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Chapter 2.4

INACTIVATION OF VIRUSES

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ABSTRACT

Viruses are infectious particles composed of nucleic acids and proteins that depend on cells for energy. Viruses invade cells where they proliferate, resulting in disease. Sterilization, disinfection, and antisepsis are important for preventing diseases derived from pathogens such as viruses. The disinfectants used for viruses are mostly chemicals including alcohols like ethanol and isopropanol. Alcohols are effective against enveloped viruses such as human immunodeficiency virus (HIV) and influenza virus but not small non-enveloped viruses such as parvovirus and poliovirus. To develop methods of sterilization, confirmation of results using appropriate samples is necessary. Towards this goal, several physical methods have recently been developed to facilitate sterilization; including the use of pulsed light, supercritical fluids, pulsed electric fields, and gas plasma. Although most of these methods have not been widely adopted, further increases in reliability, convenience, and suitability should contribute to the spread of their applicability. In this review, we describe viruses, conventional means of disinfection, trends in the development of new methods of sterilization and potential applications of these methods.

CHARACTERISTICS OF VIRUSES AND DISINFECTANTS

A virus is a particle composed of nucleic acids surrounded by proteins. Viruses cannot survive and multiply without host cells because they cannot produce energy by themselves [16]. They are very small (<0.5μm-size) and invisible to the naked eye. To recognize their shape, an electron microscope is needed. Viruses include (i) bacteriophages, which infect

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bacteria, (ii) viroids, which are only RNA, devoid of proteins, and infect higher plants causing crop diseases, and (iii) animal viruses. In animal viruses, the recent emergence of previously unrecognized viruses and re-emergence of known viruses has been reported. The viruses include severe acute respiratory syndrome-associated (SARS) coronavirus, avian and swine influenza A viruses, West Nile virus, human metapneumovirus, Ebola virus, and hantaviruses [26]. Importantly, 75% of emerging human infectious diseases are zoonotic, i.e., the pathogens can be transmitted from animals to humans [51]. Besides viruses, prions (proteinaceous infectious particles) [32] have been studied in the field of virology, because they cause prion diseases, which belong to slow virus diseases. Prion is an important pathogen in terms of inactivation because it is the most difficult pathogen to inactivate.

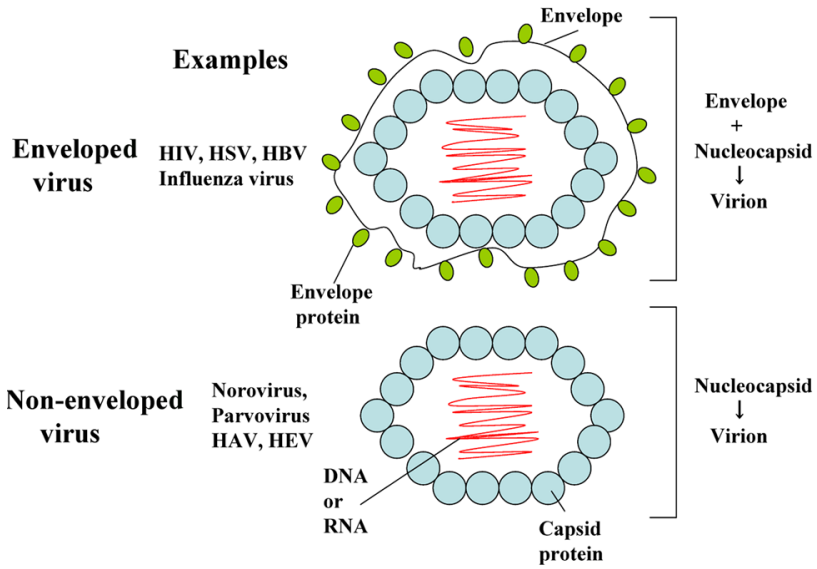

Figure 1. Structure of enveloped and non-enveloped viruses. Non-enveloped viruses are composed of capsid protein and nucleic acid (DNA or RNA), viz. nucleocapsid., which constitute an infectious unit, the virion, whereas enveloped viruses are composed of an envelope and nucleocapsid. HAV: hepatitis A virus; HBV: hepatitis B virus; HEV: hepatitis E virus; HIV: human immunodeficiency virus; HSV: herpes simplex virus.

Before discussing sterilization, disinfection, and antisepsis, we should mention the structure of viruses. The basic structure is a nucleocapsid complex composed of capsid proteins and nucleic acids (RNA or DNA) (Fig. 1). In terms of resistance to biocides, mammalian viruses are mainly divided into two types, enveloped viruses and non-enveloped viruses. Enveloped viruses, which include human immunodeficiency virus (HIV), hepatitis B virus (HBV), influenza viruses, and herpes simplex virus (HSV), are considered more sensitive to biocides. Their characteristics depend on structure including the external lipid bilayer envelope, which contains proteins (usually glycoproteins, or proteins with linked carbohydrate groups). The infectious unit of a virus is the virion, envelope + nucleocapsid. Enveloped viruses are easily destroyed by agents affecting lipids such as alcohols, ether, 2-phenlphenol, cationic surfactants, and chlorhexidine [5, 17-19, 21, 31, 42, 52] (Table 1). Studies have shown that disinfection with alkaline glutaraldehyde (2%) effectively destroys the hepatitis C virus (HCV) [7, 37], HBV and HIV [3, 12, 17]. HIV can be inactivated by

70% isopropanol + 0.5% chlorhexidine gluconate (CHG), 40% CHG [28], chloroxylenol [55], and benzalkonium chloride [55]. In contrast, non-enveloped viruses, which include the norovirus, poliovirus, and human hepatitis A virus (HAV), are composed of a nucleocapsid without an envelope. In these viruses, the virion is the nucleocapsid itself. Non-enveloped viruses are more resistant than enveloped viruses and not inactivated by alcohols. However, several reports have suggested that alcohols at high concentrations reduce the viral titers of relatively large non-enveloped viruses such as rotavirus [1, 2], adenovirus [39], rhinovirus [39], and hepatitis A virus (HAV) [54]. HAV is resistant to ether and acids and not inactivated by heating at 60°C for 60 min but is inactivated by heating at 70°C for 30 min and 100°C for 5 min. The resistance of small non-enveloped viruses is generally greater than that of Gram-positive and -negative bacteria and vegetative fungi (Fig. 2).

Table 1. The representative results of virudal activity of reagents

Envelope	Virus name	RNA/DNA	Effective disinfectants
+	Vaccnia virus	DNA	4.8% chloroxylenol
+	Vaccinia virus	DNA	95% ethanol
+	SARS-associated coronavirus	RNA	60°C, 30min
+	RSV	RNA	35% isopropanol or 4% CHG
+	PRRSV	RNA	40% methanol or 10% propylene glycol
+	HSV	DNA	75% ethanol, 95% isopropanol, or 70% ethanol + 0.5% CHG
+	HIV	RNA	70% ethanol
+	HIV	RNA	70% isopropanol + 0.5% CHG or 4% CHG
+	Avian influenza virus	RNA	0.1% soap (lifebuoy®), 0.2% detergent (surf excel®), or 0.3% alkali (caustic soda)
+	Human influenza virus	RNA	95% ethanol
+	HBV	DNA	70% isopropanol
+	HBV	DNA	80% ethanol
+	HCV	RNA	2% glutaraldehyde
+	HCV	RNA	2% glutaraldehyde
-	Rotavirus	RNA	70% isopropanol
-	Poliovirus	RNA	1% Virkon®
-	Parvovirus B19	DNA	60°C
-	Parvovirus B19	DNA	60°C
-	Ljungan virus	RNA	90°C, 20min
-	HEV	RNA	56°C, 30min
-	HEV	RNA	56°C or 60°C, 60 min
-	HEV	RNA	70°C, 10min
-	HAV	RNA	70% ethanol, 4% CHG, or 0.3% triclosan
-	Adenovirus	DNA	0.9% Virkon®

CHG: chlorhexidine gluconate
HAV: hepatitis A virus
HBV: hepatitis B virus
HCV: hepatitis C virus
HEV: hepatitis E virus
HIV: human immunodeficiency virus
HSV: herpes simplex virus
PRRSV: porcine reproductive and respiratory syndrome virus
RSV: respiratory syncytial virus
SARS: severe acute respiratory syndrome
Virkon®: Peroxygenic acid (Antec International Limited, Sudbury, Suffolk, UK)

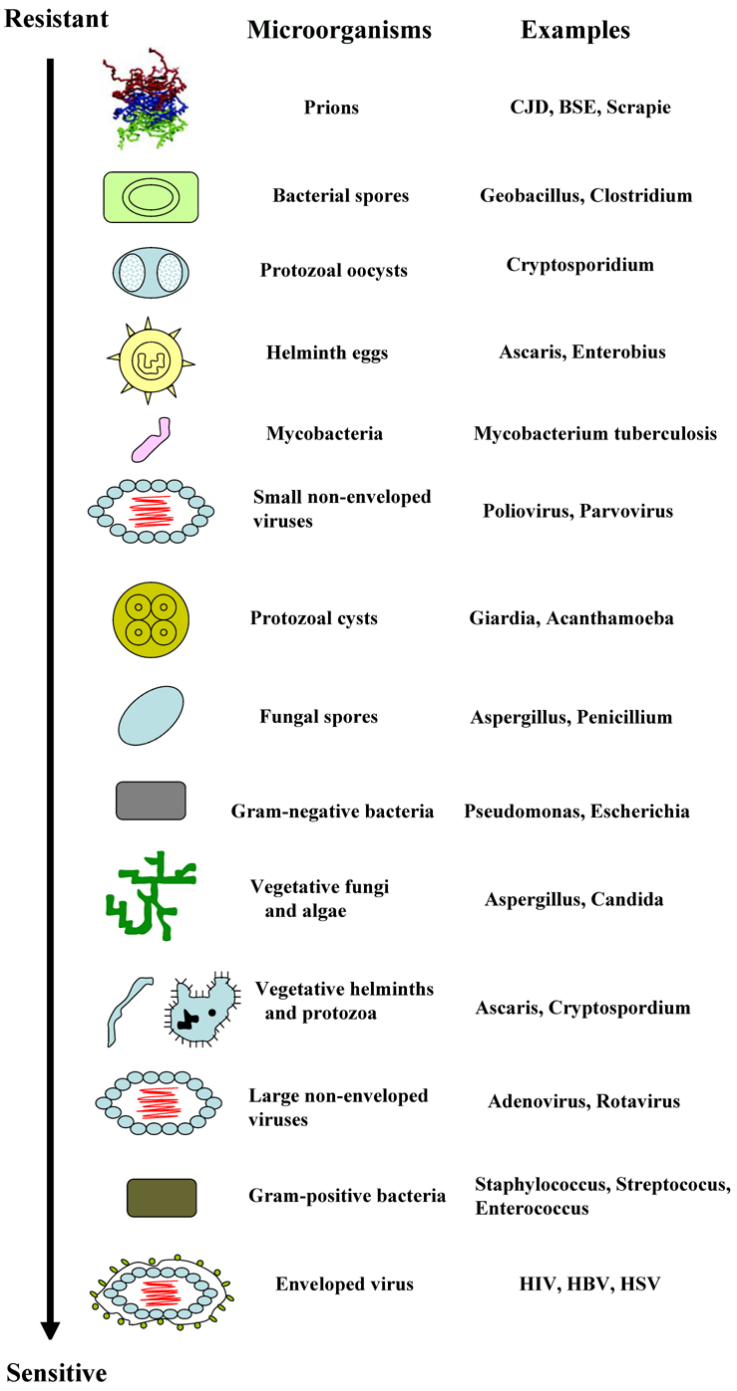

Figure 2. General order of resistance against biocides and biocidal process. It should be noted that this order can change depending on the biocide and biocidal process. Modified from Fig. 2 and Fig 1.17 in Nakamura HK et al. [29] and McDonnell et al. [25] with permission from Elsevier and ASM Press, respectively. BSE: bovine spongiform encephalopathy; CJD: Creutzfeldt-Jakob disease; HBV: hepatitis B virus; HIV: human immunodeficiency virus; HSV: herpes simplex virus.

The recent emergence and re-emergence of pathogens represent a threat to public health. Studies have proven that most of these pathogens with the exception of prions, HPV, and norovirus, are sensitive to commercial disinfectants [35]. Although outbreaks of rotavirus-caused gastroenteritis have been reported in pediatric clinics, agents confirmed to be effective against rotavirus include 95% ethanol, 70% isopropanol, 2% glutaraldehyde, 0.35% peraceutic acid, phenol, and quaternary ammonium [22, 40, 41, 48, 49]. SARS coronavirus is completely inactivated by treatment with 70% ethanol + povidone iodide, 2.5% glutaraldehyde [15]. There are no reports on the disinfection of human papillomavirus (HPV) and norovirus, neither of which can be made to proliferate *in vitro* using current technologies, although both viruses are very important for public health. A substitute for norovirus is the feline calicivirus (FCV), which is a related species and can proliferate *in vitro*. Bleaching agent (1,000 ppm), accelerated hydrogen peroxide (5,000 ppm), chlorine disinfectant (1,000 ppm), chlorine dioxide, quaternary ammonium (2,470 ppm), 0.1% quaternary ammonium + 79% ethanol, and 75% ethanol are all effective in inactivating FCV [38].

Reagents such as glutaldehyde, hypochlorite, phenol, ethylene oxide and hydrogen peroxide, and treatments such as ultraviolet (UV) light, radiation, and heating have a broad spectrum of effect for the inactivation of viruses. Such treatments attack DNA, RNA, proteins, and/or lipids and affect the nucleocapsid complex. As the nucleocapsid complex is the basic structural unit of viruses, its destruction causes a reduction in infectivity even in non-enveloped viruses. However, the effectiveness of heating varies possibly due to the secondary and tertiary structure of viral capsid proteins. Therefore, confirmation of the effectiveness of each disinfectant against viruses using a reliable standardized system is required.

ROUTE OF VIRAL INFECTIONS AND ASSOCIATED DISEASES AND ENVIRONMENTS

Each virus has tissues and organs where they prefer to proliferate. This information is important for preventing infections and analyzing the risk of transmission. Similarly, viruses have a preferable infection route, which is related to the distribution of cellular receptors for viral infections. For example, HIV infects *via* blood and mucosa because leukocytes especially helper T cells and macrophages express CD4, a cellular receptor for HIV [16]. The proliferative region and site of initial infection are not always the same. The human influenza virus infects the respiratory tract where it proliferates. However, HSV invades the skin or mucosa, undergoes retrograde transport to ganglions, and then becomes latent [16]. In response to reactivation stimuli, HSV moves to the skin and mucosa where it proliferates and causes illness with skin eruptions. Although the rabies virus initially invades skin *via* acetylcholine receptors [16], it undergoes retrograde transneuronal transport from peripheral to central nerves where it proliferates and causes disease.

In terms of infections, the host has a close relationship to viruses. The condition of the host influences the efficiency of infection. This means that immunological reactions contribute to the prevention of viral infections. Moreover, some hospitalized patients are immunocompromised and decreased resistance to viral attacks due to aging, treatment with anti-cancer or immunosuppressive drugs, or the use of catheters can enhance bacterial infections. Therefore, one should pay attention to such patients to prevent infections. In

addition, people at hospitals such as doctors, nurses, and visitors may also contribute to the spread of infections. Race, gender, nutrition and fatigue are also related to the efficacy of infections. Environmental factors such as population density, infrastructure, customs, and presence of vectors also affect the efficiency of infections.

Viruses normally interact with external materials including proteins, lipids, salts and cell debris etc. In addition, conditions favorable to viruses are related to resistance to virucides. Such environments include blood, serum, spinal fluid, and saliva. Soils prevent drying and/or stabilize the viral structure, extending the survival time of viruses. In other cases, some viruses cause the aggregation of host cells [16] or make aggregates themselves [56], what is called clumping, resulting in a reduction of the virucidal effect.

STERILIZATION, DISINFECTION, AND ANTISEPTICS AND THEIR CONFIRMATION

Any discussion of biocides and the biocidal process first requires a correct definition of sterilization, disinfection, and antisepsis. Sterilization means the complete removal of microbes including bacterial spores, which is basically the "gold standard" for evaluating the sterilization process. Therefore, detailed investigations are required to ensure the killing of viable organisms at a sterility assurance level (SAL) of 10^{-6} [25]. When the exposure time for the process is doubled at an initial population of 10^{6}, a reduction to 10^{-6} is assumed and considered to mean the complete removal of microbes. There have been limited numbers of studies on sterilization processes for viruses. This is because there is a common assumption that sterilization processes effective for bacterial spores are also effective for viruses. Therefore, most scientists think that analyses using viruses are not necessary if effectiveness against bacterial spores is confirmed. However, as the condition of the host and soil around viruses affect the efficiency of disinfection and sterilization, the order of resistance to biocides may change dependent on the environment. On the other hand, disinfection does not mean complete death but a reduction to an appropriate level for safe handling. Pasteurization, sanitization, and high-, intermediate- or low-level disinfection are included in "disinfection". Antisepsis means the destruction or inhibition of microorganisms in living tissue, e.g., on the skin. Therefore, toxic products cannot be used for antisepsis. Standards and guidelines for sterilization, antisepsis and disinfection are given in Chapter 2.1.

The recent emergence and re-emergence of viruses requires confirmation of viral inactivation by conventional methods. However, this is nearly impossible due to the rapid increase in variety. An understanding of inactivation mechanisms and the resistance of viruses to such biocides would help the effectiveness of each treatment.

PHYSICAL STERILIZATION AND GAS PLASMA STERILIZATION

Recently, several methods have been developed for physical sterilization. Although steam, dry-heating, ionizing radiation and filtration are conventionally used for physical sterilization, pulsed light, supercritical fluids, pulsed electric fields, and gas plasma have recently been used [26].

Pulsed light sterilization (PLS) uses high energy light of short-duration (0.01 to 50 J/cm^2) [20]. PLS has not been widely used, although broad spectrum activity is estimated, because the mechanism of action is similar to that of non-ionizing radiation targeting nucleic acids. Another method is to convert substances into supercritical fluids, which have the combined properties of a liquid and gas, achieved using high pressure and high temperature [45]. Another approach takes advantage of pulsing an electric field, which breaks membranes, forms pores, and disrupts the cytoplasm [47]. Considering the mechanism of action, the effects on spore and non-enveloped viruses are limited. Reports of supercritical fluids and pulsed electric fields regarding biocidal activity are very limited.

Sterilization using gas plasma, the fourth state after solid, liquid, and gas is also promising [46]. The gas plasma is generated by removing electrons and producing a highly excited mixture of charged nuclei and free electrons. The process is achieved by the application of sufficient energy, in the form of heat or an electromagnetic field, to gas. Currently, there is only one commercial gas plasma sterilizer, STERRAD® (Johnson & Johnson K.K). Inactivation of HIV [34], hepatitis A virus [34], respiratory syncytial virus (RSV) [34], vaccinia virus [34], HSV [34], poliovirus [34], and duck hepatitis B virus [53] by this machine has been reported. However, the mechanisms of action in STERRAD® mainly seem to be due to the effect of vaporized hydrogen peroxide, not gas plasma. In STERRAD®, gas plasma seems to contribute to the elimination of remaining toxic vaporized hydrogen peroxide after the reaction. There have also been reports of gas plasma sterilization using oxygen, nitrogen, peracetic acid, aldehyde, and noble gases (Argon and Helium). As some of these gases do not have a biocidal effect themselves, it is reasonable that the biocidal effect of the gas plasma process contributes to sterilization. Such a gas plasma-based approach is useful for sterilization of fragile medical devices, especially endoscopes, thus facilitating the safe re-use of equipment.

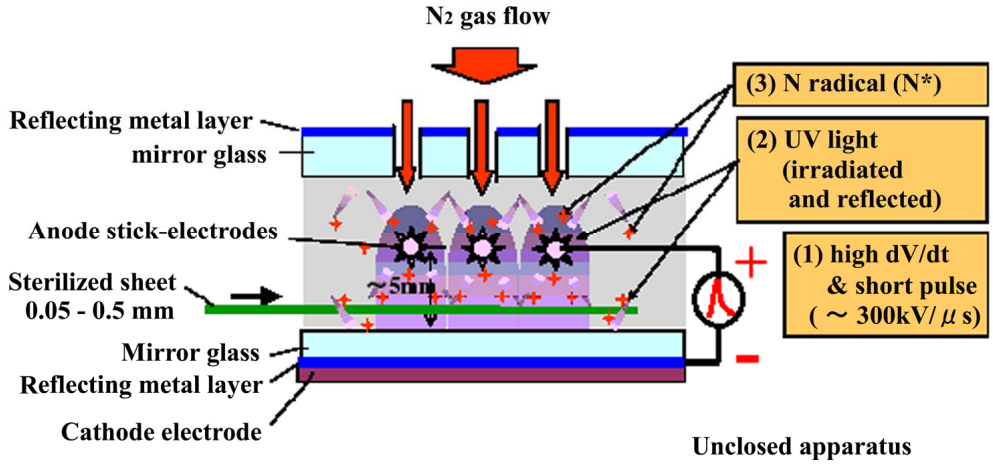

Figure 3. N_2 gas plasma sterilization system. Possible sterilization mechanisms are also shown. (1) N radical (2) UV light, (3) high dV/dt and short pulse. Citied from Fig. 1(a) in Shintani H et al. [46] with permission from Society for Antibacterial and Antifungal Agents, Japan. UV: ultraviolet.

The mechanisms of action of gas plasma sterilization remain unclear. At least three major mechanisms (radical production, UV light exposure, and high dV/dt and short pulse) are

presumed in this system [46]. Examples of N_2 gas plasma are shown in Fig. 3. Etching and cleaning effects may also contribute. However, the mechanisms would depend on the types of gases used to generate the gas plasma.

Using similar methods, gas plasma has been recently used for the disinfection of air in air-conditioners of Japanese companies [8, 30, 44]. Regretfully, we could not sufficiently ensure the effectiveness of disinfection and mechanisms of action.

Further details of gas plasma sterilization are given in Chapter 3.1-3.9.

CONCLUSION

The effectiveness of disinfection, sterilization and other measures against viruses depends on viral structure. The physicochemical properties of viruses affect how they are transmitted and survive in various environments. This is important when considering disinfection and sterilization because one has to choose safe procedures for preventing infections and the spread of viruses in laboratories and public areas. Therefore, a correct evaluation of procedures for the inactivation of virus-containing or -contaminated samples is important.

Recently, improvements in methods of sterilization have been achieved. The further development of methods to sterilize microorganisms including prions is more important with increasing emphasis of reducing microorganism-related iatrogenic risks recognized in terms of public health. Recent developments have also suggested the importance of confirming the efficacy of each method with a reliable standardized system.

Gas plasma sterilization is a promising method potentially effective against all microorganisms including prions. This approach would offer profound advantages over previous methods. By introducing appropriate gases into the system, certain gases critical for sterilizing each microorganism may be elucidated. In addition, a combination of gases and recently developed methods of generating plasma would also enhance the effectiveness of sterilization. These new technologies would contribute to infection prevention and contamination control such as the provision of safe drinking water and blood, production and preservation of products, sterilization of medical devices, and decontamination of surfaces.

Finally, the authors note that the information in this book is based on scientific publications at the time of preparation. Therefore, the authors and publisher have no responsibility for any consequences of the application of any of the information in this book by any reader. In addition, it was not their intention to include or exclude any particular products, instruments, reagents, or methods. Recently, the Centers for Disease Control and Prevention (CDC) guidelines for “Disinfection and sterilization in healthcare facilities, 2008” [36] have been published. For clinical issue in disinfection and sterilization, the CDC book is appropriate for consultation.

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