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Interdisziplinäres Hautkrebszentrum Ostbayern
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Investigation of the antiviral qualities of UVB irradiation filtered with Sunexx filter foils or Sunexx filter glasses in a bacteriophage bacteria system

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1. Summary

New antiviral strategies and treatments are needed to counteract ongoing or future pandemics. One promising strategy is the use of solar UV irradiation to inactivate viruses. In this project, two major aims have been achieved. First, the development and the application of a safe and versatile system to test the antiviral effects of different treatments, second to show the antiviral effect of UV irradiation filtered by Sunexx filter foils or Sunexx filter glasses.

To achieve the first aim, we modified the plaque assay technique, in which treated viruses (bacteriophages), have to show their ability to infect their host (bacteria). The viruses, which have received severe damages from the treatment, cannot infect their hosts successfully and this effect could be observed in this assay.

We treated viruses with UV irradiation, filtered by Sunexx foils or Sunexx glasses and showed with our modified plaque assay, that UV irradiation filtered by Sunexx foils or glasses does have significant antiviral effects.

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2. Introduction

The effective solar UV radiation reaching the Earth's surface, can be divided in UVA (320 nm – 400 nm) and UVB (280 nm – 320 nm) irradiation. Concerning the total solar energy of UV radiation on Earth's surface, UVB radiation is only a small part of this energy (for example according to standard reference spectra for energy applications ASTM G173-03 at an Air Mass (AM1.5) with ~900 W/m² the total solar energy reaching the Earth's surface, the portion of UV irradiation ($280 \leq \lambda$ (nm) < 400) – is approximately 30.663 W/m² and the portion of UVB has approximately according to ISO 21348 or DIN EN ISO 15858, 24442, 24443, 24444, 26369 ($280 \leq \lambda$ (nm) < 320) 0.799 W/m² or according to DIN 5030/5031 or CIE S 017 or DIN EN ISO 18369 or IEC 62471 ($280 \leq \lambda$ (nm) < 315) = 0.353 W/m²). This also corresponds to approx. 0.09% and 0.04%, respectively, of the solar energy reaching the Earth's surface (figure 1). Due to the lack of a standardized definition of solar radiation spectral categories in national and international standards (such as DIN, EN, ISO, CIE, etc.) and the highly sensitive wavelength-specific biological effects, this study is based on the exact recording and emission of wavelengths, whereby the spectral categories for understanding only provide a rough framework.

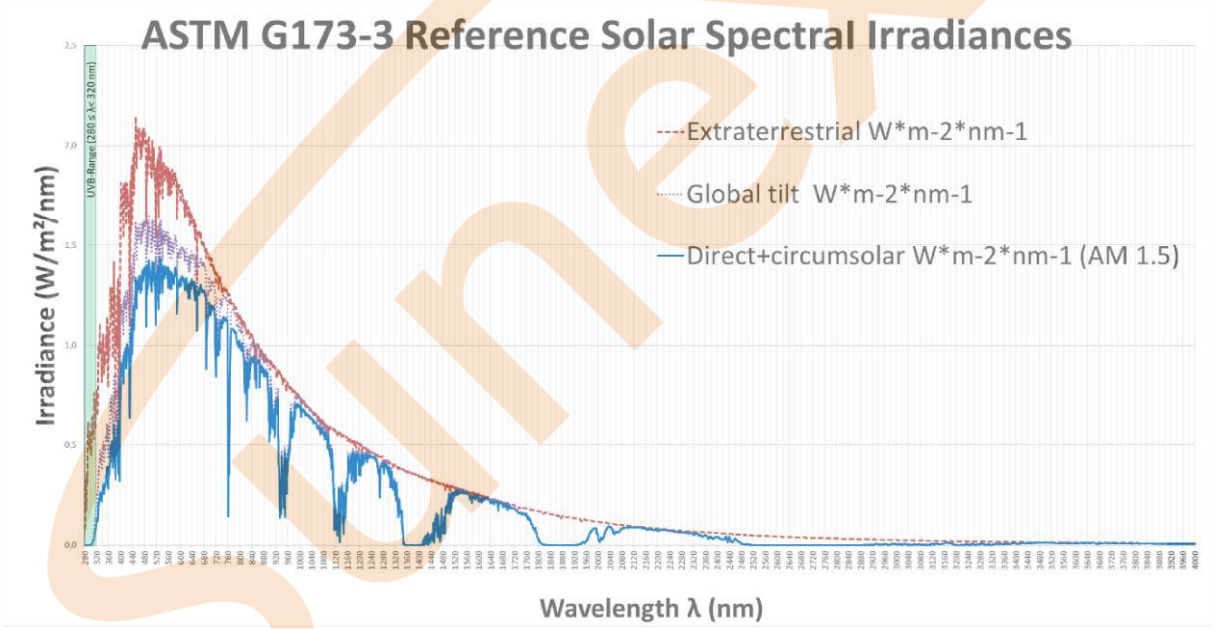

Figure 1 ASTM G173-G Reference Solar Spectral Irradiances

Despite this, UVB irradiation can be highly mutagenic and threatening to life (Wang PW, Hung YC et al. 2019). Most organisms have adapted to this threat by evolving efficient repair mechanisms (Yu and Lee 2017), or antioxidant defense systems (Ivanova I, Bogner

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C et al). However, viruses do neither have a DNA repair system nor a complex antioxidant defense system. This makes them vulnerable to UV irradiation (Weyersberg L et al. 2023).

The effect of UV irradiation on RNA or DNA viruses is subject to intensive research and it has been shown, that UV irradiation can have strong inactivating properties on the SARS-CoV2 Virus (Corona Virus) (Nicastro et al. 2021; Herman et al. 2021; Biasin et al. 2022) and many other types of viruses. The handling of corona viruses in a laboratory is difficult due to strict safety regulations and many bureaucratic obstacles. Therefore, a reliable viral test system with lower safety classification, is needed to achieve fast and reliable results. Very common for these purposes is the host virus system with the host bacteria Escherica Coli and the bacteriophage (virus) Escherichia coli bacteriophage MS2. The corona viruses as well as the bacteriophage MS2 have their genes encoded on RNA instead of DNA. This fact and the fast and safe handling of E. coli and MS2 system makes them well suited as surrogate for experiments with Corona viruses.

The Sunexx filter foils or Sunexx glasses are permissive to a specific spectrum and intensity of UV irradiation. These Sunexx filter foils or Sunexx glasses have been designed for the purpose to optimize physiological and psychological beneficial effects of solar irradiation and to shield against most of the negative side effects (damaging of skin cells, which can promote cancer and skin aging). Concerning the biological safety, we showed that protective Sunexx filter foils or Sunexx glasses do ameliorate the damaging side effects) of solar irradiation (Ivanova et al. 2022).

The measurements of the transmissions of the Sunexx filter foils or Sunexx glasses were carried out using a Varian Cary 500 UV/VIS/NIR spectrophotometer with double beam scanning in the wavelength range from 200 nm to 3300 nm in one nanometer measuring steps (figure 2 and 3).

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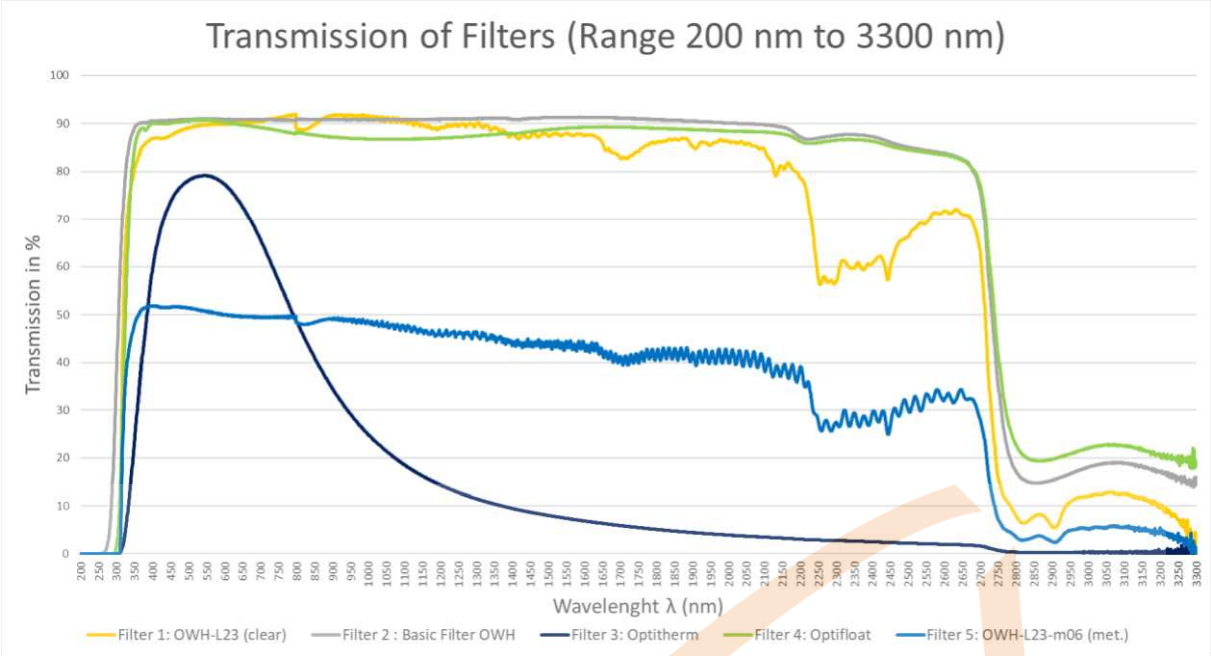

Figure 2 Transmission values of Sunexx filter foils or Sunexx glasses (filters) in the range from 200 to 3300 nm measured by spectrophotometer Cary 500

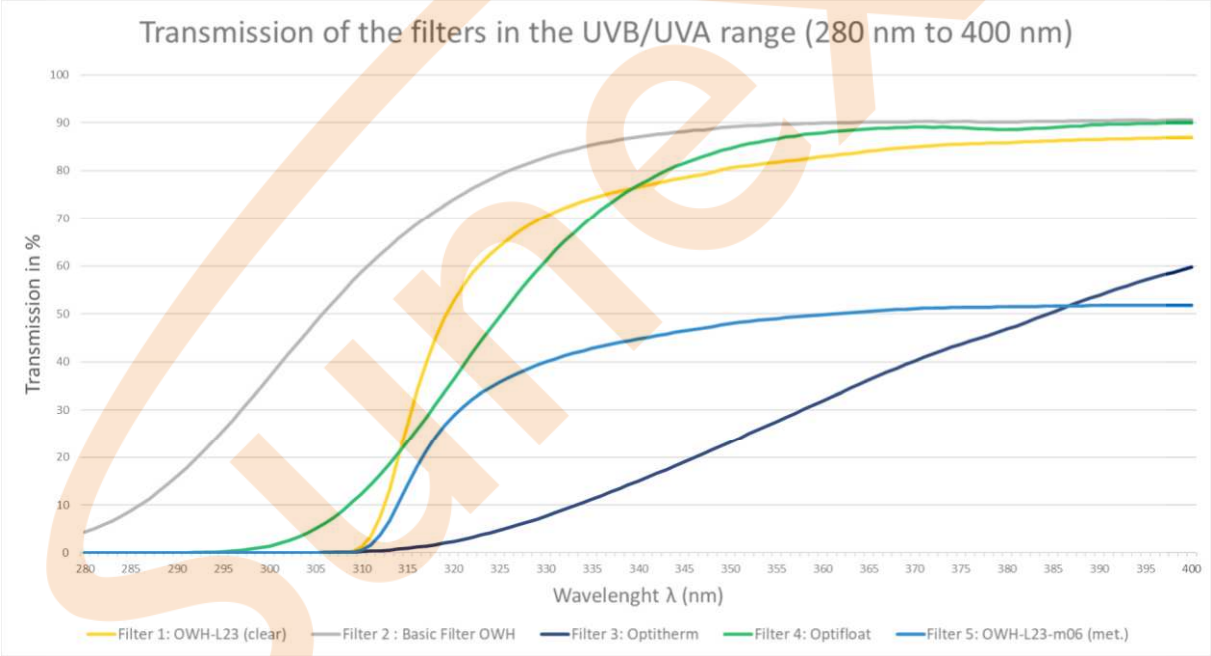

Figure 3 Transmission values of Sunexx filter foils or Sunexx glasses (filters) in the UVB/UVA range from 280 to 400 nm measured by spectrophotometer Cary 500

For a reliable, fast and safe detection of antiviral effects, the virus -host system (Escherica Coli and the bacteriophage Escherichia coli bacteriophage MS2) is well suited. These viruses (bacteriophages) are considered to be harmless for healthy persons and allow a

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safe handling due to low safety level (Sicherheitsstufe 1/Safety level 1). In addition to this, this system is a well-accepted alternative for experiments with Corona viruses.

A functional MS2 bacteriophage can infect its specific host bacterium (E. coli) and, after reproduction in this host, the copied viruses are released, resulting in the lysis of the bacterium. Due to the specifity of the host-phage-interaction it has a very low hazardous potential for humans and animals. Thus, this system can be handled very easy with minimal safety precautions and less bureaucratic obstacles.

One of the most versatile and reliable methods to quantify the number of functional bacteriophages is the plaque assay. In this assay, a diluted suspension of bacteriophages is applied on a petri dish, which is completely covered with bacteria (host of bacteriophages). A single functional bacteriophage can infect a bacterium. After infection of the initial host, the surrounding bacteria are infected, leaving a so-called lytic plaque at the site of infection (Strauss et al. 1963; Gaush et al. 1968) (figure 4) caused by copies of one initial functional phage. Therefore the number of plaques on the petri dish gives information how many functional bacteriophages have been in the suspension.

We modified this assay to test the efficiency of antiviral strategies (filtered UVB irradiation). Here viral suspensions were exposed to antiviral treatments (filtered UVB irradiation) and afterwards the ability of these viruses to infect bacteria was tested. The more efficient this antiviral treatment was, the less efficient could these treated viruses infect bacteria (because of the damage they received during the treatment).

In our modified assay, we treated suspensions with and without UVB irradiation and with and without UVB irradiation filtered with Sunexx foils or glasses.

Employing this technique, we found that UVB irradiation, which passes through basic filters, m06 filters as well as L23 filters have viricide effect on MS2 phages.

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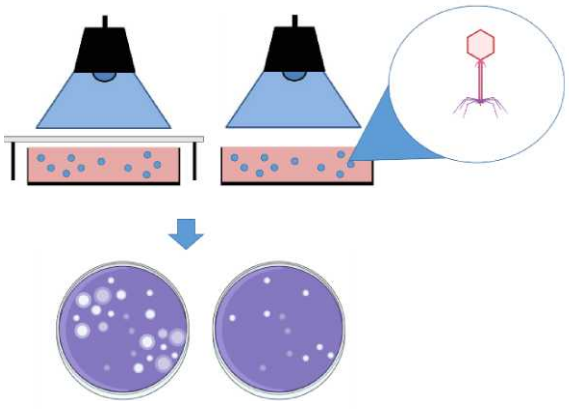

Figure 4 Investigation of Sunexx foils or glasses

Investigation of antiviral qualities (viricide effects) of Sunexx foils and glasses using a modified plaque assay. The figure was prepared using BioRender.

The project was funded by the funding programme Innovation Vouchers (as well as Hightech) of the Ministry of Economic Affairs, Labour and Tourism in Baden-Württemberg and by Sunexx GmbH. (The client is Sunexx GmbH, Zollernring 32, 72186 Empfingen, Germany.)

3. Material and Methods

3.1. Bacteria and viruses

The host bacteria and bacteriophages (Escherichia coli bacteriophage MS2) have been purchased form Leibniz Institute DSMZ (Escherichia coli (https://www.dsmz.de/collection/catalogue/details/culture/DSM-5695); Escherichia phage MS2 (https://www.dsmz.de/collection/catalogue/details/culture/DSM-13767)). The E.coli bacteria and viruses have been grown at 37 °C with the recommended medium NZCYM Medium (Roth, catalog number X974.1).

The bacteria were inoculated overnight with 5 mL NZCYM medium. On the following day the suspension was centrifuged for 5 min. at 5000 rpm. The medium was discarded and the pellet was resuspended with fresh medium.

For the phages propagation, the bacteria were inoculated overnight and then the bacterial optical density (OD) was measured at 600 nm wavelength, using Specord 50 Plus

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spectrometer from Analytik Jena GmbH (Jena, Thüringen, Germany). The bacteria were diluted until an OD of 0.2 was reached. Afterwards, a 100 µl of the phages were added to 10 mL bacterial suspension. The suspension was incubated at 37 °C on a shaker until the solution turned clear. Subsequently, the lysate, containing functional bacteriophages, was centrifuged at 10 000 rpm for 10 min. and filtered using 0.2 µm Steriflip filter (Millipore, catalog number SCGP00525). The phages suspension was stored at 4 °C for up to a week.

3.2. UV irradiation

The lysate containing the phages was tempered to room temperature. Subsequently, 200 µl of the suspension was pipetted on a cell culture dish (Corning Primaria, catalog number 353801). The dishes were irradiated using different UV lamps – UVA Sellamed 1200 lamp with a peak at 375 nm, UVB Biometra lamp with peak at 312 nm and UVC lamp with peak at 254 nm.

3.3. Measurement of the radiation transmitted by the Sunexx filter foils or Sunexx glasses (filters)

The radiation transmitted by the Sunexx filter foils or Sunexx glasses (filters) was measured using an UVpad spectrometer from Opsytec Dr. Gröbel GmbH (Ettlingen), Germany in accordance with the manufacturer's instructions.

3.4. OD (Optical Density) assay

The bacteria were cultured overnight. On the following day, the suspension were centrifuged and the old medium was discarded. The bacteria were resuspended in fresh medium and diluted until a starting OD of 0.2 was reached. One hundred and fifty µl of bacteriophage lysate (which have been treated with or without UVB irradiation, with/without UVC irradiation, with/without UVA irradiation and with/without UVB+Sunexx foils/glasses were added to 10 ml of bacterial suspension and incubated at 37 °C on a shaker. The OD was measured every 45 min.

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3.5. Drop cast assay

The E.coli bacteria were cultured overnight. The next day the suspension was centrifuged and the old medium was discarded. The bacteria were resuspended in fresh medium and diluted until a starting OD of 0.3 was reached. One mL of this suspension was platted on NZCYM-agar-petri dishes and incubated overnight at 37 °C to allow a bacterial lawn to grow. On the following day, the phages were treated with/without UVB irradiation and with/without UVB irradiation filtered with Sunexx foils or glasses (table 1 and table 2). The treated phages´ lysate samples were diluted in NZCYM Medium in a dilution series (from undiluted to 10^{-15}), mixing 100 µl lysate with 900 µl medium. After the preparation of the dilution, 5 µl of the diluted lysate was plated on the bacterial lawn and incubated overnight at 37 °C. Afterwards, the number of lytic plaques was photographed and counted (figure 5).

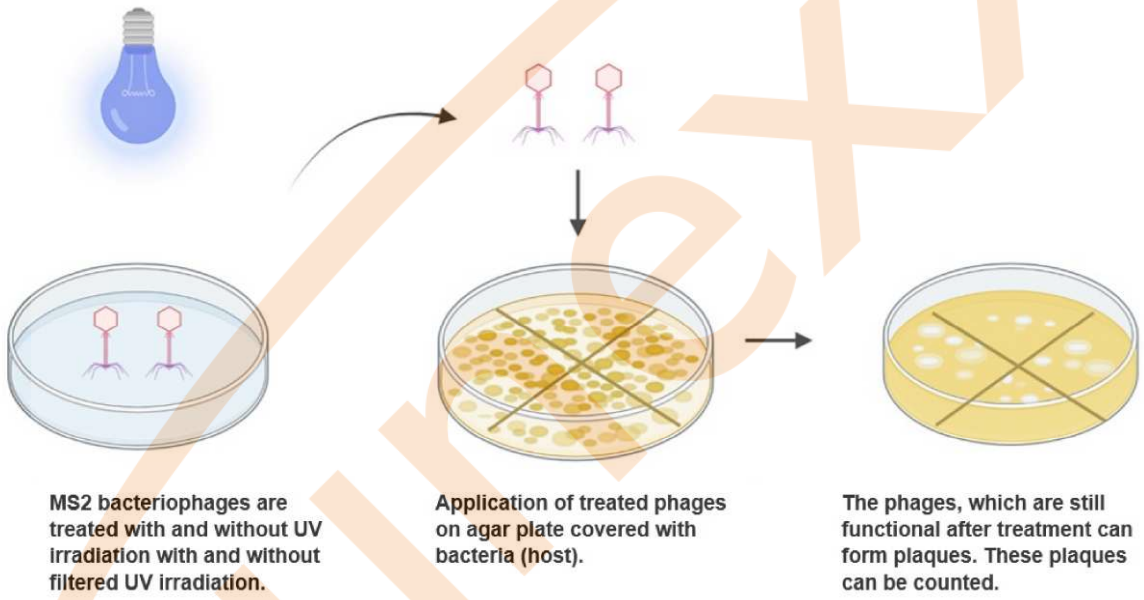

Figure 5 Drop cast assay

Fig.5 shows: MS2 bacteriophages are treated with and without UV irradiation or and with and without filtered UV irradiation. Afterwards the treated phages are applied on sections of prepared agar plates, which are covered with bacteria (host). The bacteriophages, which are still functional after treatment can form plaques. These plaques can be counted. The figure was prepared using BioRender.

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Filter	description of filter – thickness 4 mm
1	UVB Sunexx – Type of filter: OWH-L23 (clear)
2	UVB Sunexx – Type of filter: basic filter/basic glass
3	UVB Sunexx – Type of filter: Optitherm
4	UVB Sunexx – Type of filter: Optifloat
5	UVB Sunexx – Type of filter: OWH-L23m06 (met.)

Table 1 description of Sunexx filter foils or Sunexx glasses (filters)

	No UVB	UVB treatment (1000 mJ/cm^2)					
		No filter	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5
Diluted bacteriophage suspensions (dilution factor 10^{-8})	x						
Diluted bacteriophage suspensions (dilution factor 10^{-8})		x					
Diluted bacteriophage suspensions (dilution factor 10^{-8})			x				
Diluted bacteriophage suspensions (dilution factor 10^{-8})				x			
Diluted bacteriophage suspensions (dilution factor 10^{-8})					x		
Diluted bacteriophage suspensions (dilution factor 10^{-8})						x	
Diluted bacteriophage suspensions (dilution factor 10^{-8})							x

Table 2 Treatment of bacteriophage suspensions for drop cast assay

Each diluted bacteriophage suspensions (here shown for the diluted bacteriophage suspension with the dilution factor 10^{-8}) have been treated as depicted in this table. The same setup has been used for further dilutions (dilution factors from 10^{-9} to 10^{-15}).

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3.6. Plaque assay

The E.coli bacteria were inoculated overnight. The next day the suspension were centrifuged and the old medium was discarded. The bacteria were resuspended in fresh medium and diluted until a starting OD of 0.6 was reached. The phages were treated with/without UVB irradiation and with/without UVB irradiation filtered with Sunexx foils/glass (table 1 and table 3). The suspensions with the treated phages were diluted in NZCYM Medium in a dilution series (from undiluted to 10^{-11}), mixing 100 µl lysate with 900 µl medium. Five hundred µl of the bacterial suspension and a hundred µl of the diluted phages (from 10^{-7} to 10^{-11}) lysate were mixed together and platted on NZCYM-agar-petri dishes. Following an overnight incubation at 37 °C, the plaques number were photographed and counted (figure 6).

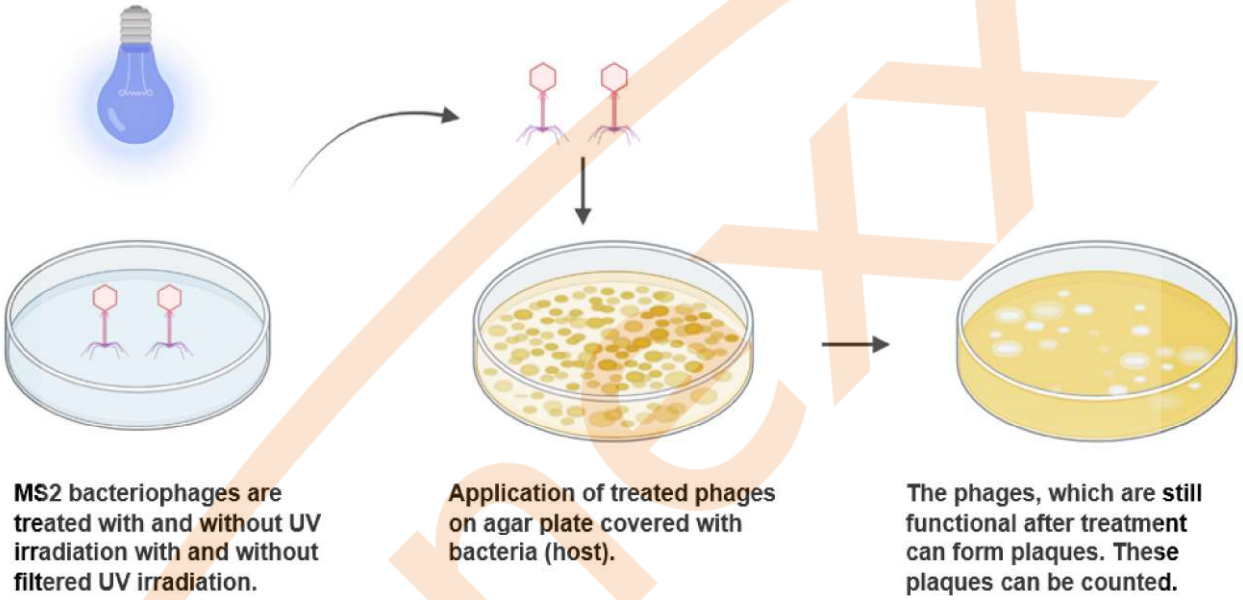

Figure 6 Plaques assay

This figure shows MS2 bacteriophages are treated with and without UV irradiation or and with and without filtered UV irradiation. Afterwards the treated phages are applied on agar plates covered with bacteria (host). The bacteriophages, which are still functional after treatment can form plaques. These plaques can be counted. The figure was prepared using BioRender.

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	No UVB	UVB treatment (1000 mJ/cm^2)					
		No filter	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5
Diluted bacteriophage suspensions (dilution factor 10^{-7})	x						
Diluted bacteriophage suspensions (dilution factor 10^{-7})		x					
Diluted bacteriophage suspensions (dilution factor 10^{-7})			x				
Diluted bacteriophage suspensions (dilution factor 10^{-7})				x			
Diluted bacteriophage suspensions (dilution factor 10^{-7})					x		
Diluted bacteriophage suspensions (dilution factor 10^{-7})						x	
Diluted bacteriophage suspensions (dilution factor 10^{-7})							x

Table 3 Treatment of bacteriophage suspensions for plaque assay

Each diluted bacteriophage suspensions (here shown for the diluted bacteriophage suspension with the dilution factor 10^{-7}) have been treated as depicted in this table. The same setup has been used for further dilutions (dilution factors from 10^{-7} to 10^{-11}).

3.7. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 10.3.1. Comparisons among groups were performed using one-way ANOVA, with Dunnett´s multiple comparisons test. The data was presented as the mean ± standard deviation of three independent experiments. $P < 0.05$ was considered to indicate a statistically significant difference.

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4. Results

4.1. Measurement of the radiation transmitted by the Sunexx filter foils or Sunexx glasses (filters)

The radiation transmitted by the Sunexx filter foils or Sunexx glasses (filters) was measured with a spectrometer from Opsytech (UVpad E portable spectroradiometer) in a range from 260 nm to 400 nm. The transmission data shows that the foils/glass have a wavelength-specific absolute and relative filtration characteristics (see figure 2 and 3), in which a specific part in a specific intensity is allowed to pass the filter (figure 7).

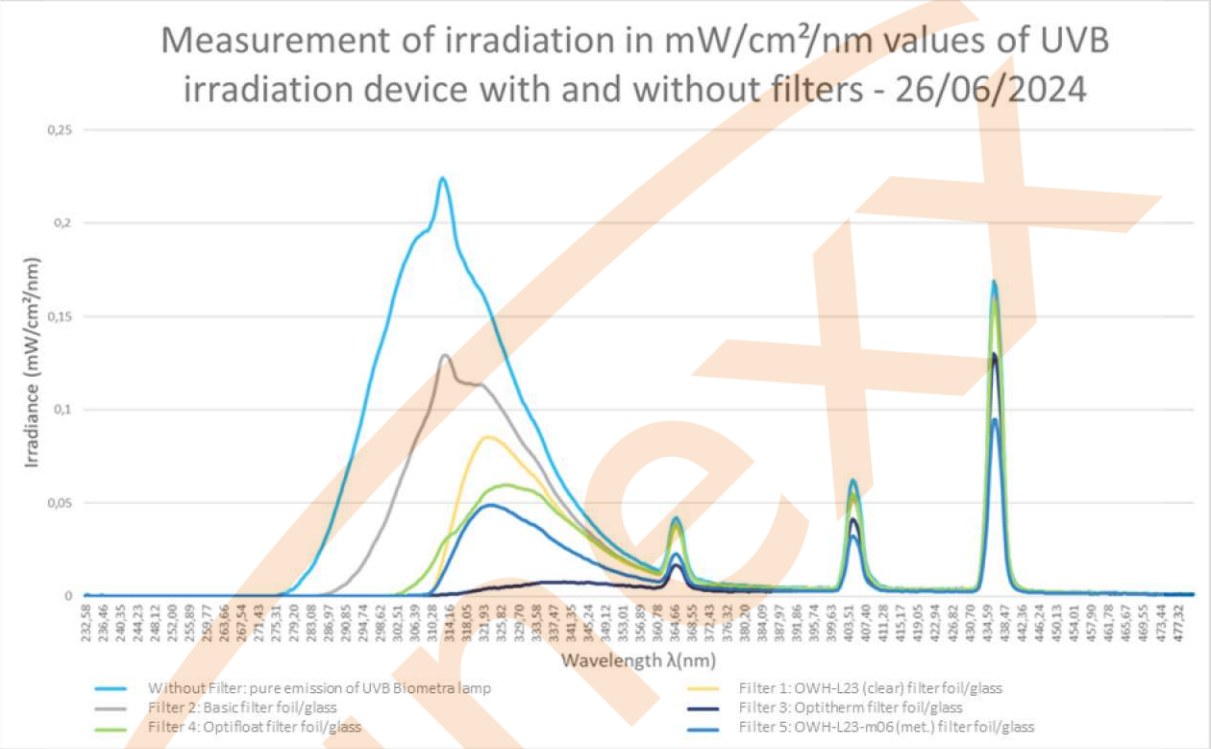

Figure 7 Measurement of irradiation of UVB irradiation device with and without Sunexx filter foils or Sunexx glasses (filters)

The spectral irradiance of UVB irradiation device was measured with and without Sunexx filter foils or Sunexx glasses (filters). The distance of the spectral radiometer to the UVB irradiation device was comparable to the distance applied with bacteriophage suspensions.

4.2. OD (optical density) measurement

The excessive growth of bacteria in medium makes the medium more or less turbid, leading to increased OD values. The OD values in this experiment depend on the concentration of bacteria in the medium. The higher the concentration, the higher the OD

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value. If bacteriophages are added to a growing bacteria population, the phages will proliferate and the concentration of bacteria will decrease leading to a lower OD value.

The irradiation treatment induces damages in the population of bacteriophages and the number of functional infectious phages is decreased, which leads to a delayed growth or damaged during radiation a growth retardation (figure 8).

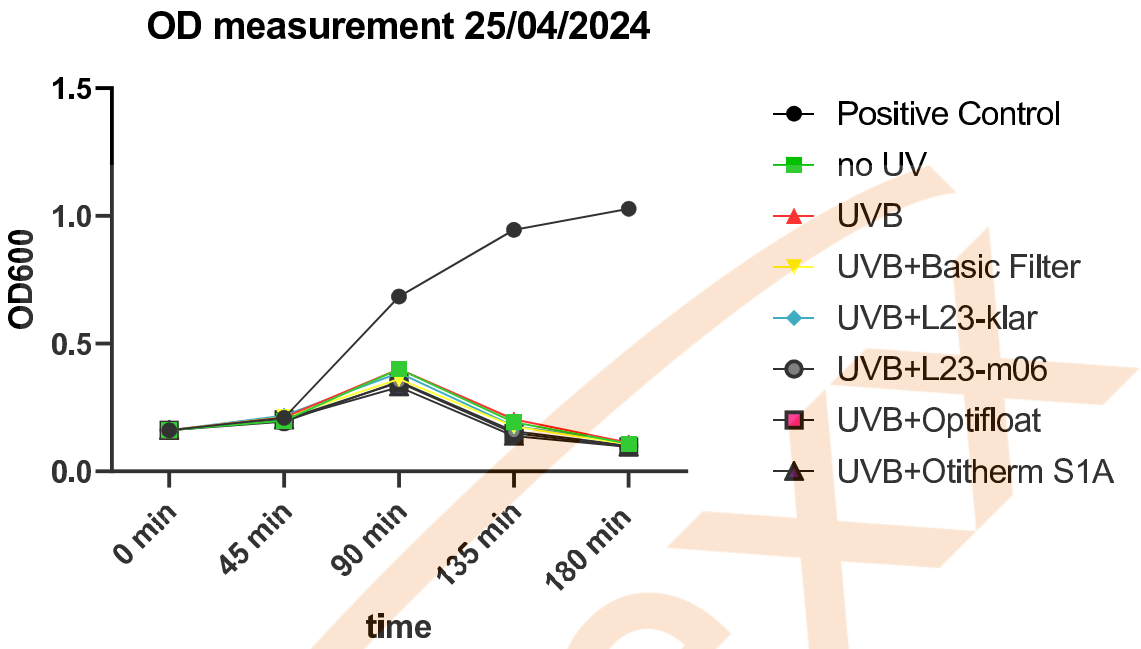

Figure 8 OD measurement with UVB irradiation with and without different filters

This figure shows the OD measurement following treatment with 30 mJ/cm² UVB irradiation (no UV; UVB 30 mJ/cm²; UVB+ Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat, UVB + Optitherm).

The OD values of no UV treated phages and the OD phages treated with UVB and UVB + filters display no significant difference, showing that filtered UV irradiation is capable to decrease virus load (figure 8).

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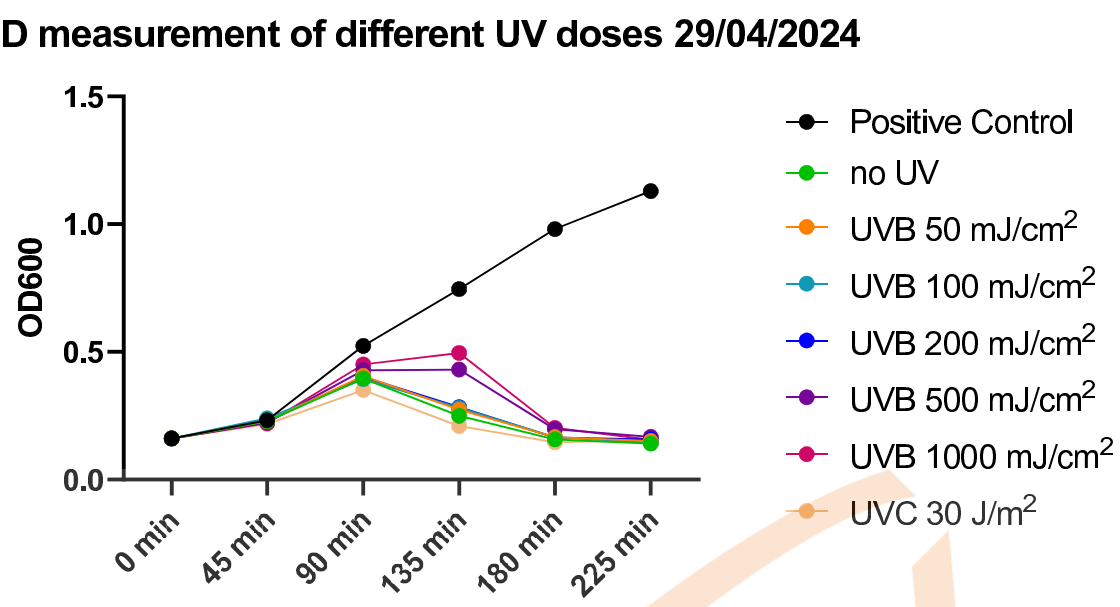

Figure 9 OD measurement with and without UVB irradiation (different doses) and with and without UVC irradiation

This figure shows the OD measurement following treatment with different doses of UVB irradiation and UVC irradiation (no UV; UVB 50 mJ/cm^2; UVB 100 mJ/cm^2, UVB 200 mJ/cm^2, UVB 500 mJ/cm^2, UVB 1000 mJ/cm^2 and UVC 30 J/cm^2).

As described previously the bacterial growth leads to a turbid medium (due to the large number of bacteria in the medium) with high OD values. If viral suspensions are added to growing bacteria, the viruses kill most of the bacteria, leading to a clearance of turbid medium with low OD values. Here the addition of viral suspensions leading to a clearance of the medium. This clearance is delayed when viral suspensions are treated with high doses of UVB irradiation (500 mJ/cm² and 1000 mJ/cm²). This indicates, a large proportion of the bacteriophage population was inactivated by the 1000 mJ/cm^2 and the number of functional phages decreased (figure 8). The remaining low number of phages needed more time to proliferate and clear the medium.

These results show, that UVB irradiation could potentially have an antiviral effect!

In an attempt to estimate the doses of UV irradiation (UVA irradiation, UVB irradiation and UVC irradiation), which is sufficient to kill the complete phage population, we increased the UV doses (18 J/cm^2 UVA irradiation; 5000 mJ/cm^2 UVB irradiation; 70 J/m^2 UVC irradiation). The phage populations, which received high UVC and UVA doses, still were able to proliferate in their hosts, leading to a clearance of the medium. In the phage population which received a high UVB dose no prominent clearance of the medium was observed in the first 135 minutes after irradiation and after 135 minutes a small difference

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to the untreated control was observed, indicating that a very low number of phages might have been still functional after the applied UVB dose (figure 8).

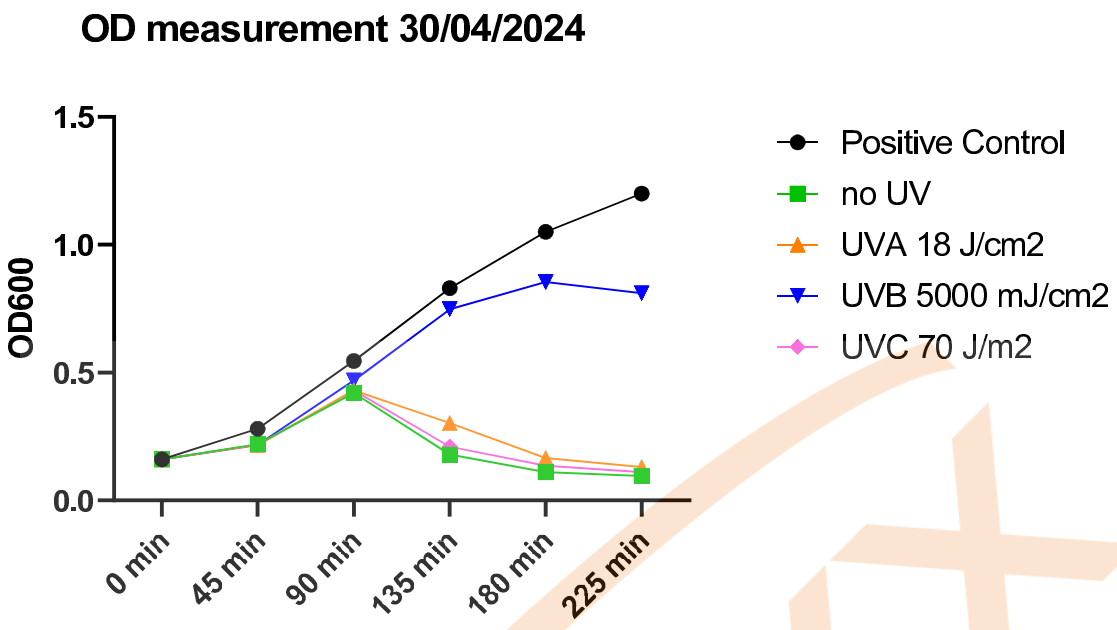

Figure 10 OD measurement with and without UVA irradiation and with and without UVB irradiation and with and without UVC irradiation

This figure represents the OD measurement following treatment with UVA 18J/cm^2, UVB 5000 mJ/cm^2 and UVC 70 J/m^2.

The OD values of UVA and UVC treated phages show no difference compared to the untreated phages (figure 9). The bacteriophages treated with 5000 mJ/cm^2 UVB show a serious increase of the OD values, meaning a notable reduction in the phage population (figure 9). Although at the end of the measurement, a drop down in the OD was visible. If one estimates an exponential growth model for phage proliferation this indicates that only a small portion of the phages was not damaged and were still viable.

These results show, high doses of UV irradiation are needed to induce serious damage to the phage populations. These irradiations needed extremely long irradiation time, which makes these high dose treatments unpractical and inapplicable in our laboratory settings.

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4.3. Drop cast assay

In this assay 1000 µl of bacterial suspension were platted on a agar plate and left overnight to form bacterial lawn. The next day, 5 µl of the phage suspension with different dilutions were applied to the bacterial plates After overnight incubation the number of spots have been photographed and counted.

As seen, the number of plaques is decreased upon treatment with filtered UV irradiation with Sunexx filters, compared to the number of plaques of the control experiment (no UV treatment) at the same time. The population of phages was damaged by filtered UV irradiation (figure 10). These results show, that filtered UV irradiation can induce an antiviral effect!

Figures 10-12 show the number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

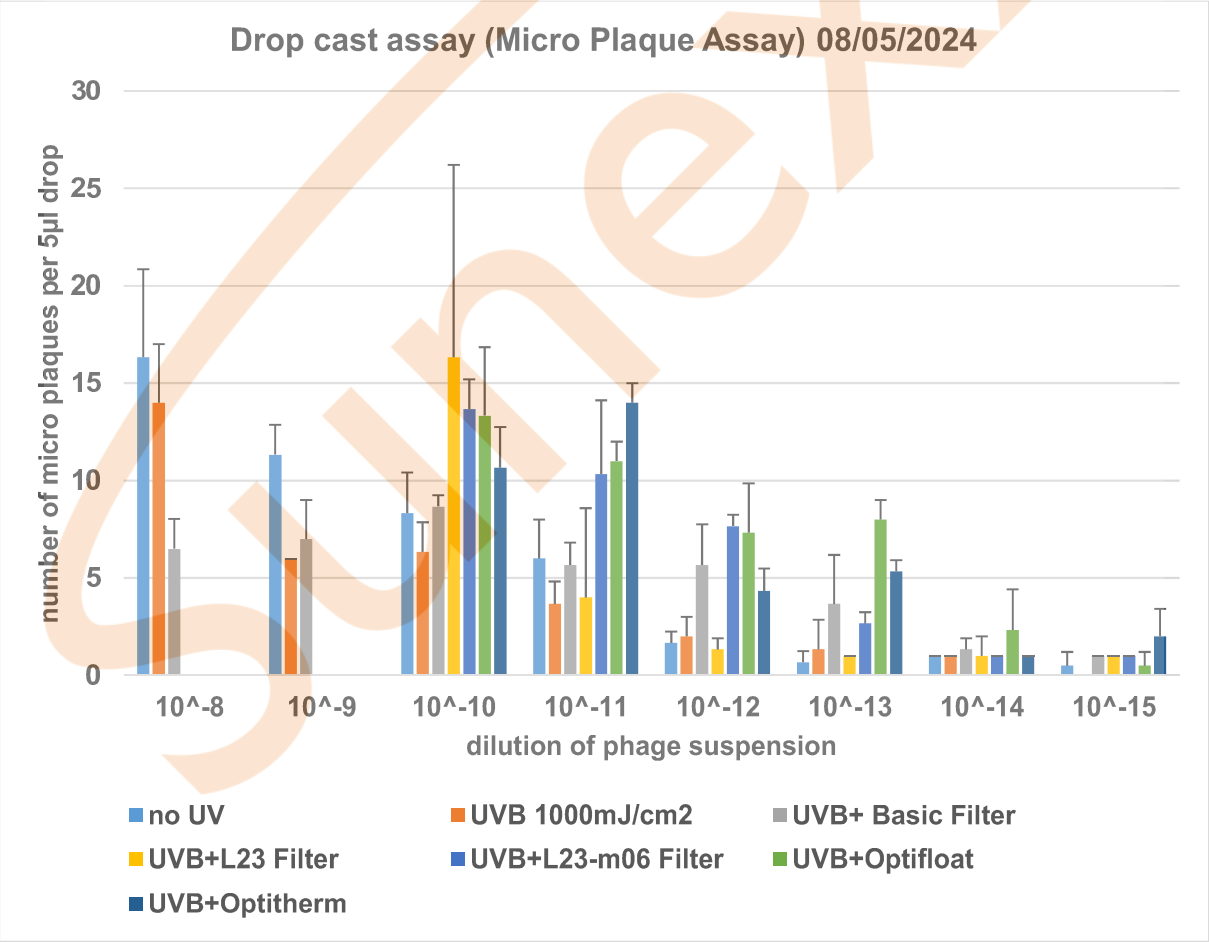

Figure 11 Drop Cast Assay (Micro Plaque Assay) first experiment

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This figure represents the drop cast experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB+L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

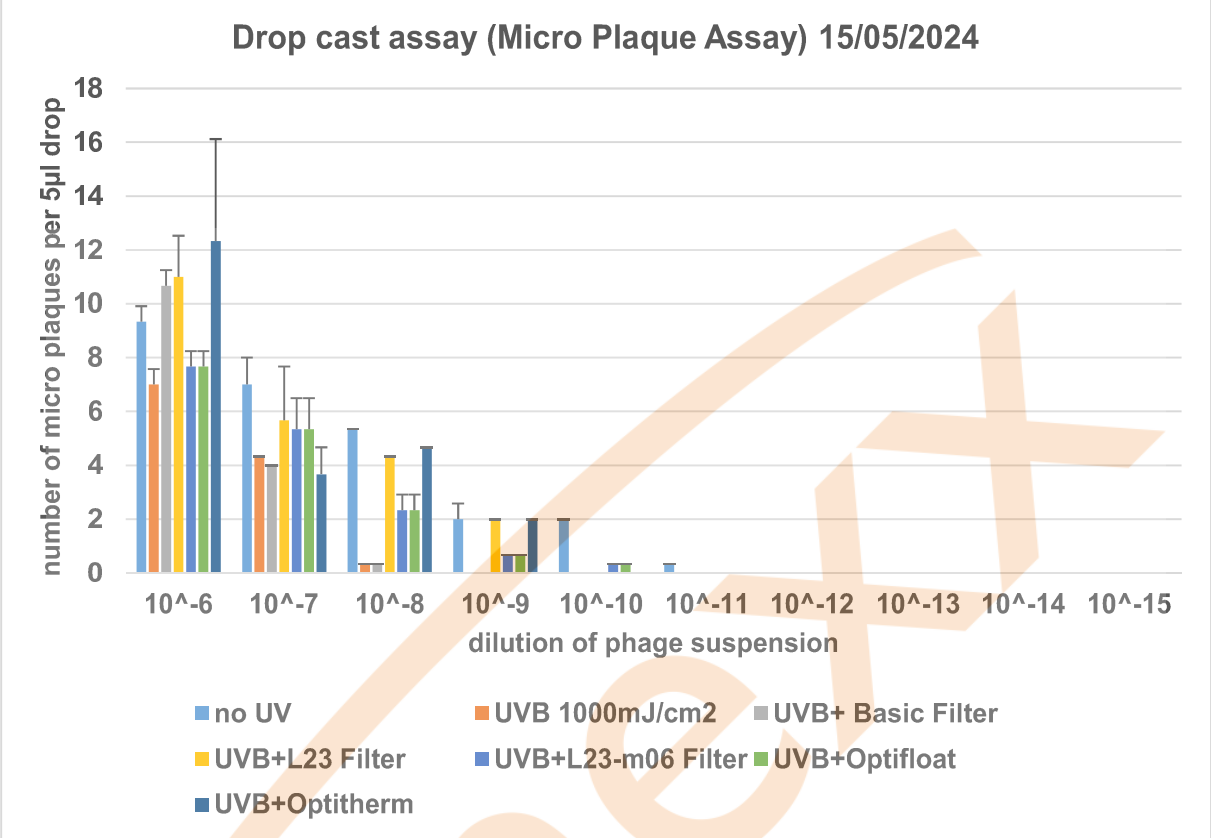

Figure 12 Drop Cast Assay (Micro Plaque Assay) second experiment

This figure represents the drop cast experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

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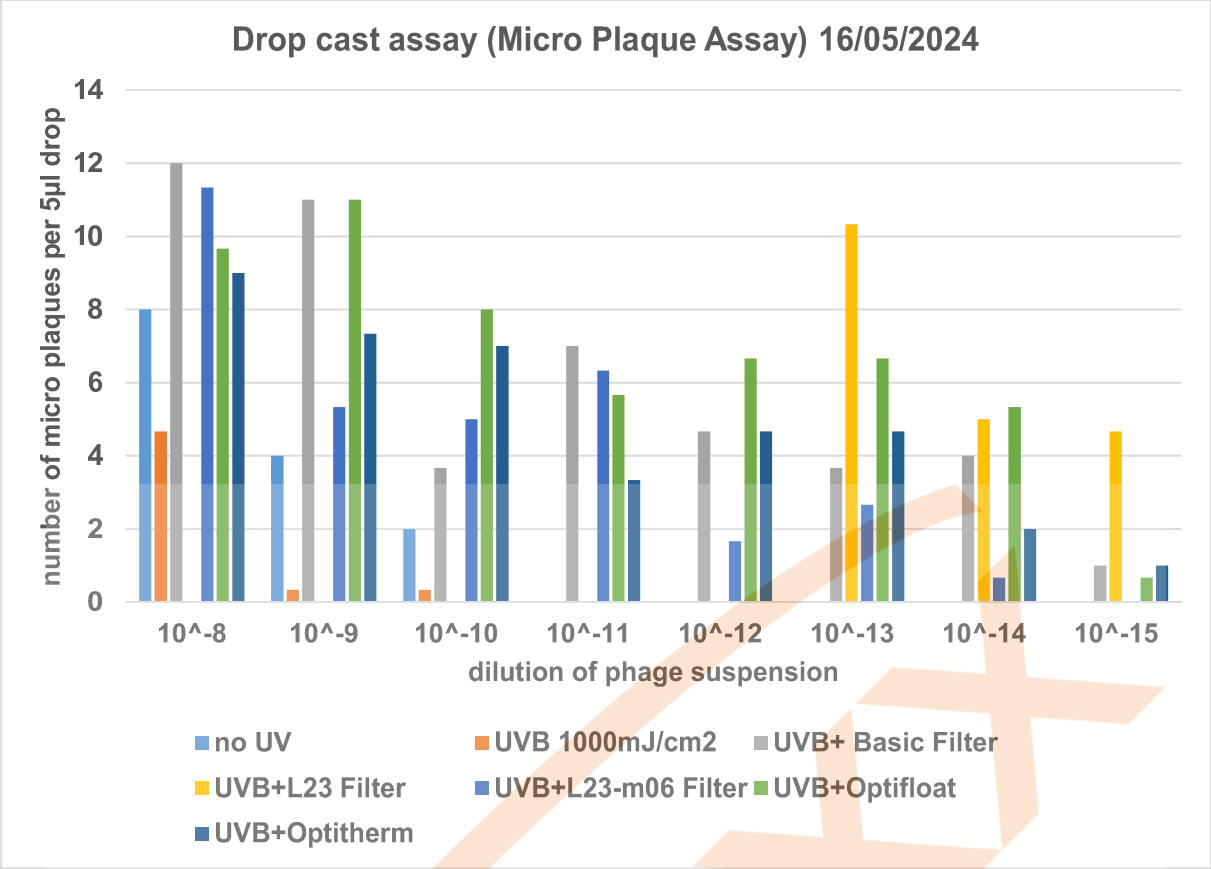

Figure 13 Drop Cast Assay (Micro Plaque Assay) third experiment

This figure displays the drop cast experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

These results show, that UV treatment influenced the growth of phages. The results of the experiments are heterogeneous and this technique seems to have high variations in our setup. Therefore, we switched to the plaque assay as a more reliable assay.

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4.4. Plaque assay

In this assay 100 µl of phage suspensions with different dilutions of the original phage suspension and 500 µl of host suspension were mixed together and applied on sections of agar plates. After overnight incubation, the plates were photographed and number of plaques and counted.

As seen, the number of plaques is decreased upon treatment with filtered UV irradiation with Sunexx filters, compared to the number of plaques of the control experiment (no UV treatment) at the same time. The population of phages was damaged by filtered UV irradiation (figures 14-19). These results show, that filtered UV irradiation can induce an antiviral effect!

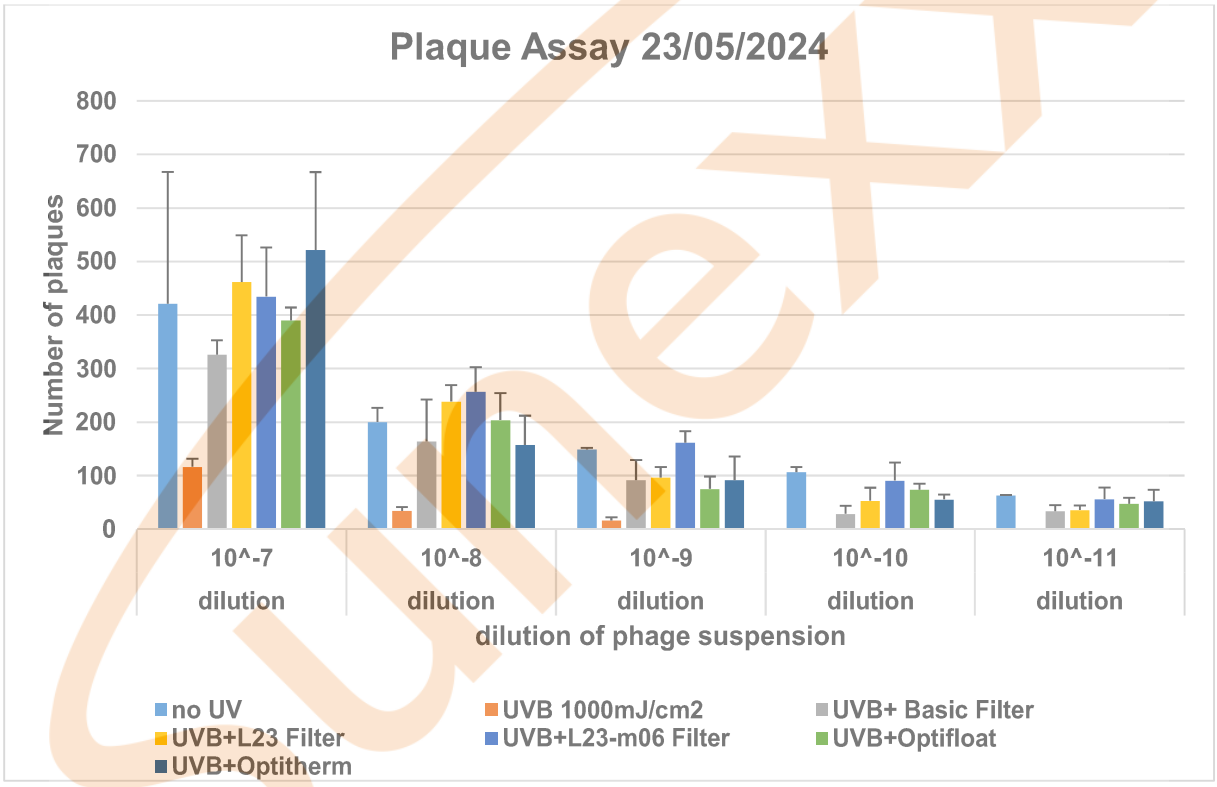

Figure 14 Plaque Assay (first experiment)

This figure shows the plaques assay experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm) The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

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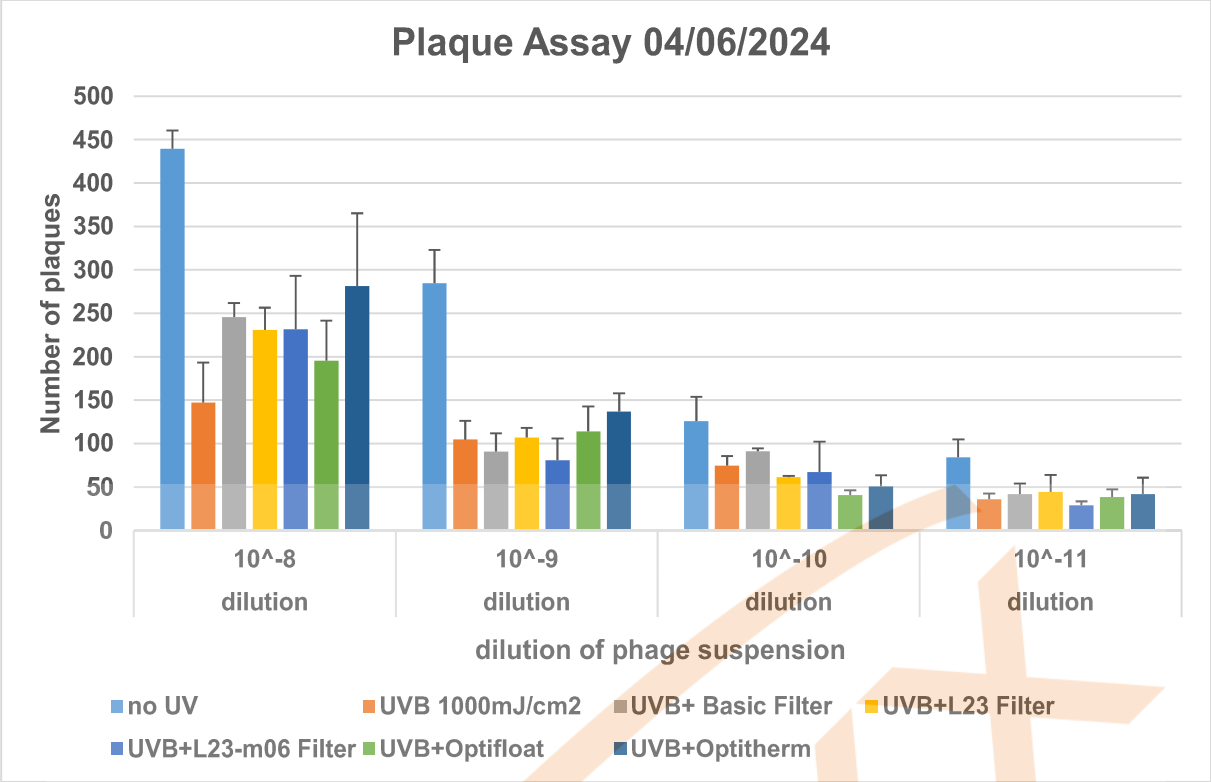

Figure 15 Plaque Assay (second experiment)

This figure shows the plaques assay experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm) The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

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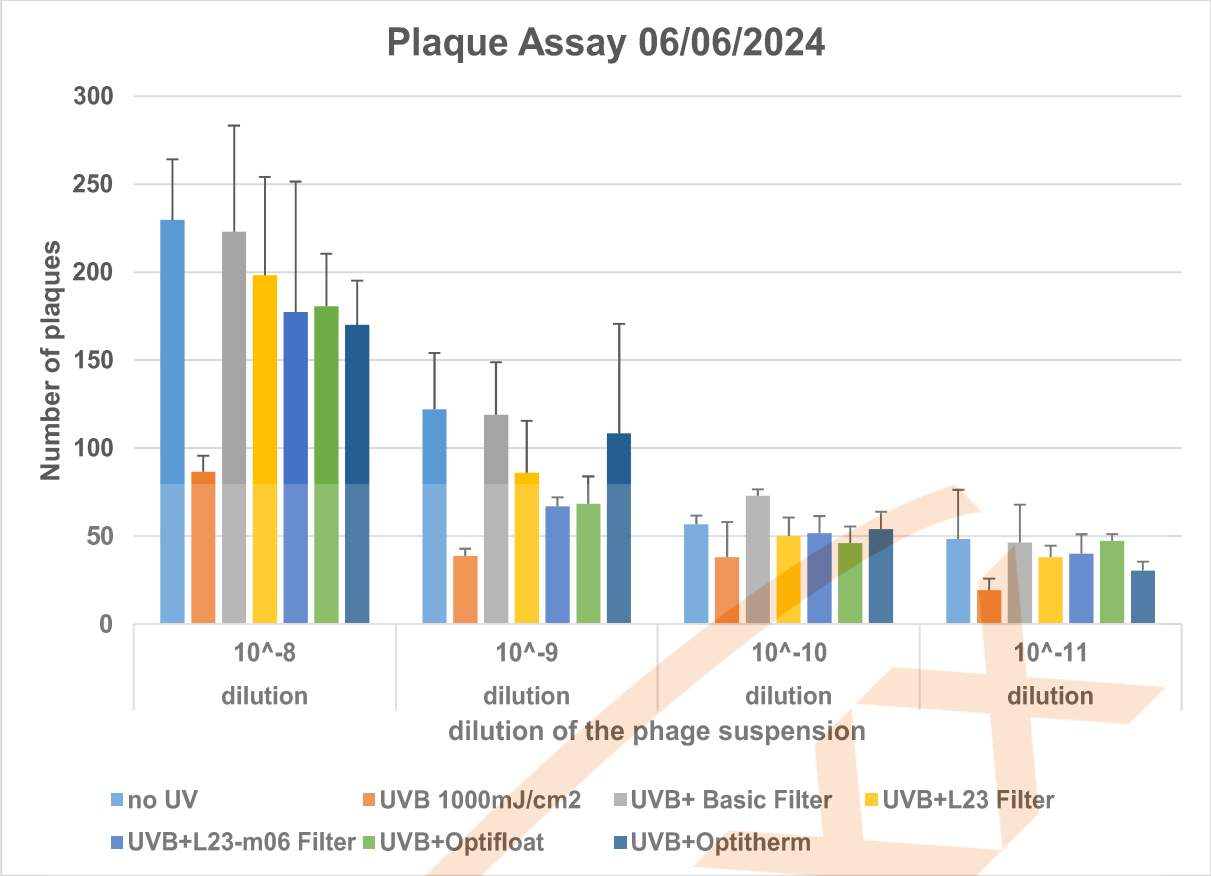

Figure 16 Plaque Assay (third experiment)

This figure shows the plaques assay experiment using different dilutions of treated phages (no UV; UVB 1000 mJ/cm²; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm) The number of plaques induced by treated phages (with UV irradiation and with and without Sunexx filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation).

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Plaque assay: Influence of UVB and filters on phage virality (n=3)

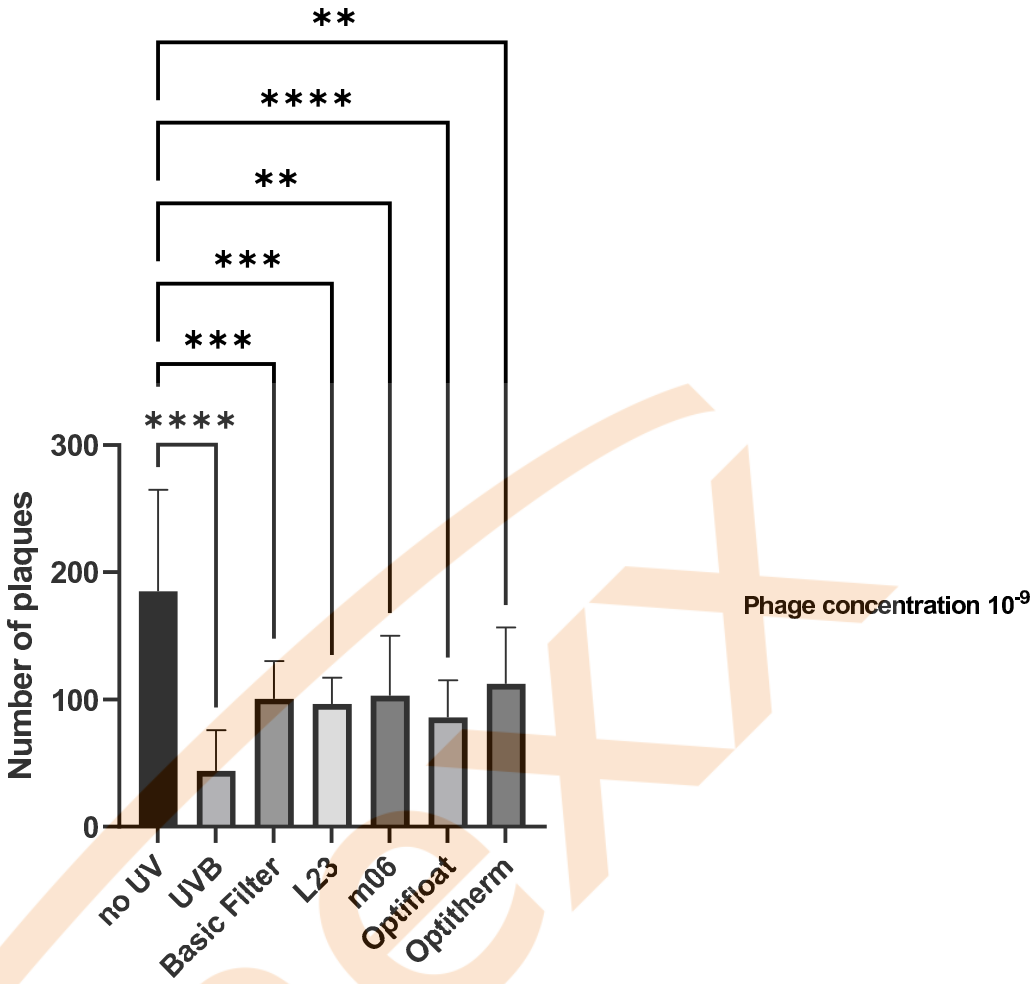

Figure 17 Plaque Assay (summary for phage concentration 10^{-9})

This figure shows the mean value of count by phage concentration 10^{-9} following treatment (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The figure shows the mean value with standard deviation of three independent experiments.

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Plaque assay: Influence of UVB and filters on phage virality (n=3)

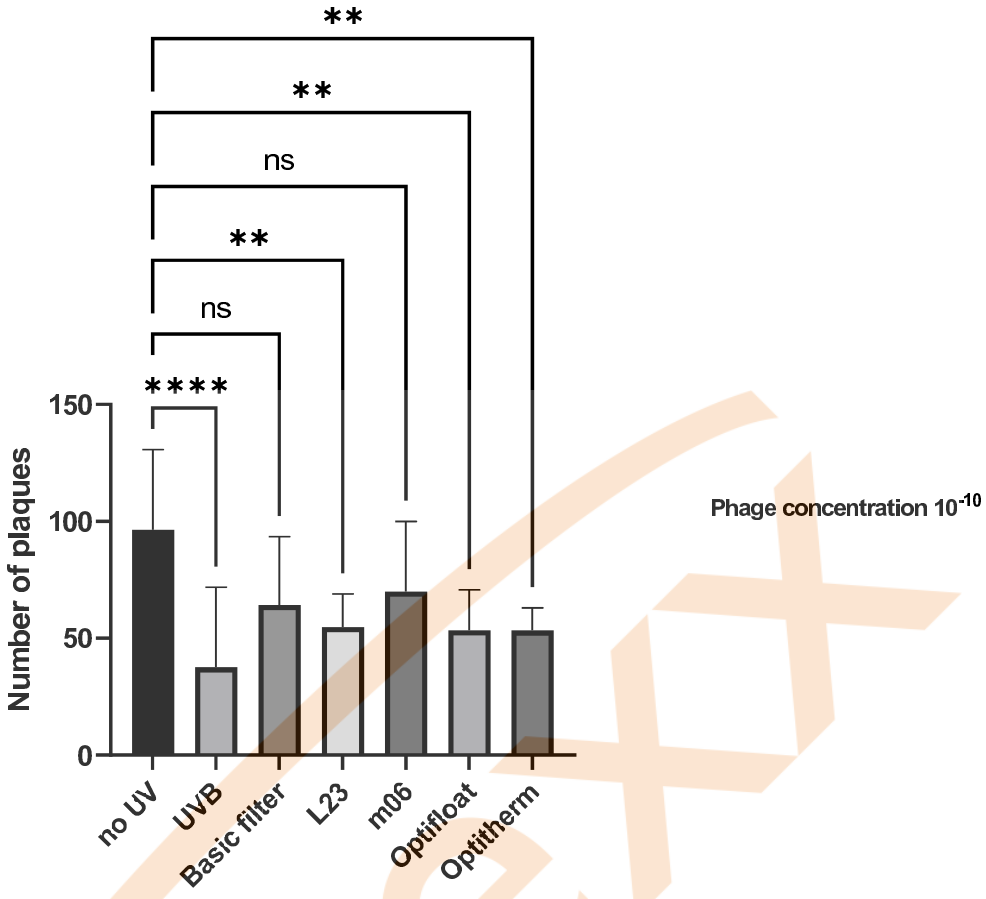

Figure 18 Plaque Assay (summary for phage concentration 10^{-10})

This figure shows the mean value of count by phage concentration 10^{-10} following treatment (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The figure shows the mean value with standard deviation of three independent experiments.

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Plaque assay: Influence of UVB and filters on phage virality (n=3)

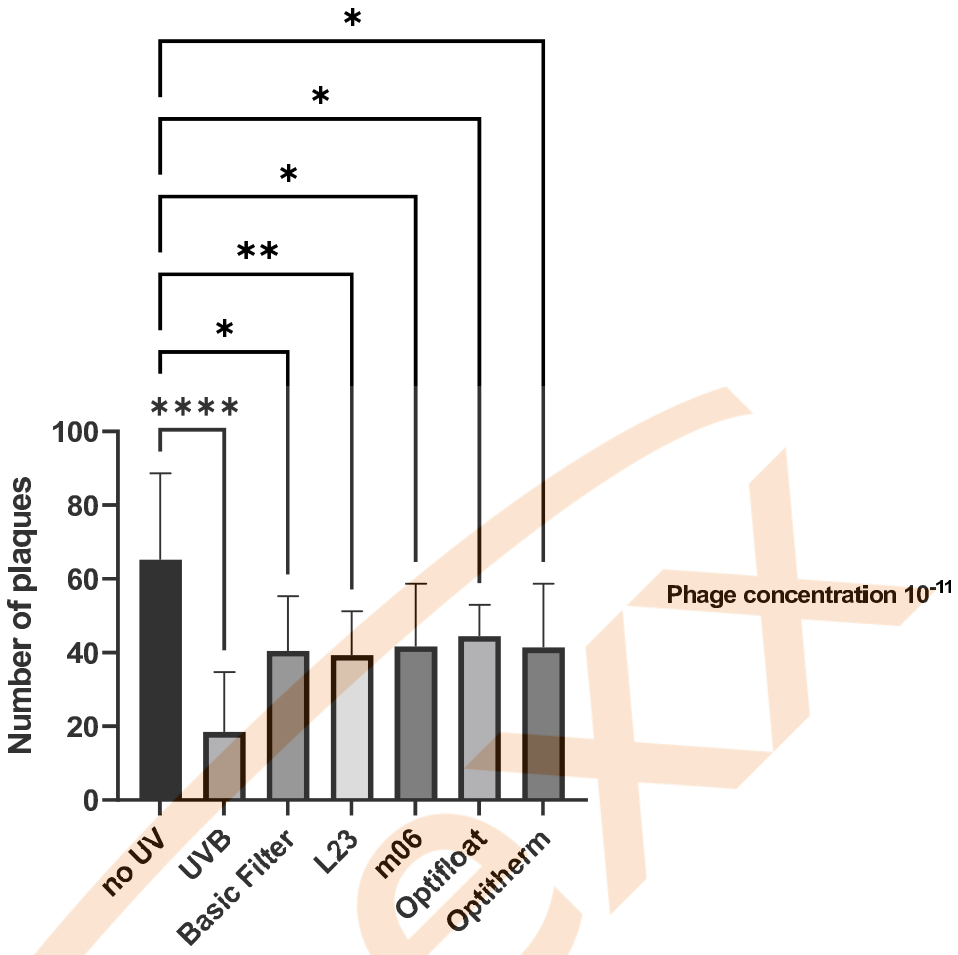

Figure 19 Plaque Assay (summary for phage concentration 10^{-11})

This figure shows the mean value of count by phage concentration 10^{-11} following treatment (no UV; UVB 1000 mJ/cm^2; UVB + Basic Filter; UVB + L23 Filter; UVB + L23-m06 Filter, UVB + Optifloat; UVB + Optitherm). The figure shows the mean value with standard deviation of three independent experiments.

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5. Discussion

It has been shown that Sunexx filters can decrease negative effects of UV irradiation in human cells (Ivanova et al.; 2022). The actual situation concerning Corona pandemic shows, that new antiviral strategies and treatments are needed to interfere with ongoing pandemics and to avoid or counteract future pandemics. The aim of this study was to test antiviral properties of UVB irradiation, filtered with Sunexx filters.

To achieve this, we modified the plaque assay test system to get a safe and versatile viral test system for the investigation of the antiviral effects of different treatments. We confirmed the antiviral effect of UVB irradiation and we could show that an antiviral effect of UVB irradiation filtered with Sunexx foils or glasses is still present.

5.1. Measurement of transmission of Sunexx foils or glasses

The measurement of the transmission values (irradiation power in $mW/cm^2/nm$) show, that the foils/glasses have specific transmission profiles with different peaks with different intensity in the range of approximately 295 nm to 330 nm quality and several peaks in similar range of approximately 364 nm, 406 nm and 436 nm with different intensities. This data reveals, that each foil/glass we investigated has a characteristic transmission profile (see figure 2 and 3). Each profile with its specific intensity for each wavelength can have different biological effects. It has been shown that treatment with infrared irradiation ameliorated UV irradiation induced apoptosis (Kimeswenger et al. 2016).

5.2. OD (optical density) measurement

The more bacteria grow in a medium the more this medium gest turbid. If bacteriophages are applied to a medium with growing bacteria, the number of bacteria is reduced due to viral reproduction and the medium gets less turbid. The clearance of the medium delays, when virus suspensions treated with high doses of UV irradiation are applied to the bacteria. This shows that different numbers of irradiation and different kinds of irradiation (UVC, UVB and UVA irradiation) can decrease the virus populations. Interestingly high doses of UV irradiation are need to achieve a delayed clearance of the medium. This is in accordance to the results of other groups (String et al. 2023), but the doses applied to the virus suspensions would lead to severe damage in human skin cells (literature). However, these results are not sufficient for the assumption that these viruses could have some protection systems against high doses of UV irradiation. The results gained by these

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experiments cannot be compared to different techniques used in human skin cells to measure UV sensitivity. If one estimates an exponential growth model for phage proliferation, even a small number of functional viruses after treatment can lead to excessive numbers of viruses (and clearance of the medium) in a short period of time.

5.3. Drop cast assay

In this assay, a bacterial lawn was treated with different dilutions of phage suspension and after incubation, the number of spots has been photographed and counted. In these experiments it can be seen, that UV irradiation with and without filters often had effects on the number of spots.

It has to be mentioned that dilutions with high numbers of phages (10^{-7}; 10^{-8};10^{-9}) sometimes yielded a high number of spots, some of them grown together. This high concentration of spots per area were difficult to count and sometimes errors can appear. As seen in most experiments with dilutions of 10^{-9} or higher dilutions the number of spots is decreased upon treatment with filtered UV irradiation with Sunexx filters, compared to the number of spots of the control experiment (no UV treatment) at the same time. The population of phages was damaged by UV treatment with or without filters (figure 8-10). These results show that filtered UV irradiation can have an antiviral effect. It has to be noted that all investigated filters (Basic Filter; L23 Filter; L23-m06 Filter, Optifloat; Optitherm) show more or less intensive this antiviral effects. But up to now only L23 filters and L23-m06 filters have been shown to have protective qualities against UV induced aging and cancer associated cellular damage in human cells (Ivanova et al. 2022).

5.4. Plaque Assay

In this assay phage suspensions with different dilutions were incubated together with their host bacteria on agar plates. After incubation and treatment (no UV; UVB 1000 mJ/cm^2; UVB+ Basic Filter; UVB+L23 Filter; UVB+L23-m06 Filter, UVB+Optifloat; UVB+Optitherm) the number of plaques have been photographed and counted. The number of plaques induced by treated phages (with UV irradiation and with filters) was decreased, compared to the number of plaques induced by untreated phages (no UV irradiation) (figures 11-13).

Similar to the results of the drop cast assay, the dilutions with a high number of phages (10^{-7}; 10^{-8};10^{-9}) sometimes yielded a high number of plaques spots, some of them grown together. This high concentration of plaques per area were difficult to count and sometimes

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errors an appear. Therefore, only dilutions in which counting was reliable were displayed. The figure shows medium values and standard deviations of three independent experiments with higher dilutions (10^{-9}; 10^{-10};10^{-11} 10^{-12}). It can be seen that UV treatment with and without filters decreased the number of functional viruses. The population of phages was also damaged with filtered UV irradiation, showing a viricide effect of filtered UV irradiation in different dilutions (figure 11-15). These results show that filtered UV irradiation can have an antiviral effect, supporting results from drop cast assays. This fact strengthens the hypothesis that UV irradiation filtered with Sunexx filters does have an antiviral effect. Similar as in the results of the drop cast assay, it has to be noted that all investigated filters (Basic Filter; L23 Filter; L23-m06 Filter, Optifloat; Optitherm) show more or less intensive this antiviral effects. As mentioned above, up to now only the two filters (L23 filters and L23-m06) have been shown to be protective against UV induced aging and cancer associated cellular damage in human cells (Ivanova et al. 2022).

6. Outlook

We treated viruses with UV irradiation, filtered by Sunexx foils/glasses and showed with our modified plaque assay, that UV irradiation filtered by Sunexx filters does have significant antiviral effects on MS2 phages. For more information regarding antiviral qualities of UV irradiation filtered with Sunexx filters some features have to be investigated.

6.1. Testing with different virus host systems

The initial experiments we have performed show that the antiviral effect is present and can be measured in a single bacteria and bacteriophage system. To gain more insight, different bacteria and phage systems have to be tested if there is a similar effect. Although MS2 phages are often used as a surrogate for the corona virus (String et al. 2023), results of one single type of virus cannot give accurate information for other types or families of viruses. Therefore, the present study should be expanded with different viruses as they have different UV sensitivity. The test with different viruses can give better information of the antiviral capacity of UVB irradiation filtered with Sunexx filters.

6.2. Testing with more detection systems

The modified plaque assay is a versatile and robust method for detection but different testing systems should be added to confirm the results of this project. It would be

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recommended to establish and use more assay systems. We could use RT PCR and real time PCR of viral RNA with and without treatment. The number of functional RNA after UV treatment can serve as an indicator for the number of functional viruses after UV treatment.

6.3. Testing with different kinds of UV irradiation and complete solar simulated irradiation

It has been shown in literature that different kinds of UV irradiation can have different effects on organisms. Some of these different kinds of UV irradiation can have opposite effects on the organism and can enhance other effects or ameliorate different processes in the cell. To get an overview of complete antiviral properties of Sunexx filters we have to test different kinds of UV irradiations (UVB and UVA irradiation) and we should test simulation of solar radiation in a range from 280 nm to 2000 nm with a solar simulator.

6.4. Protective ozone shield, climate change and local UV intensity

The natural ozone layer filters extraterrestrial solar radiation (figure 1) and absorbs the high-energy UVC and parts of the UVB radiation, thus preventing harmful solar radiation from reaching the earth's surface. Humans, animals and plants have adapted to solar radiation on earth's surface over the course of evolution. Due to the general depletion of the ozone layer, e.g. due to climate change, the proportion of this ground-level UVB radiation is increasing. For humans, the harmful UVB rays are not directly recognizable or predictable without technical aids. However, local and temporary ozone mini-holes are particularly dangerous (Martinez-Lozano et al. 2011). The shorter the wavelength and thus the higher the energy of the UV radiation, the more viruses can be destroyed and provitamin D3 formed; however, the risk of erythema with lasting cell damage also increases. These action spectra are very close together (Bernard et al. 2023). Therefore, filters are also essential for protection, which reliably filter absolute and relative wavelengths of sunlight – at least similar to those of an optimally functioning natural ozone shield – prevent or reduce harmful additional exposure, but at the same time trigger a large number of natural photobiological effects and enable complex interaction – as well as hormesis effects. In particular, science still has a great deal of research to do on the complex natural interaction of the individual effects, which can amplify or reduce them or trigger further effects altogether, which humans need for a healthy/healthier natural life.

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