The remaining infectious virus is recovered from the carriers and is measured and, again, a $\log_{10}$ reduction value and corresponding D value may be determined.

A

Heat treatment
(x°C, y min)

Carrier   add virus   Add neutralizer   Scrape off
                 and dry   mixture

B

Heat treatment
(x°C, y min)

Virus suspension   Add neutralizer; mix   Assay for virus
in tube

Scheme 1. High-level flow diagrams for carrier (A) and liquid (B) inactivation study design.

There have been relatively few studies that have evaluated heat inactivation of viruses on carriers [5–11], and we are aware of only a single study directly comparing liquid and carrier heat inactivation in a side-by-side format [11]. The prevailing opinion has been that viruses are more susceptible to heating in liquid than when deposited on surfaces and that dry heat efficacy is related to residual moisture or relative humidity [7, 9–12]. In order to clarify the relative susceptibilities of model enteroviruses to liquid and carrier inactivation, we have evaluated poliovirus-1 (PV-1; family Picornaviridae) and adenovirus type 5 (Ad5; family Adenoviridae) inactivation in two liquid matrices (medium containing 5% serum [medium] or undiluted fetal bovine serum [serum]) or when deposited on two carrier materials (stainless steel [Steel] or glass). The two enteroviruses may be transmitted by the fecal-oral route and therefore ability to inactivate viruses dried onto surfaces following deposition from contaminated water is of public health interest. See **Box 1** for information about poliovirus, adenovirus and associated disease.

Box 1. Poliovirus, adenovirus, and associated disease. The majority of PV-1 infections result in an abortive flu-like prodrome or are asymptomatic. In ~5% of infections, a meningitic phase follows the prodrome as the virus displays a predilection for the nervous system [13]. Spinal poliomyelitis with varying degrees of flaccid weakness follows shortly in some cases, while a bulbar form with minimal limb involvement but higher mortality can also occur. Interestingly, the "summer plague" of poliomyelitis that was experienced between 1916 and the advent of vaccination in the mid-1950s has been attributed in part to improvements in community sanitation [13] occurring around the turn of the century. The herd immunity that previously existed due to early infection coinciding with presence of maternal antibodies was lost when sanitation improved. Acquisition of the infection later in childhood was associated with a greater chance for poliomyelitis. Poliomyelitis still occurs in certain underdeveloped regions of the world, despite efforts at global eradication.

Adenoviruses can cause respiratory and gastrointestinal infections. Adenovirus types 40 and 41 represent common cases of infantile gastroenteritis, although most of the 41 types of adenovirus may be recovered from the feces of patients. These enteroviruses may be spread by the fecal-oral route. Contamination of water supplies and fomites (environmental surfaces) can lead to transmission of the enteritis from infected to noninfected individuals [14].

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2. Materials and methods

2.1. Viruses

Poliovirus type 1 (PV-1), strain Chat, was propagated in rhesus monkey kidney LLC-MK2 derivative cells (American Type Culture Collection CCL-7.1). The virus was diluted in Roswell Park Memorial Institute (RPMI) medium supplemented with 5% newborn calf serum (NCS, source: ThermoFisher Scientific, Waltham, MA) and added to T-75 flasks of the LLC-MK2 cells. The flasks were incubated at $36 \pm 2°C$ with $5 \pm 1\%$ CO_2 for 90 min to allow for viral adsorption, after which they were refed with growth medium. Incubation was continued at $36 \pm 2°C$ with $5 \pm 1\%$ CO_2 until 90% of the cells exhibited viral cytopathic effect (CPE). The flasks were frozen at $-80°C$ and then thawed at room temperature. The medium from the flasks was collected and clarified by centrifugation at 2000 rpm for 15 min and the resulting

supernatant was aliquoted and stored at −80°C until use. The certified titer of the stock PV-1 was determined to be 6.79 $\log_{10}$ tissue culture infective dose$_{50}$ per mL (TCID$_{50}$/mL) in MA-104 cells (Charles River Laboratories, Germantown, MD).

Adenovirus type 5 (Ad5), strain Adenoid 75, was propagated in human lung epithelial A549 cells (American Type Culture Collection CCL-185). The virus was diluted in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS, source: ThermoFisher Scientific, Waltham, MA) and added to T-75 flasks of the A549 cells. The flasks were incubated at 36 ± 2°C with 5 ± 1% CO_2 for 90 min to allow for viral adsorption, after which they were refed with the growth medium. Incubation was continued at 36 ± 2°C with 5 ± 1% CO_2 until 100% of the cells exhibited viral CPE. The flasks were frozen at −80°C and then thawed at room temperature. The medium from the flasks was collected and clarified by centrifugation at 2000 rpm for 15 min and the resulting supernatant was aliquoted and stored at −80°C until use. The certified titer of the stock Ad5 virus stock was determined to be 7.01 $\log_{10}$ TCID$_{50}$/mL in A549 cells.

2.2. Carriers and liquid matrices

Glass carriers consisted of 4-in^2 area of a sterile glass Petri dish. Steel carriers consisted of brushed stainless steel discs of 1 cm in diameter. The serum matrix consisted of undiluted FBS, while the medium matrix consisted of RPMI medium containing 5% NCS for PV-1 and DMEM medium containing 5% FBS for Ad5.

2.3. Evaluation of heat inactivation (duplicate replicates)

Virus was spread onto the glass carriers (0.4 mL virus suspension) or steel carriers (0.05 mL virus suspension) and allowed to dry at room temperature (20–21°C) per ASTM International (ASTM) standard E1053 [15]. For liquid inactivation, 0.2 mL of virus suspension was added to 1.8 mL of serum or medium in glass tubes per ASTM standard E1052 [16].

Carriers containing virus were placed into a hot-air oven (Isotemp™ General Purpose, Fisher Scientific Catalog No. 151030509) set at one of three test temperatures (46, 56 and 65°C) for 5, 20, or 60 min. The relative humidity of the oven was not measured.

Glass tubes containing virus/medium or virus/serum solutions prepared as described earlier were placed into a hot air oven set at one of three test temperatures (46, 56 and 65°C) for 5, 20, or 60 min. The relative humidity of the oven was not measured.

Following the heating times, 4 mL of neutralizer (FBS) was added to the virus film on the glass or steel carriers and used to remove the film from the surface with cell scrapers. The liquid heat inactivation conditions were neutralized following heating by addition of 2 mL of cold neutralizer.

Post-neutralization samples were serially diluted and selected dilutions were inoculated onto the proper host cells for each virus (8-wells per dilution in 96-well plates). A virus recovery

control (VRC) was included to determine the relative loss in virus infectivity as a result of drying and neutralization. Virus was applied to the carriers (glass or steel) or added to liquids (serum or medium) and held at room temperature ($20 \pm 1°C$) for the longest contact time evaluated (60 min). The resulting $TCID_{50}$/mL titer results for the VRC were then compared to heat-treated titers for the corresponding carrier/matrix type to calculate the reduction in infectivity caused by heat treatment. The various 96-well plates were incubated at $36 \pm 2°C$ with $5 \pm 1\%$ CO_2 for 6–9 days (PV-1) or 11–14 days (Ad5). Following incubation, the plates were scored for CPE. The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) was calculated using the Spearman-Kärber formula [17].

2.4. Calculation of D and z values and power function analysis

Decimal reduction (D) values were estimated from the most linear portions of the inactivation versus time curves for the various set temperatures (not shown). The plots included both replicate values for any given temperature and time point, therefore represent an analysis of the pooled replicate data, with a single D value being generated. Rapid deviation from linearity in these plots was noted as complete inactivation of virus occurred rapidly at the higher temperatures. We acknowledge that a certain degree of error is associated with the D value estimation process. Such errors do not detract from the validity of the comparisons to be made between carrier and liquid inactivation results, since comparison of the raw inactivation versus time results obtained leads to similar conclusions.

The z value (°C per $\log_{10}$ change in D) for a given data set was obtained from plots of $\log_{10}D$ versus temperature (not shown), evaluated using the linear regression function of Excel. The z value is obtained as 1/slope (m) from the linear fit equation (Eq. (1)):

$$y = mx + b \tag{1}$$

where y = $\log_{10}D$, x = temperature, m = slope and b = y-axis intercept.

Plots of D versus temperature were evaluated using the power function of Excel to obtain the line fit equation (Eq. (2)):

$$y = ax^{-b} \tag{2}$$

where y = D, x = temperature and a and b are constants unique to each line fit equation. This equation allows one to extrapolate the D value at any given inactivation temperature and can also be rearranged to solve for temperature, as shown in (Eq. (3)).

$$temperature\ (°C) = \left(\frac{D}{a}\right)^{-\frac{1}{b}} \tag{3}$$

allowing one to estimate the inactivation temperature required to achieve a desired D value [18] (see also discussion later).

3. Results

3.1. Carrier and liquid heat inactivation results for PV-1

Replicate results for heat inactivation of PV-1 on carriers or in solutions are displayed in **Table 1**. Three exposure times (5, 20 and 60 min) and three temperatures (46, 56 and 65°C) were evaluated.

Mode	Inactivation matrix	Inactivation time (min)	Log_{10} reduction at inactivation temperature		
			46°C	56°C	65°C
Carrier inactivation					
	Glass	5	−0.25[a]	0.00	0.25
		5	−0.50	1.50	0.50
		20	−0.25	≥ 4.86	5.21
		20	0.00	≥ 5.72	≥ 5.10
		60	4.26	≥ 4.85	≥ 4.97
		60	4.71	≥ 5.72	≥ 5.10
	Steel	5	−0.25	0.25	0.25
		5	0.37	0.87	0.50
		20	1.63	≥ 4.97	≥ 4.72
		20	1.37	≥ 5.22	≥ 4.85
		60	≥ 4.35	≥ 4.97	≥ 4.72
		60	≥ 4.22	≥ 5.22	≥ 4.85
Liquid inactivation					
	Medium	5	0.00	0.00	0.12
		5	0.00	−0.13	0.75
		20	0.13	2.25	≥ 5.22
		20	0.13	2.12	≥ 5.60
		60	1.13	≥ 5.10	≥ 5.22
		60	0.88	≥ 4.22	≥ 5.60
	Serum	5	−0.25	0.37	0.00
		5	0.13	0.00	0.50
		20	0.00	2.12	≥ 5.22
		20	−0.12	2.00	5.38
		60	1.38	≥ 4.97	≥ 5.22
		60	1.50	≥ 4.35	≥ 5.47

[a]The values indicate the log_{10} reduction (log_{10} titer heated – log_{10} titer for VRC) for two replicates per time point. Values shown as "≥" indicate complete inactivation.

Table 1. Heat inactivation data for PV-1.

The results of a virus recovery control for the virus stock have been subtracted from the $\log_{10}$ reduction values displayed in this table. This corrects for any loss of infectivity associated with drying of the virus stock and recovery after a 1-h hold at room temperature. A striking difference in carrier versus liquid inactivation was noted for the 46°C study. The PV-1 heated on steel carriers was completely inactivated (≥ 4.2 $\log_{10}$) in 60 min.

On glass carriers, 4.3–4.7 $\log_{10}$ PV-1 inactivation occurred in 60 min. During this time frame, less than 1.5 $\log_{10}$ inactivation of PV-1 occurred when liquid heating was compared. In the 56°C study, greater inactivation occurred on carriers by 20 min, compared to virus heated in solution. In the 65°C study, similar inactivation occurred for virus heated on carriers or in solution, regardless of the inactivation time.

In order to reduce the heat inactivation data for PV-1 to a form usable for comparisons between viruses and between matrices/carriers, D values (minutes required for 1 $\log_{10}$ titer reduction) were estimated from the most linear portions of the inactivation versus time curves for the various set temperatures. The D values, displayed in **Table 2**, were then used to generate $\log_{10}D$ versus temperature curves from which z values (°C per $\log_{10}$ change in D) were obtained. Plots of D versus temperature (**Figure 1**) depict a surface along which the D required for 1 $\log_{10}$ inactivation at any given heating temperature is displayed.

3.2. Carrier and liquid heat inactivation results for Ad5

Replicate results for heat inactivation of Ad5 on carriers and in solutions are shown in **Table 3**. These studies involved the same temperatures and exposure times used for the PV-1 studies described earlier. The $\log_{10}$ reduction values have again been corrected for the virus recovery control. In the case of Ad5, differences in susceptibility to heat inactivation on glass carriers, relative to steel carriers, were noted at each temperature, with greater inactivation at any

Temperature	D values (min)				
	Glass		Steel	Medium	Serum
46°C	15.2		14.0	64.1	47.4
56°C	3.9		4.1	12.5	10.1
65°C	4.0		4.4	3.9	9.3
	z values (°C per $\log_{10}$ change in D)				
	32		37	16	27
	Power function coefficients				
a	6×10^7		8×10^6	2×10^{15}	5×10^9
b	4.02		3.50	8.11	4.87

Table 2. Estimated D, z and power function values for PV-1.

exposure time being observed on steel carriers. In general, heat inactivation on carriers was found to be similar to that observed in solutions, with no clear differences noted between temperature dependence and time kinetics.
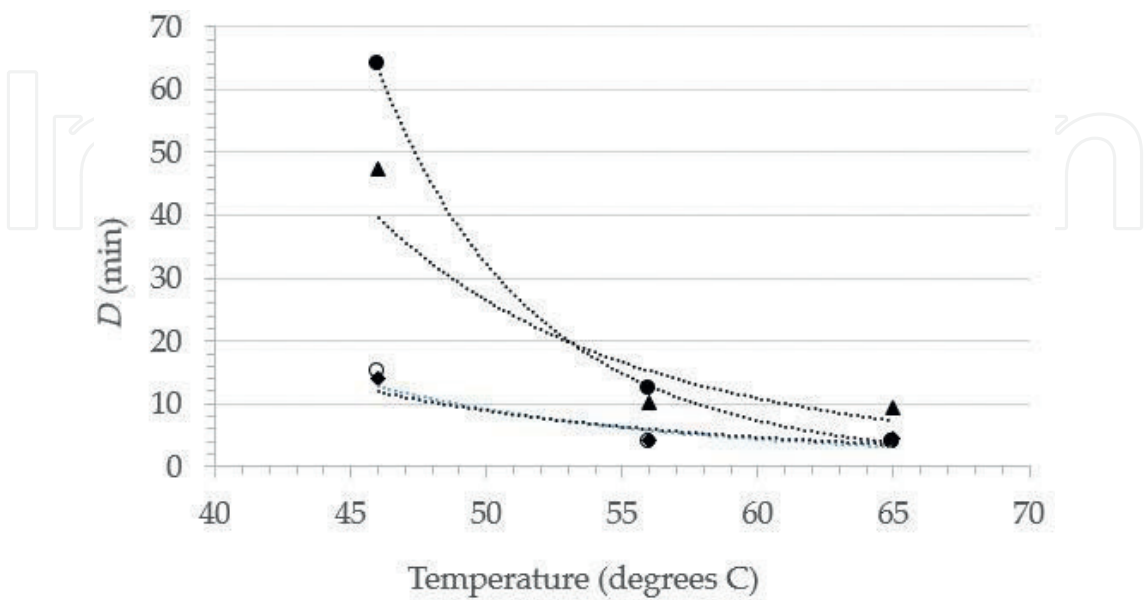

Figure 1. *D* vs. temperature relationships for heat inactivation of PV-1 on Steel (◆) or Glass (O) carriers and Medium (●) or Serum (▲) liquid matrices. All points along the fit lines represent 1 log$_{10}$ inactivation of PV-1.

Mode	Inactivation matrix	Inactivation time (min)	Log$_{10}$ reduction at inactivation temperature		
			46°C	56°C	65°C
Carrier inactivation					
	Glass	5	1.12[a]	1.25	1.63
		5	0.50	1.00	2.00
		20	2.00	1.63	2.88
		20	0.88	1.00	2.75
		60	2.37	4.85	≥4.10
		60	1.13	4.47	≥4.10
	Steel	5	0.62	−0.25	0.75
		5	0.88	−0.13	−0.37
		20	2.25	3.20	3.85
		20	1.63	3.12	3.10
		60	3.10	≥ 3.97	≥ 3.85
		60	2.86	≥ 4.22	≥ 3.10

Mode	Inactivation matrix	Inactivation time (min)	Log$_{10}$ reduction at inactivation temperature		
			46°C	56°C	65°C
Liquid inactivation					
	Medium	5	0.25	−0.12	0.63
		5	0.38	−0.25	−0.25
		20	0.37	1.13	4.10
		20	0.63	1.75	3.35
		60	1.37	4.10	≥ 4.10
		60	1.75	4.10	≥ 3.35
	Serum	5	0.50	−0.37	0.62
		5	0.63	0.25	0.25
		20	0.38	2.25	4.22
		20	1.00	1.25	3.85
		60	2.63	4.10	≥ 4.22
		60	3.25	4.35	≥ 4.85

[a]The values indicate the log$_{10}$ reduction (log$_{10}$ titer heated – log$_{10}$ titer for VRC) for two replicates per time point. Values shown as "≥" indicate complete inactivation.

Table 3. Heat inactivation data for Ad5.

Temperature	D values (min)			
	Glass	Steel	Medium	Serum
46°C	29.2	18.2	38.3	20.8
56°C	12.9	6.8	14.7	14.0
65°C	6.5	6.0	5.6	5.1
	z values (°C per log$_{10}$ change in D)			
	29	39	23	32
	Power function coefficients			
a	5×10^{8}	5×10^{6}	6×10^{10}	8×10^{7}
b	4.34	3.28	5.51	3.95

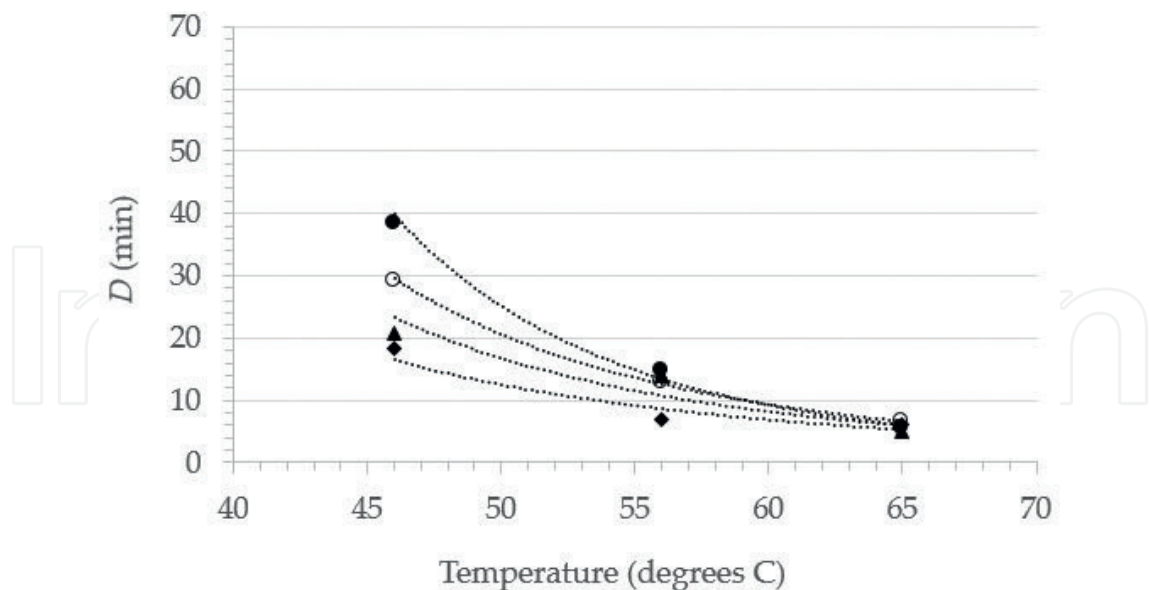**Table 4.** Estimated *D, z* and power function values for Ad5.

Figure 2. *D* vs. temperature relationships for heat inactivation of Ad5 on Steel (◆) or Glass (O) carriers and Medium (●) or Serum (▲) liquid matrices. All points along the fit lines represent 1 $\log_{10}$ inactivation of Ad5.

This conclusion may also be reached through examination of the calculated *D* and *z* values (**Table 4**) and the power function curves displaying the relationship between *D* and temperature (**Figure 2**). In no case was complete inactivation of the virus observed in exposure times under 60 min and with the exception of heating on steel carriers, complete inactivation was not observed at temperatures under 65°C.

4. Discussion of study results

A recent paradigm shift in virology has been the recognition of the important role of fomites (environmental porous and nonporous surfaces) in disseminating infectious virus (reviewed in [19, 20]). With this recognition has come a movement toward the conduct of carrier studies (in lieu of solution inactivation studies) to evaluate survival of viruses on typical fomite surfaces (glass, stainless steel, plastic, Formica, etc.) and to determine the efficacy of inactivation approaches for disinfection of contaminated fomites. This is not to say that carrier studies were not performed previously (e.g., [21]), but the literature for carrier inactivation of viruses was relatively sparse prior to the turn of the century. Arguments for and methodologies for conduct of carrier studies have become more common within the past two decades (e.g., [22, 23]) and a literature data base for viral inactivation on carriers is now accumulating. As mentioned within the introduction, however, side-by-side comparisons of inactivation efficacy in solutions versus on carriers are lacking. This is true in particular for thermal inactivation.

On the basis of the prevailing opinion [7, 9–12], our assumption going into these comparison studies was that we would confirm the expected increased resistance of viruses to dry heat inactivation as compared to heating in solutions. Although the humidity associated with carrier heating was not measured in our studies, this was expected to be low for a dry heat

oven. This condition was predicted, on the basis of previous work [7, 11], to further reduce the effectiveness of the carrier heating approach, relative to liquid heating. Our side-by-side studies clearly did not confirm these expectations. For instance, PV-1 exhibited markedly reduced D values when subjected to dry heating at the relatively low temperature of 46°C, indicating increased susceptibility of this enterovirus, relative to liquid heating. This difference is not attributed to experimental artifact, since our liquid heating results compare reasonably well with previous results obtained for hepatitis A virus (another enterovirus from the Picornavirus family) inactivation in culture medium [24] and food homogenates [25, 26] (**Figure 3**; see also review by Bozkurt et al. [27]).

Our carrier results indicate a much greater sensitivity of PV-1 to dry heat than was determined by Sauerbrei and Wutzler [9]. These authors observed 4.3 $\log_{10}$ inactivation after 60 min at 75°C, providing an approximate D value of 13 min at this temperature. The differences may be due to methodology, as these authors also reported much different results for Ad5 relative to our results (see below). The impact of organic load on heat inactivation of PV-1 in our study was minimal, as shown by the similarity in D values and D versus temperature curves for liquid inactivation in culture medium vs. bovine serum. This is in marked contrast to our findings [6] for the flaviviruses Zika virus, bovine viral diarrhea virus and West Nile virus, where dry heating at 56°C was much more effective in the absence compared to the presence of a high organic load.

There have been few reports on heat inactivation of adenovirus. Maheswari et al. [28] evaluated liquid heat inactivation and observed over a 7.5 $\log_{10}$ reduction in titer following 10 min heating at 70°C. This corresponds to a D of ~1.3 min at this temperature. Tuladhar et al. [29] examined liquid heating of Ad5 in the presence of organic load (1% stool) and in culture medium. The D values at 73°C were 0.53 and 0.40 min, respectively [29]. This indicated a minor impact of organic load on heat inactivation, as we found in the present study.
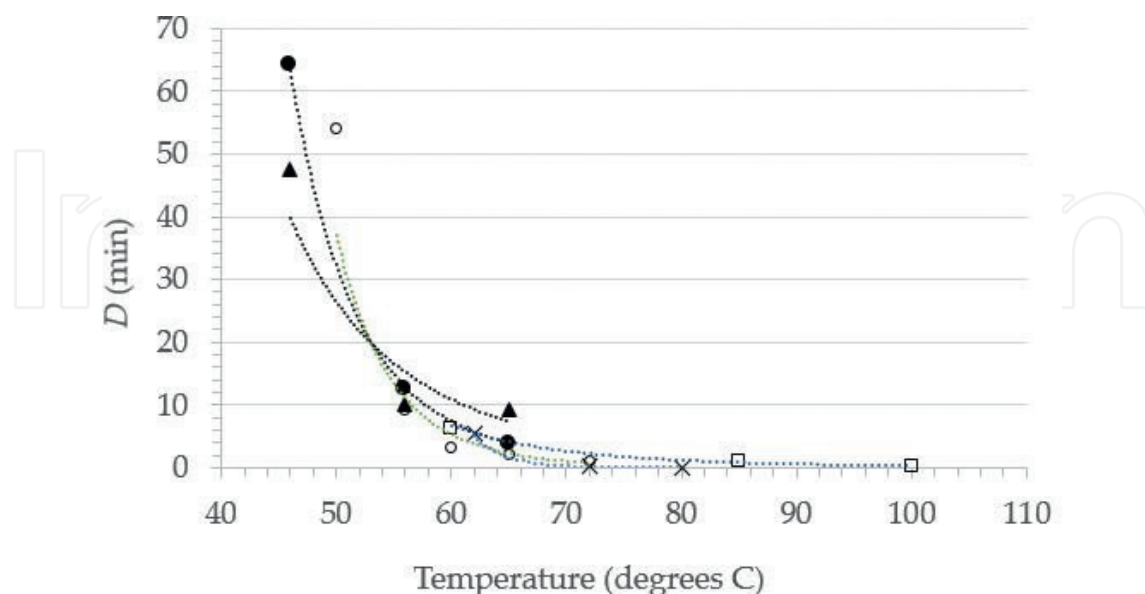

Figure 3. D vs. temperature relationships for heat inactivation of PV-1 in Medium (●) or Serum (▲) liquid matrices; comparison to hepatitis A virus inactivation in culture medium (✕, Ref. [24]) or in homogenates of mussels (O; Ref. [25]) and (□; Ref. [26]).

Comparisons between carrier and liquid heat inactivation for adenoviruses have not been reported. Sauerbrei and Wutzler [9] found Ad5 to be relatively resistant to dry heating. Their data indicate a D value of 67 min at 75°C [9]. This is very discrepant from our carrier results for Ad5. The reason is not clear, although the time kinetics for inactivation were not studied in detail in the previous study (time points included 60 and 120 min only). In our study, clear differences between liquid heating and dry (carrier) heating were observed primarily at 46°C, as the time kinetics were relatively similar for the higher temperatures evaluated.

Questions regarding the impact of organic load and carrier versus liquid heating on the efficacy of thermal inactivation of enteroviruses spread by the fecal-oral route are relevant in achieving adequate disinfection of surfaces in healthcare settings where such viruses might be present in organic-containing physiological substrates (blood, sputum, feces, etc.). It has been shown that transfer of infectious virus from contaminated fomites to humans can result in acquisition of disease [30, 31]. It is important therefore to collect information on the utility of different inactivation approaches, whether these are chemical or physical that might be used to disinfect contaminated fomites. Our results with two enteroviruses from different nonenveloped families suggest that the efficacy of heat inactivation assessed in a liquid versus carrier test format varies according to the virus under evaluation. If extent of heat inactivation is dependent more on the protein composition of the virus than the presence or absence of a lipid envelope, perhaps the differences observed for these two enteroviruses are not unexpected. The variability observed, even among these two nonenveloped viruses, suggests that extrapolation of carrier versus liquid inactivation efficacy should not be made across virus families. As a result, we are now conducting similar studies with a wider range of viruses to more fully characterize the requirements for heat inactivation under these varied conditions.

5. Our interpretation of heat inactivation data

Historically, the relationship between D and temperature has been displayed in plots of $\log_{10} D$ versus temperature (e.g., **Figure 4**). The slope of the (typically) linear relationship thus generated is equivalent to $-1/z$. The z value so obtained can then be used to predict D values at other (nonmeasured) temperatures, using the rather cumbersome formula shown in Eq. (4):

$$\log_{10} D_{predicted} = \log_{10} D_{ref} - \frac{T_{predicted} - T_{ref}}{z} \tag{4}$$

where $T_{predicted}$ is the temperature at which D is to be predicted and T_{ref} is the temperature at which D_{ref} was actually measured [32]. On the other hand, the plotting of D versus temperature is much more straightforward and intuitive and is occasionally seen in the inactivation literature (e.g., [29]).

The utility of the plot of D versus temperature is greatly enhanced when the power function line fit is added to the plots, as has been done in **Figures 1–3**. The resulting fit lines may be viewed as surfaces along which any temperature and D-value pair is associated with 1 $\log_{10}$ inactivation. The extrapolation of D to nonempirical temperatures that requires some effort using the z values therefore becomes quite easy and straightforward using the D vs. temperature power curve plots.
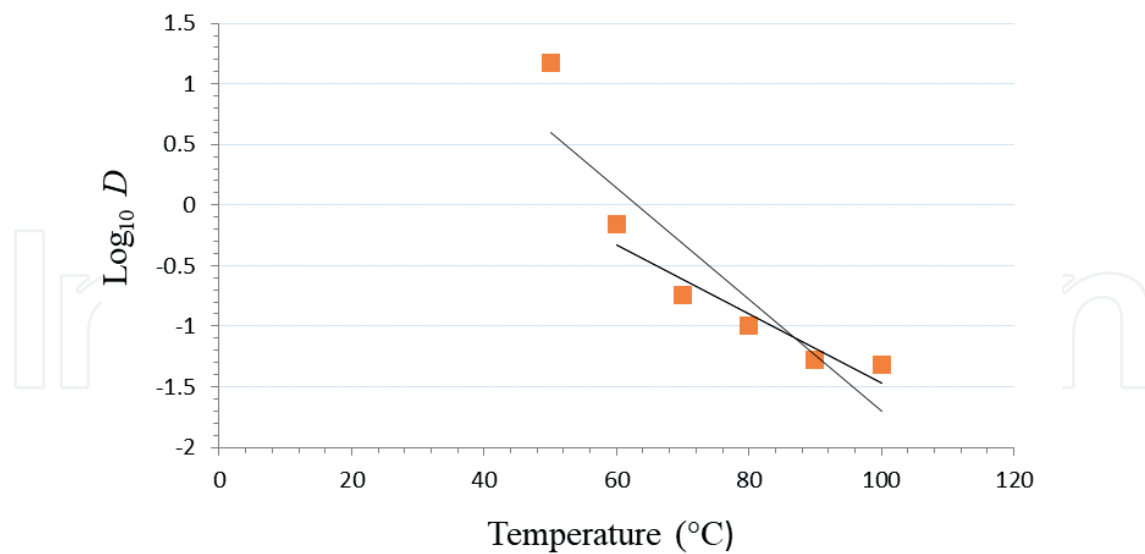
Figure 4. A plot of $\log_{10} D$ vs. temperature for heat inactivation of the OPN strain of the Picornavirus foot and mouth disease virus (Figure from [18], data are from reference [33]).

The nonlinear relationship displayed in the D versus temperature plot (**Figures 1–3**), with the steep portion of the curve at relatively lower temperatures followed by a flattening out at higher temperatures, is more informative also from a mechanism of inactivation point of view than the $\log_{10} D$ versus temperature plot. If heat inactivation is attributed to capsid opening followed by nuclease destruction of genomic material [2, 3], then the steep portion of the curve may represent reaching a threshold temperature required for capsid opening. Once this threshold temperature has been reached, relatively small incremental increases in temperature result in dramatic decreases in the time required for 1 $\log_{10}$ inactivation. Differences between carrier and liquid heat inactivation observed at the lower end of the D versus temperature plot might then correspond to differences in extent or kinetics of heat exchange or other factors to be described below.

There are frequent errors associated with calculation of D values and our own results are not immune to this, as we acknowledged in the methods section earlier. Some might argue that the concept behind the D value for heat inactivation is not always correct. The implication behind D values is that heat inactivation at a given temperature is first order with respect to time, such that a constant $\log_{10}$ inactivation occurs within a given unit of time. In reality, the time frames over which linear behavior is observed experimentally are very short at high temperatures and are limited by the titers of the virus stocks being inactivated. At lower temperatures, extended contact times are required to obtain several $\log_{10}$ of inactivation, so again the determinations of D values can be challenging. In addition, there is always a degree of error associated with the measurement of virus titers before and after heat treatment. D values at three or more different temperatures are required for calculation of power function coefficients and for determining z values, so thoroughly characterizing heat inactivation efficacy in this manner is a rather complicated endeavor.

In general, experimental error associated with calculation of D values translates to poorer linear line fits (i.e., lower coefficients of determination or R^2 values) in the $\log_{10} D$ versus temperature

curves. Since the D versus temperature relationship is merely a transformation of the $\log_{10}D$ versus temperature relationship, we have routinely noted that deviations from linearity for the $\log_{10}D$ versus temperature plots (such as those shown in **Figure 4**) are associated with poorer power function fits for the D versus temperature curves generated from the same inactivation results. In **Figure 4**, the R^2 value for the line fit to all six points is 0.82, while the R^2 value for the line fit only to the higher five points is 0.90. The corresponding R^2 values for the power function fits are 0.89 (for all six points) and 0.94 (for the highest five points). The two constants (*a* and *b*) from the power function equation (Eq. (2)) are derived from the *y*-intercept and slope, respectively, from the linear line equation (Eq. (1)) of the corresponding $\log_{10}D$ versus temperature plots.

In sum, regardless of the method used for the analysis of heat inactivation results, it is the D value itself that is the source of most error. However, the conclusions made above regarding efficacy of heat inactivation applied to viruses in solution versus viruses dried on carriers, or the impact of organic load on heat inactivation, can be made directly by evaluation of the raw inactivation data itself. Therefore, the difficulties associated with the appropriateness or accuracy of the D value concept do not detract from our overall conclusions regarding heat inactivation of these two enteroviruses.

6. Executive summary

- Virus inactivation by chemical and physical means may be evaluated either in liquid studies or in carrier studies.

- Liquid inactivation studies are relevant to a barrier or clearance process intended to reduce the viral titer of a solution, while carrier inactivation studies are relevant for surface disinfection approaches.

- A greater volume of virus inactivation data exists in the literature for liquid, relative to carrier, inactivation. Very few studies have compared liquid and carrier inactivation in a side-by-side design.

- Prevailing opinion has been that viruses are less susceptible to heat inactivation in the carrier format relative to the liquid format. Our studies have not confirmed this.

- We found that PV-1 was much more susceptible to inactivation at 46°C on carriers than in liquids, while the susceptibility to inactivation at 65°C was similar for both test formats.

- We found that Ad5 was only slightly more susceptible to inactivation at 46°C on carriers than in liquids, while the susceptibility to 65°C was similar for both test formats.

- Regardless of study format (liquid or carrier) complete inactivation of PV-1 occurred within 20 min at 65°C, while 1 h was required at this temperature to completely inactivate Ad5.

- The presence or absence of increased organic load in the liquid inactivation matrix did not impact heat inactivation efficacy for either PV-1 or Ad5.

- The decimal reduction value (*D*) versus temperature relationship is described well by a power function line fit and the resulting line fit equation may be used in a straightforward

manner to extrapolate $\log_{10}$ reduction in virus titer from empirically tested temperatures to other temperatures of interest.

7. Future perspectives

Inactivation studies performed in solutions have been useful in providing comparative efficacy data for different physical and chemical inactivation approaches targeting a given virus or for comparing the intra- and inter-family susceptibilities of different viruses to a given inactivation approach. The current rankings of viruses in terms of susceptibilities to such approaches (e.g., [34, 35]) have largely been derived from liquid inactivation studies. The results of liquid inactivation studies should not be extrapolated to inactivation of viruses on surfaces, however. This is because differences in presentation of the virus to the active, in diffusion of the active through the liquid or virus film (for chemical approaches) or in penetrability of radiation to the viruses or in kinetics of heat exchange (for physical approaches), almost certainly exist. Such differences may favor inactivation in one or the other of the liquid or carrier formats. Generalizations on the relative sensitivities of viruses to inactivation on carriers versus in liquids should not be made in the absence of data. Side-by-side carrier and liquid inactivation studies such as the ones described in this chapter are needed to elucidate the possible differences in efficacy for the various chemical and physical inactivation approaches. This aspect of the inactivation literature is in its infancy, but with time it is expected that the database will continue to grow.

As more sophisticated thinking about the relationship between our environmental microbiome and public health has been evolving, arguments have been made that the current approach to surface disinfection should change. In other words, there is a viewpoint that advocates replacement of the current "sterilization approach' with the use of "smart" antimicrobial agents that target the pathogens while sparing the nonpathogenic population [36]. Heat is, in some regards, capable of serving as a targeted inactivation approach. This is due to the rather striking differences in heat inactivation sensitivity of various viruses or, indeed, various microorganisms in general. At least for the moment though, and especially where viruses are concerned, it would appear that our current "sterilization" approach to heat inactivation will prevail, as we are not overly concerned about the possibility of nonpathogenic viruses competing with pathogenic ones.

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