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28/10/2019

ADDENDUM

to certificate

"Inactivation of bacteria, viruses and other pathogens by UV-C radiation in the Leica cryostat product family"

(10 December 2010)

The disinfection efficacy of UVC radiation in the cryostats LEICA CM1850UV, CM1860UV, CM1900UV, and CM1950 had been validated in instruments equipped with germicidal UVC lamps UVC 2018-2K and UVC 2020-2K (sterilAir AG, Weinfelden, Switzerland), respectively.

Recently, the above mentioned lamps have been exchanged by equivalent types provided by orca GmbH, Kürten, Germany.

Technical data relevant to UV-C output and lamp geometry, thus of germicidal efficacy, of all lamps are summarized in Table 1. Pairwise comparison of the lamps UVC 2018-2K and UVpro K18-4PL as well as UVC 2020-2K and UVpro K20-4PL, respectively, shows almost identical specifications.

It can thus be safely expected that exchange of the lamps has no significant or may even have a positive effect on disinfection efficacy.

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Supplier:	steril/	Air AG	orca (GmbH
Lamp/ Specification:	UVC 2018-2K	UVC 2020-2K	UVpro K18- 4PL	UVpro K20- 4PL
Input power ¹ [W]	18	18	18	20
UVC output ¹ [W _{253,7 nm}]	5.6	7.1	6	7.2
Intensity ^{1,2} [µW _{253,7nm} /cm ²]	_3	71	62	71
Operating temperature [°C]	_3	0-15	0-15	0-15
Radiation arc [mm]	_3	320	295	340
Tube length [mm]	357	400	357	400
Quartz glass, emitter tube, Ø x thickness [mm]	_3	15 x 1.2	15 x 1.2	15 x 1.2
Quartz glass, sleeve tube, Ø x thickness [mm]	25 x - ³	24.5 x 1.5	24.5 x 1.5	24.5 x 1.5

Table 1: Technical data of the UVC lamps

¹ depends on ballast

² distance to radiation source 1 meter

³ not given

References:

- Maier, I. Certificate. Inactivation o bacteria, viruses and other pathogens by UV-C irradiation in the Leica cryostat product family. Updated version, Dec 10, 2010, 21 pp.
- Technical data sheet / specification UVC 2018-2K, 08/2001, sterilAir AG, supplied by Leica Biosystems ID No. DB047140422
- Technical data sheet / specification UVC 2020-2K, 09/2007, sterilAir AG, supplied by Leica Biosystems ID No. DB047743192
- Technical data sheet / specification UVpro K18-4PL, orca GmbH, supplied by Leica Biosystems ID No. DB047157384
- Technical data sheet / specification UVpro K20-4PL, orca GmbH, supplied by Leica Biosystems ID No. DB047157385

Amtzell, 28/10/2019

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CERTIFICATE

Inactivation of bacteria, viruses and other pathogens by UV-C irradiation in the Leica cryostat product family

1. Summary

UV-C radiation is effective in disinfecting surfaces and air within the irradiated working space of the cryostats Leica CM1850UV, CM1860UV, CM1900UV and CM1950 at -20 °C (Table 1).

For high-level disinfection, irradiation for three hours (CM1850UV/CM1860UV/CM1950) and four hours (CM1900UV) is recommended. Vegetative bacteria including *Mycobacterium tuberculosis*, bacterial spores (*Bacillus* sp.) and fungi are inactivated within this period of time. Viruses are also inactivated by at least 4 log₁₀ units (99,99 %), including resistant species like hepatitis viruses.

Intermediate level disinfection can be achieved by short-term irradiation of 30 minutes (CM1850UV/CM1860UV/CM1950) and 40 min (CM1900UV). This reduces vegetative bacteria including *Mycobacterium tuberculosis* and sensitive viruses like *Influenza A virus* (including influenza A type H5N1 and H1N1 viruses) and *Poliovirus* by at least 5 log₁₀ units (99,999%).

UV-C irradiation within the working space of the cryostats can provide safe and effective surface and air disinfection and significantly reduces infection risk.

It is recommended to wipe off visible contamination in the cryostat with an alcohol-based disinfectant before using the UV lamp. The germicidal effect of radiation is restricted to directly illuminated areas and pathogens not shielded by other material. Therefore, UV-C irradiation cannot replace regular chemical disinfection of the cryostat chamber.

10 December 2010

Ingo Maier, PhD, PD

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Table 1: Predicted UV-C disinfection efficacy¹ for selected pathogens in the cryostats Leica CM1850UV/CM1860UV/CM1950 and CM1900UV²

Species	Irradiatio	Irradiation time ²			
	30 min / 40 min	3 h / 4 h			
Bacteria ³					
Bacillus ssp.(vegetative)	+	+			
Bacillus sp. (spores)		+			
Burkholderia pseudomallei	+	+			
Enterobacter faecium	+	+			
Escherichia coli	+	+			
Klebsiella pneumoniae	+	+			
Mycobacterium tuberculosis	+	+			
Proteus mirabilis	+	+			
Pseudomonas aeruginosa	+	+			
Salmonella ssp.	+	+			
Staphylococcus aureus	+	+			
Vibrio cholerae	+	+			
Yersinia pestis	+	+			
Yeasts ³ and molds ³					
Aspergillus fumigatus (spores)		+			
Candida albicans	+	+			
Cryptococcus neoformans		+			
Viruses ⁴					
Adenoviruses		+			
Hepatitis A virus	+	+			
Hepatitis B virus		+			
Herpes viruses	+	+			
Influenza viruses	+	+			
Poliovirus	+	+			
SARS coronavirus		+			
Simian virus 40		+			
Vaccinia virus	+	+			

+: disinfection achieved

- ¹: valid only for conditions equivalent to those in the tests
- ²: 40 min/4h irradiation periods apply to the cryostat CM1900UV-series
- ³: reduction by 5 log₁₀ units (bacteria, fungi incl. yeasts) ⁴: reduction by 4 log₁₀ units (viruses)



2. Experiments

Leica Biosystems Nussloch GmbH (formerly Leica Microsystems Nussloch GmbH) contracted *ecoscope* (Amtzell, Germany) in 2004 to evaluate the surface disinfection efficacy of ultraviolet irradiation in the Leica cryostat CM1850. The apparatus was equipped with a low-pressure mercury arc lamp (sterilAir GmbH, Kürten, Germany).

The evaluation consisted of determining the inactivation by UV-C (254 nm) irradiation of test bacteria and viruses on stainless steel surfaces in the cryostat chamber at -20 °C.

In a first project, the bacterium *Staphylococcus aureus* ATCC 6538 was used as a biodosimetry strain. Bacteria were dried onto stainless steel plates from suspensions in distilled water. The germ carriers were placed into different, defined positions within the cryostat chamber. It was demonstrated that under the specified test conditions, UV irradiation was capable of inactivating *S. aureus* by >5 log₁₀ units after 15 to 30 min irradiation, depending on the position in the cryostat (14).

In two independent experimental series, the inactivation of the test virus *Simian virus 40* (*SV 40*, a *Polyomavirus*) exposed to UV-C for different periods of time was investigated. Viruses suspended in cell culture medium containing 2 % bovine fetal serum were dried onto stainless steel plates. The virus carriers were placed in a fixed position in the cryostat chamber. After irradiation, the viruses were rinsed off and in blind tests applied to monkey kidney cell cultures (CV-1) for virus propagation. After 12 to 14 and 15 to 18 days incubation, the cell cultures were examined for virus-specific cytopathic effects and the infectivity titers were determined. The results were presented in a separate test report (15). It was shown that the inactivation of *SV 40* by >4 \log_{10} units was achieved by UV irradiation for 95 to 180 minutes.

Leica Biosystems supplied comparative measurements of UV-C intensities at different positions in the working spaces of the cryostats CM1850UV, CM1950 and CM1900UV. The dimensions of the working spaces of the cryostats CM1850UV and CM1860UV are essentially the same. The UV-C lamps are identical. The experimental results obtained on the cryostat CM1850UV can thus be applied also to the other instruments mentioned above.

On the basis of these experimental results and available scientific information the inactivating effect of UV-C irradiation in the cryostats on pathogenic microorganisms and viruses could be assessed (Table 1). A selection of literature data on UV-C (254 nm) radiation doses required for the inactivation of various microorganisms and viruses is summarized in the appendix.

3. The mechanism of UV damage

Low-pressure mercury arc lamp radiation is essentially monochromatic with a peak output at 253.7 nm, close to the absorption maximum of nucleic acids (DNA, RNA), the carrier of genetic information. Absorption of UV photon energy damages the genetic material of microorganisms by formation of lesions, in particular through dimerization of adjacent pyrimidines in nucleic acids. Accumulated lesions may overwhelm the cellular capacity for repair, induce mutations, inhibit replication and thus finally kill the organism (16, 21).



4. Influence of nucleic acid conformation on UV resistance

Nucleic acids in viral genomes may have different conformations: single-stranded DNA resp. -RNA (ssDNA resp. ssRNA) or double-stranded DNA resp. -RNA (dsDNA resp. dsRNA). In inactivation experiments on ssRNA and dsRNA obtained from the same virus it was shown that ssRNA is more sensitive against UV-C radiation than dsRNA (5, 39, 49). The same is true for ssDNA and dsDNA (1, 24, 39, 40, 48). DNA viruses are more sensitive than RNA viruses. These results are supported by available data on UV inactivation: ssRNA viruses like Caliciviridae, Orthomyxoviridae, Picornaviridae and Togaviridae are entirely highly sensitive, whereas dsRNA-and dsDNA viruses of comparable genome size (Adenoviridae, Reoviridae, Polyomaviridae) are clearly more resistant to UV-C (Table 2). The differences in sensitivity most probably reflect different capacities for host cell repair.

5. Kinetics of UV-C inactivation in microorganisms

Germicidal UV irradiation inactivates pathogens according to the standard decay equation $S = \exp(-k*I*t)$ (first order kinetics). S represents the fraction of the original population that survives exposure at time t, and I the light intensity. Mathematical modeling of UV decay curves has been reviewed by (23) and (26).

The rate constant k and lethal ultraviolet dosages (I*t) have been determined experimentally for a large number of bacteria, fungi, viruses and protozoa in numerous studies. UV-C doses required for the inactivation of a selection of microorganisms, especially viruses, are given in the appendix. Although the results vary depending on experimental design, UV measurement and state of the biological material, a conclusive picture of relative UV-C sensitivities has been obtained. By use of biodosimetry test strains, conclusions about UV-C dosages can be drawn and predictions made on their effect on other organisms (10, 27).

6. *Staphylococcus aureus* as a test bacterium

Staphylococcus aureus ATCC 6538 has been chosen as test strain, because it is one of the listed test strains in standardized disinfection testing and a potential pathogen in humans. In addition, data on the UV-C sensitivity of *S. aureus* are available from the scientific literature (Appendix).

7. SV 40 as a surrogate virus in disinfection testing

A surrogate virus employed in the testing of disinfection methods should respond to the disinfectant in question in a similar way as the pathogen against which it was designed. At best, the surrogate virus should be somewhat more resistant. Among the different virus groups, small dsDNA viruses show the highest resistance against UV-C.

SV 40 was chosen as test virus because of several advantageous properties. It is a relatively small virus (ca. 50 nm) possessing a small genome of dsDNA (5.2 kbp) and a very high resistance to UV-C radiation (see Appendix). *SV 40* propagates in mammalian cells (monkey, man). It is classified in risk class 2 and can thus be handled at reasonable expense. The virus and a suitable test system (monkey kidney cells) are available.



SV 40 is biochemically and genetically well characterized. Moreover, *SV* 40 is one of the test viruses in the standardized testing of chemical disinfectants against viruses (8, 30). Contrary to the European standard (6, 17), the German Federal Health Office (Robert Koch Institute) demands tests on *SV* 40 in addition to *Poliovirus* and *Adenovirus* because it proved to be more resistant in some investigations (33).

SV 40 is among the viruses most resistant to UV-C, surpassing vegetative bacteria and bacterial spores (see appendix). Scientific data shows that ssRNA- and ssDNA viruses are inactivated faster, as well as large dsDNA viruses like herpes viruses and *Vaccinia virus*, for example. Among the small dsDNA viruses, adenoviruses are more sensitive to UV-C than polyomaviruses including *SV* 40. *Hepatitis B virus* is of similar size as *SV* 40 (Table 2) and therefore, a similar or higher sensitivity to UV-C is postulated. Presence or absence of an viral envelope is irrelevant in relation to UV-C sensitivity. In conclusion, *SV* 40 is regarded as a suitable surrogate for pathogenic viruses in UV-C inactivation studies.

Table 2: Overview on important viruses infecting humans and predicted UV-C sensitivity

Virus family	Genome type	Enve- lope	Genome size (kb/kbp)	D ₉₀ (mWcm ⁻²)	Virus
Adenoviridae	dsDNA	no	28-45	27-49	Human adenovirus A to F
Arenaviridae	ssRNA	yes	10-11	3.5	Lassa virus
Astroviridae	ssRNA	no	6.8	10-12	Astrovirus
Bunyaviridae	ssRNA	yes	11-12	2.0-3.5	California encephalitis virus Hantaan virus
Caliciviridae	ssRNA	no	7.5	9.7-11	Norwalk virus (NoV)
					Hepatitis E virus
Coronaviridae	ssRNA	yes	30	0.7-1.1	SARS coronavirus
Deltaviridae	ssRNA	yes	1.7	22	<i>Hepatitis D virus</i> (assoc. to HBV)
Filoviridae	ssRNA	yes	19.1	2.0	viruses causing haemorrhagic fevers: <i>Marburg-, Ebola virus</i>
Flaviviridae	ssRNA	yes	10-12	6.8-8.4	Hepatitis C virus
		-			Yellow fever virus
					Tick-borne encephalitis
					virus
Hepadnaviridae	dsDNA	yes	3.2	3.8-4.1	Hepatitis B virus (HBV)
Herpesviridae	dsDNA	yes	125-235	3.5-7.0	Herpes simplex virus 1, 2
					Varicella zoster virus
					Cytomegalovirus
					Epstein Barr virus
					Human herpes virus 6, 7
					Human herpes virus 8
Orthomyxoviridae	ssRNA	yes	13.6	2.0-3.0	Influenza viruses A-C



Papovaviridae	dsDNA	no	5-8	68-103	Polyomavirus
					Papillomavirus (warts)
Paramyxoviridae	ssRNA	yes	15-16	3.0	Measles virus
_					Mumps virus
					Parainfluenza virus
					Human respiratory syncytial
					virus
Parvoviridae	ssDNA	no	5.5	2.1-3.2	Parvovirus B19
Picornaviridae	ssRNA	no	7-8	12-14	Hepatitis A virus (HAV)
					Poliovirus
					Coxsackievirus
					Echovirus
					Rhinovirus
Poxviridae	dsDNA	yes	130-375	1.8-4.3	Smallpox virus, molluscum contagiosum
Reoviridae	dsRNA	no	16-27	19-32	Reovirus Human rotavirus A, B
Retroviridae	ssRNA	yes	7-11	18-30	Human immunodeficiency virus (HIV) types 1 and 2
					Human T-lymphotropic viruses (HTLV-1, -2)
Rhabdoviridae	ssRNA	yes	12	0.9-1.2	Rabies virus
Togaviridae	ssRNA	yes	10-12	4.9-6.5	Rubella virus

Morphological characters apply to the respective virus family.

Abbreviations

 D_{90} : UV-C dose required for 90% inactivation (1 log_{10} unit reduction) dsDNA: double-stranded desoxyribonucleic acid dsRNA: double-stranded ribonucleic acid kb/kbp: x 1000 (kilo) bases resp. basepairs nm: nanometer = 10^{-9} m ssDNA: single-stranded desoxyribonucleic acid ssRNA: single-stranded ribonucleic acid

The list of viruses was compiled according to a corresponding list published by the Robert Koch Institute in cooperation with the German Association for the Control of Virus Diseases and the German Society for Hygiene and Microbiology (33), according to the U.S. Departments of Health and Human Services/Centers for Disease Control and Prevention (www.cdc.gov/ncidod/dvrd/index.htm), the ICTVdB Index of Viruses (www.ncbi.nlm.nih.gov/ICTVdb/Ictv/ICD-10.htm) and the ICTVdB Universal Virus Database (www.ncbi.nlm.nih.gov/ICTVdb/index.htm). The predicted values for UV-C sensitivity were adopted from (27).



8. Disinfection: definitions

The guidelines of the German Society for Hygiene and Microbiology (12) for the evaluation of chemical surface disinfectants provide that bacteria and fungi are usually inactivated by a factor of at least 5 log₁₀ units. This corresponds to the European standard EN 1040 on the testing of chemical disinfectants in suspension tests (13).

For the certification of virus disinfection, the guidelines of the German Association for the Control of Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (30)) for chemical surface disinfection require a reduction in infectivity by minimum 4 log₁₀ units.

In addition, the following classification scheme of disinfection levels is used:

1. <u>Low-level disinfection</u> can kill most bacteria, some viruses, and some fungi, but it cannot be relied on to kill resistant microorganisms such as *Mycobacterium tuberculosis* or bacterial spores.

2. <u>Intermediate-level disinfection</u> inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses, and most fungi, but it does not necessarily kill bacterial spores.

3. <u>High-level disinfection</u>: Destruction of all microorganisms, with the exception of high numbers of bacterial spores.

4. Sterilization: Complete elimination of microorganisms and viruses.

These definitions are used by the U. S. Department of Health and Human Services and the Association for Professionals in Infection Control and Epidemiology (35), the WHO (42) and others.

The standards refer to the effectiveness of chemical disinfectants. In analogy, they are applied to surface disinfection by UV-C irradiation in the following.

9. Destruction of bacteria and fungi by UV-C irradiation

Inactivation experiments with *Staphylococcus aureus* ATCC 6538 showed that the number of viable bacteria was reduced by more than 5 log₁₀ units after irradiation for 30 minutes in the cryostat CM1850UV. The disinfection efficacy corresponded to the guideline of the German Society for Hygiene and Microbiology (12) for surface disinfection methods and to an intermediate-level disinfection as defined above.

Disinfection of vegetative bacteria (\geq 5 log₁₀ units reduction) including *Staphylococcus aureus* is achieved by UV-C dosages of \leq 80 mWs cm⁻² (Appendix). No literature data are available for *S. aureus* ATCC 6538. However, it is not to be expected that the test strain differs significantly in sensitivity from other *S. aureus* strains. This applies also to *Mycobacterium tuberculosis* and other vegetative bacteria that have potential to pose a severe threat to public health and safety (biothreat agents, (10, 34)). The similarity in the UV response allows the prediction that 30 minutes or 40 minutes UV irradiation achieves disinfection of all vegetative bacteria listed in table 1.

Spore-forming bacteria like *Bacillus subtilis* and *B. anthracis*, however, are 5 - 10 times more resistant to UV-C than their corresponding vegetative cells.



While the UV-C sensitivity of the yeast *Candida albicans* compares to that of vegetative bacteria, *Aspergillus* spores and the melanized form of *Cryptococcus neoformans* are highly resistant to UV irradiation (Appendix).

10. Inactivation of viruses by UV-C irradiation

Within the given experimental conditions, an inactivation of the test virus *SV* 40 by minimum 4 log_{10} units was achieved by UV irradiation for 95 minutes and longer in the cryostat CM1850UV. This inactivation level corresponds to the accepted guideline of the German Association for the Control of Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (30)).

An account of the, compared to other pathogens, high resistance of the test virus *SV* 40 to UV-C (appendix), it can be assumed according to the scientific state of knowledge that other resistant viruses including *Hepatitis B virus* and fungal spores are inactivated to the same extent within the same irradiation time and also that vegetative bacteria including *Mycobacterium tuberculosis*, bacterial spores, fungi, and most viruses are destroyed with higher efficiency, as long as they are directly subjected to irradiation on surfaces or in the air (Table 1). The inactivation effect can be classified as high-level disinfection as defined above.

The test results showed that the effect of irradiation was considerably affected by components of the cell culture medium and the addition of 2 % bovine fetal serum: Irradiation periods longer than 95 minutes did not result in significantly increased inactivation. This has to be seen as an approximation to practical situations. It is recommended to remove visible contamination in the cryostat by wiping with disinfectant before using the UV lamp. For this purpose, an alcoholic based disinfectant recommended by the cryostat manufacturer should be used.

Dependent on the position in the cryostat, the radiation dose received by a surface area can be less than that in the test position used with SV 40. For disinfecting these areas the irradiation time has to be increased proportionally. In consideration of results from the study on *S. aureus* (14) and with inclusion of an additional safety margin, a factor of 1.5 + 0.4 = 1.9 is proposed for prolongation of the irradiation period. Therefore, irradiation for 3 hours is recommended for high-level disinfection in directly irradiated areas of the cryostat chamber of the CM1850UV. Comparative measurements showed that the incident UV-C radiation on surfaces of the cryostat chamber of the CM1950 reaches the same intensity as that in the CM1850UV. The dimensions of the working spaces in the cryostats CM1850UV and CM1860 UV are almost identical. Accordingly, irradiation for 30 min is also recommended for an intermediate level of disinfection in the CM1860UV and CM1950 UV , and 3 hours for high-level disinfection. The UV-C radiation intensity on surfaces of the cryostat chamber of the CM1950 UV , and 3 hours for high-level disinfection. The UV-C radiation intensity on surfaces of the cryostat chamber of the CM1900UV is by 25 % lower than in the CM1850UV. Therefore, 40 min resp. 4 hours irradiation are recommended for an intermediate resp. high-level disinfection in the CM1900 UV (Table 1).

The test results and assessment of disinfection efficacy refer to the full radiation output of a lamp such as employed in the test.

Disinfection at the predicted level is restricted to directly illuminated air and surface areas. Organic material may shield pathogens from UV-C radiation.



11. Influenza viruses

General characteristics

Influenza A viruses represent a continuous pandemic threat and are of current international concern. Influenza ("flu") viruses are classified into types A, B or C. All three types can infect humans. Influenza A viruses can infect people, birds, pigs, horses, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes based on two proteins on the surface of the virus called hemagglutinin (H) and neuraminidase (N). There are 14 hemagglutinin subtypes and 9 neuraminidase subtypes of influenza A viruses, and potentially all H/N-combinations are possible.

Influenza viruses are members of the family Orthomyxoviridae. Their genome consists of eight segments of linear, single-stranded RNA with a total length of 13,600 nucleotides. Influenza viruses are enveloped viruses. In spherical forms, the virion diameter is 80-120 nm (Table 2).

Influenza viruses are readily transmitted by aerosols or by direct contact. Viable virus particles can survive at least 48-72 h on contaminated surfaces (3, 4).

Novel influenza H1N1

In spring 2009, human infections caused by a new type of influenza A/H1N1 virus were identified in Mexico and the United States. After its discovery, the virus spread rapidly throughout the world. Three months later, about 95,000 confirmed cases and 429 deaths were reported. On 11 June 2009, the World Health Organization (WHO) raised the worldwide pandemic alert level to Phase 6 which reflected the fact that there were community level outbreaks in multiple parts of the world (9, 46, 47).

The virus originates from a swine influenza A (H1) that has been circulating in American pigs years before recognition in humans. The virus contains genes originating from American and European pig influenza and from bird and human viruses (this is called a "reassortant virus") and is readily transmitted between humans. Because influenza A/H1N1 has never before circulated among humans and most people have no or little immunity, it could cause more infections than seasonal flu. There are concerns that the virus may reassort with seasonal human influenza giving rise to even more transmissible or more pathogenic viruses (2, 18, 22, 28, 29, 37, 38).

Highly pathogenic avian influenza H5N1 ("bird flu")

Avian influenza ("bird flu") is an infectious disease of poultry caused by influenza type A/H5N1 viruses. An unprecedented epidemic of highly pathogenic avian flu (HPAI) spreads across large populations of domestic birds and migratory water fowl in Asia since 2003 and has reached Europe in late 2005, Africa in early 2006 (19, 42, 43, 45).

Influenza viruses that infect birds are called "avian influenza viruses". Only influenza type A viruses infect birds. To date, all outbreaks of HPAI have been caused by influenza A viruses of subtypes H5 or H7. HPAI is usually associated with high mortality in poultry.

Avian influenza viruses do not normally infect humans. However, there are serious concerns that the highly pathogenic avian flu virus evolves human-to-human transmission through the acquisition of genetic material from the H1N1 or H3N2 subtypes circulating in human populations. This could result in a influenza pandemic with massive fatalities worldwide (11, 20, 22, 25, 41, 44, 50).



UV-C inactivation of influenza virus

Viruses like influenza virus with genomes comprised of single-stranded RNA are particularly sensitive to UV-C radiation. This is supported by available data on UV inactivation (Table 2, Appendix). Accordingly, the decimal UV-C (254 nm) inactivation dose for influenza virus strains has been predicted as low as 2.0 - 3.0 mWs cm⁻² (7, 27, 36). It is thus in the same range as that for vegetative bacteria like *Escherichia coli* and *Staphylococcus aureus*. The susceptibility of influenza virus to UV-C disinfection has also been noted in (31, 32).

The morphology, general structure and genome organization is practically the same in all influenza viruses. Data on UV-C sensitivity of tested human influenza virus strains are thus equally applicable to other human and animal influenza subtypes.

It is concluded that a 30 min period of germicidal UV-C irradiation in the cryostats CM1850 UV/CM1860UV/CM1950 and a 40 min period in the CM1900UV results in an inactivation of *Influenza A virus* by at least 5 log₁₀ units. This corresponds to high-level disinfection.

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APPENDIX

UVC (254 nm) radiation dose required for the inactivation of selected microorganisms and viruses (at room temperature, mWs cm⁻²)

Species							
	1	2	3	4	5		
	90	99	99.9	99.99	99.999		
VEGETATIVE BACTERIA							
Bacillus anthracis	1.2-28	8.7-42	8.7-56	2.6-70	15-84	(3), (44), (47), (67), (20)	
Bacillus subtilis	3.7-12	6-18	9-14	11-15	13-18	(3), (44), (47), (63), (82), (103), (16)	
Brucella melitensis	2.8-3.7	5.3-5.8	7.8			(75)	
Brucella suis	1.7-2.7	3.6-5.3	5.6-7.9	7.5-10.5		(75)	
Burkholderia mallei	1.0-1.2	2.4-2.7	3.8-4.1	5.2-5.5		(75)	
Burkholderia pseudomallei	1.4-4.4	2.8-3.5	4.3-5.5	5.7-13		(75), (20)	
Escherichia coli	1.3-5.1	2.8-10	4.1-16	5.0-28	7.7-36	(3), (4), (6), (8), (14), (17), (27), (36), (37), (41), (44), (47), (49), (60), (70), (83), (85), (92), (90), (91), (94), (95), (96), (104), (107), (84), (16)	
Francisella tularensis	1.3-1.4	3.1-3.8	4.8-6.3	6.6-8.7		(75)	
Klebsiella pneumoniae			15	11-31	29-39	(60), (96), (107)	
Mycobacterium tuberculosis	0.5-2.3	1.0-6.0	1.5-10	2.0-13	2.4-17	(3), (25), (46), (47), (51)	
Mycobacterium avium	5.7-6.4	7.9-9.4	10-12	12-24		(84), (38)	
Mycobacterium intracellulare	7.4-7.8	11	13-15	16-19		(38)	
Mycobacterium terrae		10.5				(50	



Proteus mirabilis	0.9	1.8	2.7	3.6	4.5	(41)
Pseudomonas aeruginosa	1.0-5.5	1.9-11	2.9 -17	3.9-22	4.8-28	(3), (44), (47), (51), (96)
Salmonella sp.	1.8-5.1	3.2-7.0	5.4-9.0	7.1-25	8.3-15	(3), (17), (44), (47), (49), (60), (95), (96), (104), (20)
Shigella sonnei	4			7.5		(20)
Staphylococcus aureus	1.9 -5.5	3.9-11	5.8-17	7.8-22	9.7-28	(3), (17), (18), (44), (47)
Vibrio cholerae	0.8-1.1	1.4-6.5	2.2-12	2.5-21	19	(3), (44), (47), (78), (95), (96), (20)
Yersinia enterocolitica	1.3			3.6-11		(20)
Yersinia pestis	1.3-1.4	2.2-2.6	3.2-3.7	4.1-4.9		(75)

BACTERIAL SPORES						
Bacillus anthracis	74	149	223	297	371	(51)
Bacillus anthracis	25-28	~40	56	62-70	84	(67), (75), (20)
Bacillus pumilus			20			(66)
Bacillus subtilis	9-39	17-38	22-58	29-80	36-121	(3), (17), (27), (44), (56), (32), (64), (67), (71), (73), (74), (82), (92), (89), (90), (103), (45), (20)

YEASTS						
Candida albicans	7.6-12	11-17	15-22	18-27	22-32	(72)
<i>Cryptococcus neoformans</i> , melanized	34	68	102	136	170	(100)
<i>Cryptococcus neoformans</i> , non-pigmented	16	32	48	64	80	(100)



FUNGAL SPORES						
Aspergillus sp.	35-67	134	99-330	117-440	147-550	(35), (44), (47), (51)
Aspergillus fumigatus	54	108	162	216	270	(35)
Epidermophyton floccosum			120			(23)
Microsporum canis					120	(23)
Trichophyton mentagrophytes			120			(23)
Trichophyton rubrum				120		(23)

VIRUSES						
Adenoviridae	27-49*					(58)
	35	69	103	138		(56)
Adenovirus 1		09	103	130		(09) (27), (26),
Adenovirus 2	40-61	78-109	119-163	160, 167	198	(33), (51),
	40-01	70-103	113-103		130	(86)
Adenovirus 2		30	50	80		(31)
Adenovirus 4	10	34	69	116		(34)
Adenovirus 5				216-240	305	(46), (99)
Adenovirus 6	39	77	115	154		(69)
Adenovirus 40	30	61	93	124	155	(62)
Adenovirus 41	24-75	53-111	82-175	112-222	141	(62), (50)
Arenaviridae	3.5*					(58)
Astroviridae	10-12*					(58)
Bunyaviridae	2.0-3.5*					(58)
Caliciviridae	9.7-11*					(58)
Canine calicivirus			20			(28)
Feline calicivirus	4.8		12	19		(28), (94)
Murine norovirus				25	30	(54)
Coronaviridae	0.7-1.1*					(58)
SARS coronavirus				91	114-162	(29), (48)
Berne virus				5		(101)
Deltaviridae	22*					(58)
Filoviridae	2.0*					(58)
	2.0					
Flaviviridae	6.8-8.4*					(58)
Hepadnaviridae	3.8-4.1*					(58)



Herpesviridae	3.5-7.0*					(58)
Epstein Barr virus	16 - 23					(39)
Herpes simplex virus 1	3.7-10	7.4-20	11	24	37	(39), (76), (102)
Herpes simplex virus 2	0.4	0.7	11	13		(102)
Equine herpes virus			7.5			(101)
Orthomyxoviridae	2.0-3.0*					(58)
Influenza A	1.8-2.5	1.3-8.2	2.0		3.3	(1), (13), (42), (77)
Papovaviridae	68-103*					(58)
Polyomavirus	47	43-94	141			(53), (97), (52)
Simian virus 40	105-300	130-261		440	551	(2), (10), (11), (12), (21), (30), (46), (79), (80), (93)
Paramyxoviridae	3.0*					(58)
						(50)
Parvoviridae Parvovirus H-1, hamster	2.1-3.2*					(58)
osteolytic virus	23	46				(22)
Porcine parvovirus					ca. 83	(19)
Murine parvovirus					<20	
Picornaviridae	12-14*					(58)
Coxsackievirus	6.9-15	14-23	20-43	30-58	41-72	(5), (33), (51), (95)
Echovirus	7.0-11	14-21	21-32	28-42	35-53	(33), (51)
Encephalomyocarditis virus	7.6	15	23	16-113	25-141	(15), (105), (9)
Foot-and-mouth disease virus	24	48	72	96	120	(68)
Hepatitis A virus	4.1-7.3	7.6-14	8.0-22	11-37	13-100	(3), (5), (19), (44), (47), (95), (98), (99)
Poliovirus	4.1-8	10-16	14-23	18-31	22-43	(3), (7), (17), (33), (37), (40) recalculated by (17), (44), (53), (59), (62), (92), (94), (95), (96), (43)
Rhinovirus					"like polio"	(42), (43)



Poxviridae	1.8-4.3*					(58)
Vaccinia virus	1.5-3.5	3.0-7.1	4.5-11	6.1	7.6	(51), (57), (76)
Reoviridae	19-32*					(58)
Reovirus	17-26	35-53	52-102	70-74	87-170	(37), (51), (61), (99), (106)
Rotavirus	7.1-11	15-46	23-69	31-92	40-115	(3), (5), (17), (44), (47), (63), (87), (92), (95), (96)
Simian Rotavirus	29	58	87	117		(55)
Retroviridae HTLV-III/LAV	18-30*	200			360	(58) (65), (81)
Rous sarcoma virus					300	(48)
Rhabdoviridae	0.9-1.2*					(58)
Vesicular stomatitis virus				19	<75	(48), (24)
Rabies virus				5		(101)
Togaviridae	4.9-6.5*					(58)
Sindbis virus			15-30	40	24-50	(99), (106)
Semliki forest virus			7.5			(101)
Venezuelan equine encephalomyelitis virus				22	33	(88)

* predicted dose range for entire virus family according to ref. (58).



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