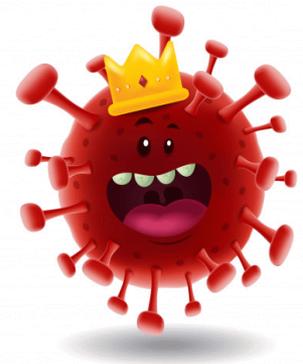
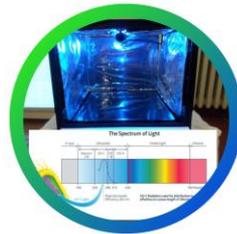
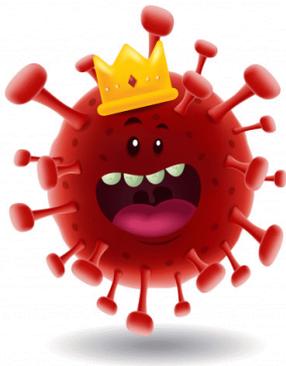


Studi di inattivazione del virus SARS-COV-2 in UV

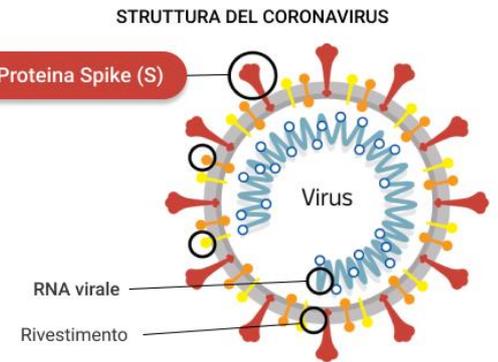
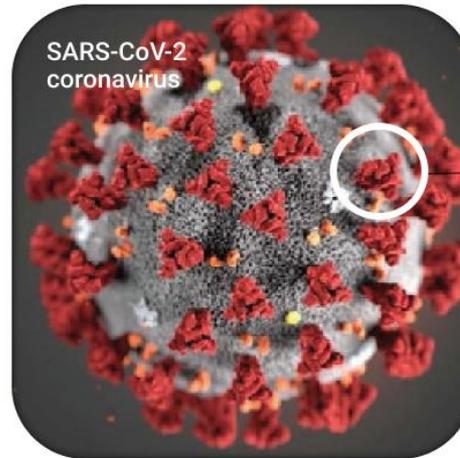
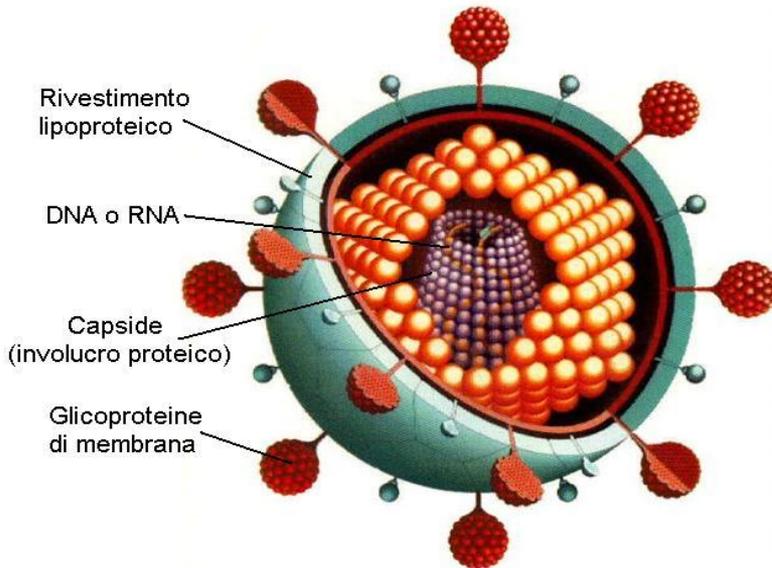
Andrea Bianco

INAF-Osservatorio Astronomico di Brera



Il virus

Gruppo di organismi di natura non cellulare e di dimensioni submicroscopiche costituiti da un acido nucleico rivestito da un involucro proteico (capside) incapaci di una sintesi proteica autonoma e perciò caratterizzati dalla vita parassitaria endocellulare obbligata.
(Treccani)



<http://www.corriere.it>

http://curba.racine.ra.it/_static/materialeStud/virus/struttura.htm

IL virus

SARS-CoV-2 (COVID-19) by the numbers

30/03/2020

Yinon M. Bar-On¹, Avi Flamholz², Rob Phillips^{3,4}, and Ron Milo^{1*}

¹Weizmann Institute of Science, Rehovot 7610001, Israel ²University of California, Berkeley, CA 94720, USA

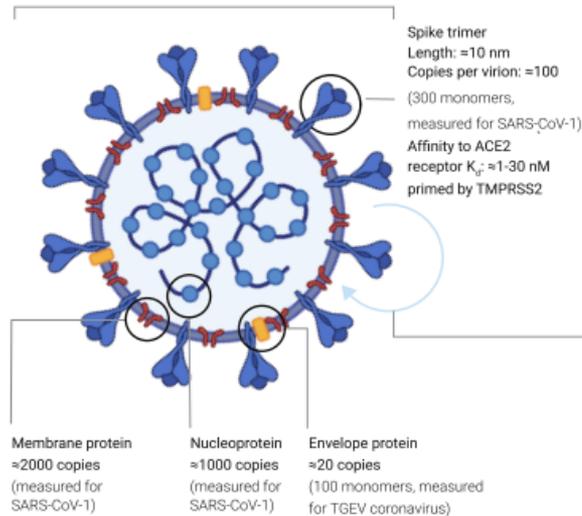
³California Institute of Technology, Pasadena, CA 91125, USA ⁴Chan Zuckerberg Biohub, San Francisco, CA 94158, USA

*Corresponding author: ron.milo@weizmann.ac.il.

Comments are welcome; this article is being updated on an ongoing basis at: <https://bit.ly/2WOeN64>

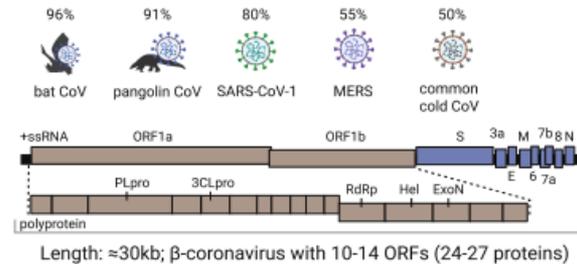
Size & Content

Diameter: ≈ 100 nm
Volume: $\sim 10^6 \text{ nm}^3 = 10^{-3} \text{ fL}$
Mass: $\sim 10^3 \text{ MDa} \approx 1 \text{ fg}$



Genome

Nucleotide identity to SARS-CoV-2



Evolution rate: $\sim 10^{-3} \text{ nt}^{-1} \text{ yr}^{-1}$ (measured for SARS-CoV-1)

Mutation rate: $\sim 10^{-6} \text{ nt}^{-1} \text{ cycle}^{-1}$ (measured for MHV coronavirus)

Replication Timescales

in tissue-culture

Virion entry into cell: ~ 10 min (measured for SARS-CoV-1)

Eclipse period: ~ 10 hrs (time to make intracellular virions)

Burst size: $\sim 10^3$ virions (measured for MHV coronavirus)

Host Cells

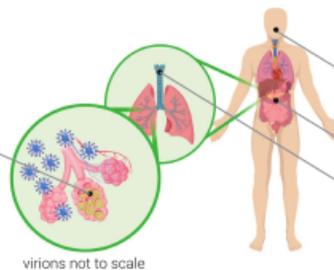
(tentative list; number of cells per person)

Type I & II pneumocytes ($\sim 10^{11}$ cells)

Alveolar macrophage ($\sim 10^{10}$ cells)

Mucous cell in nasal cavity ($\sim 10^9$ cells)

Host cell volume: $\sim 10^3 \mu\text{m}^3 = 10^{-3} \text{ fL}$



Concentration

maximal observed values following diagnosis

(Woelfel et al. 2020; Kim et al. 2020; Pan et al. 2020)

Nasopharynx: $10^6\text{-}10^9$ RNAs/swab

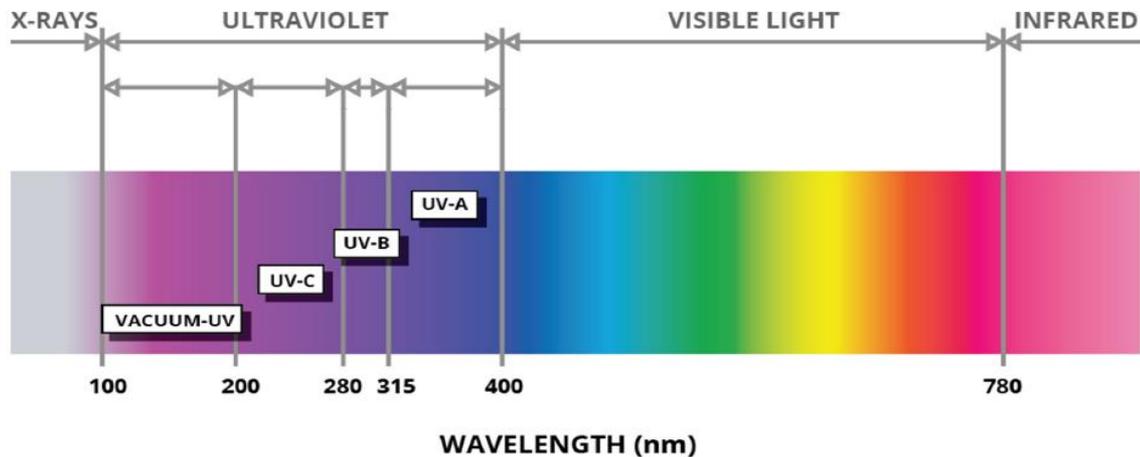
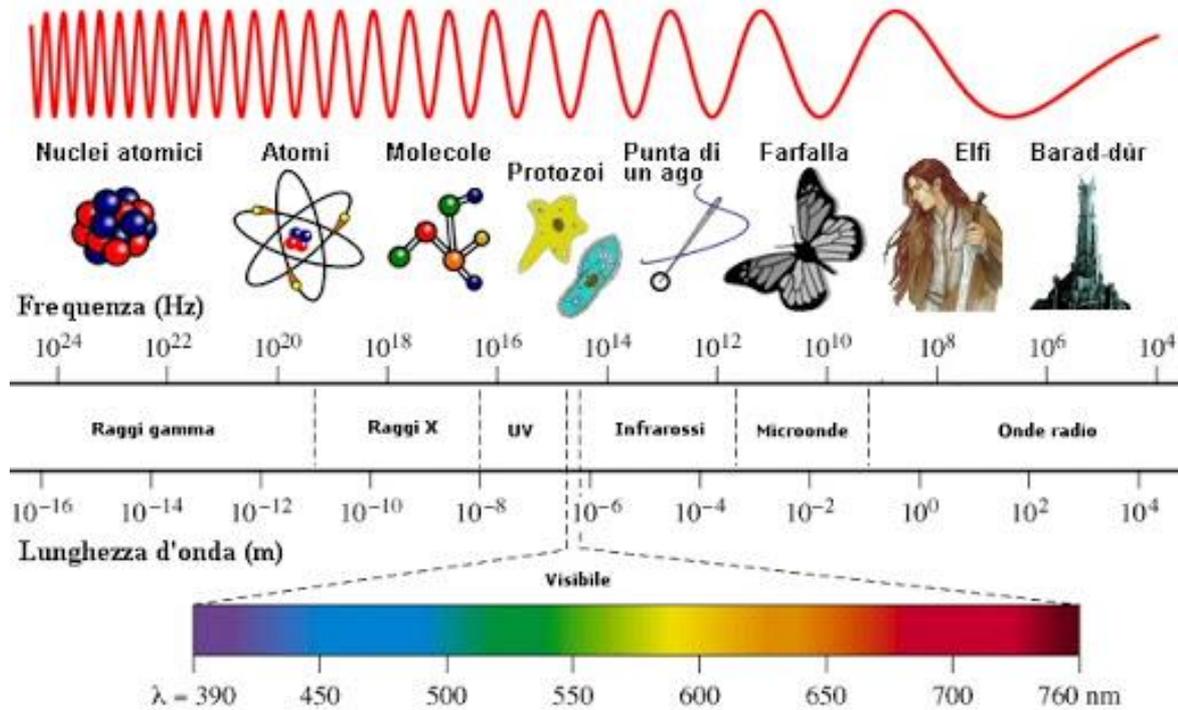
Throat: $10^4\text{-}10^8$ RNAs/swab

Stool: $10^4\text{-}10^8$ RNAs/g

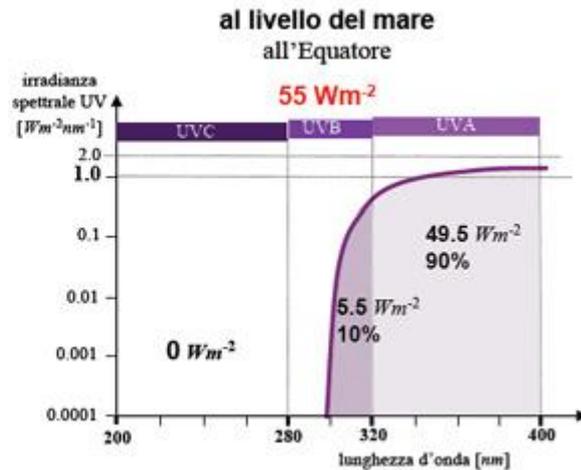
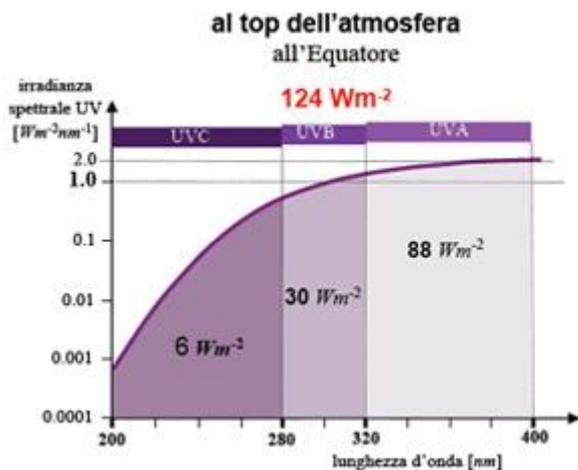
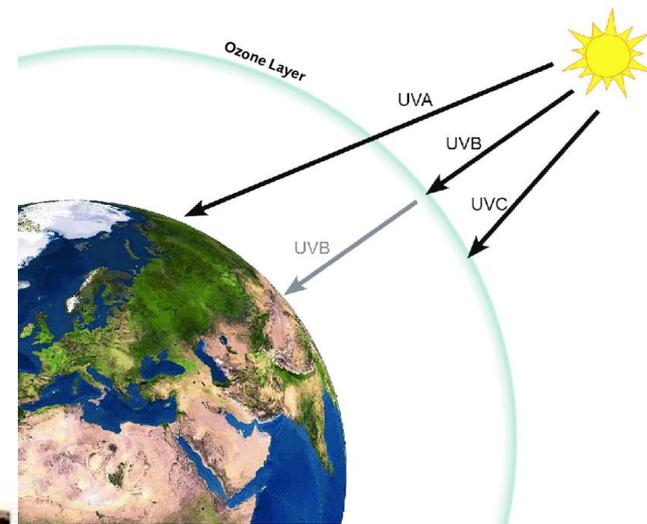
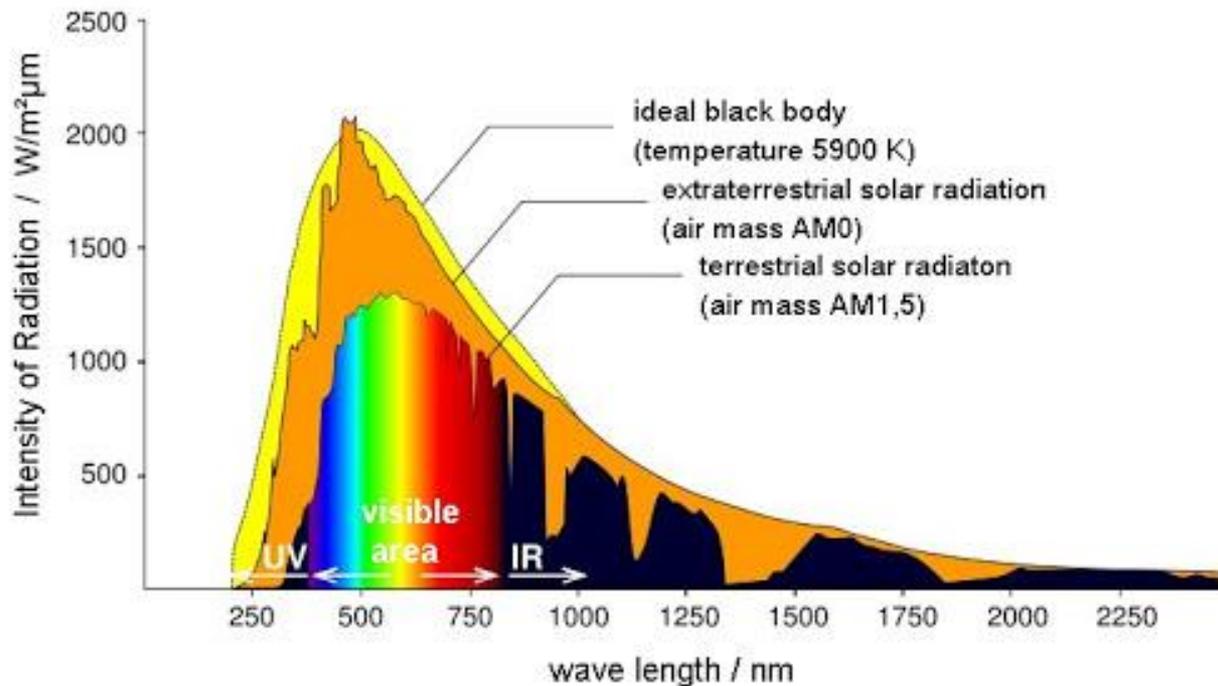
Sputum: $10^6\text{-}10^{11}$ RNAs/mL

RNA counts can markedly overestimate infectious virions

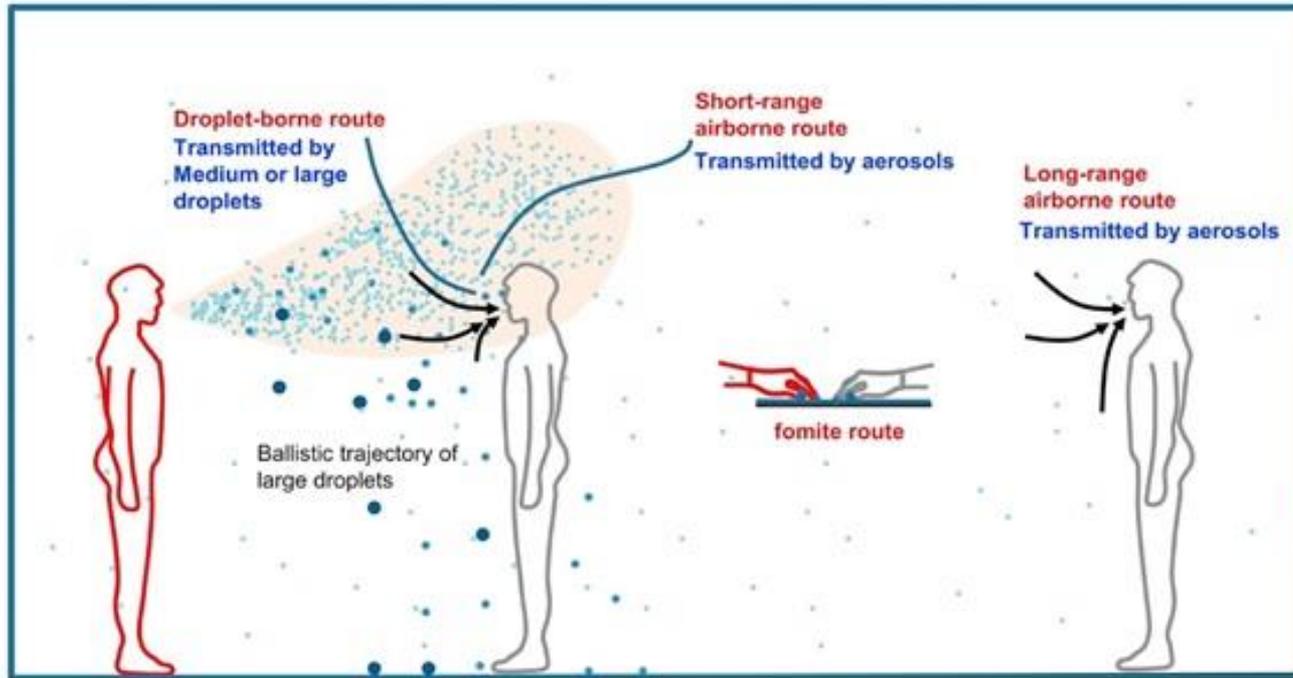
Radiazione UV



Il sole come sorgente di raggi UV



In che situazione ci troviamo



- Large droplets ($>100 \mu\text{m}$): Fast deposition due to the domination of gravitational force
- Medium droplets between 5 and 100 μm
- Small droplets or droplet nuclei, or aerosols ($< 5 \mu\text{m}$): Responsible for airborne transmission

Ci sono diversi «veicoli» per il SARS-CoV-2:

- Goccioline di saliva;
- Aerosol;
- Superficie contaminate.

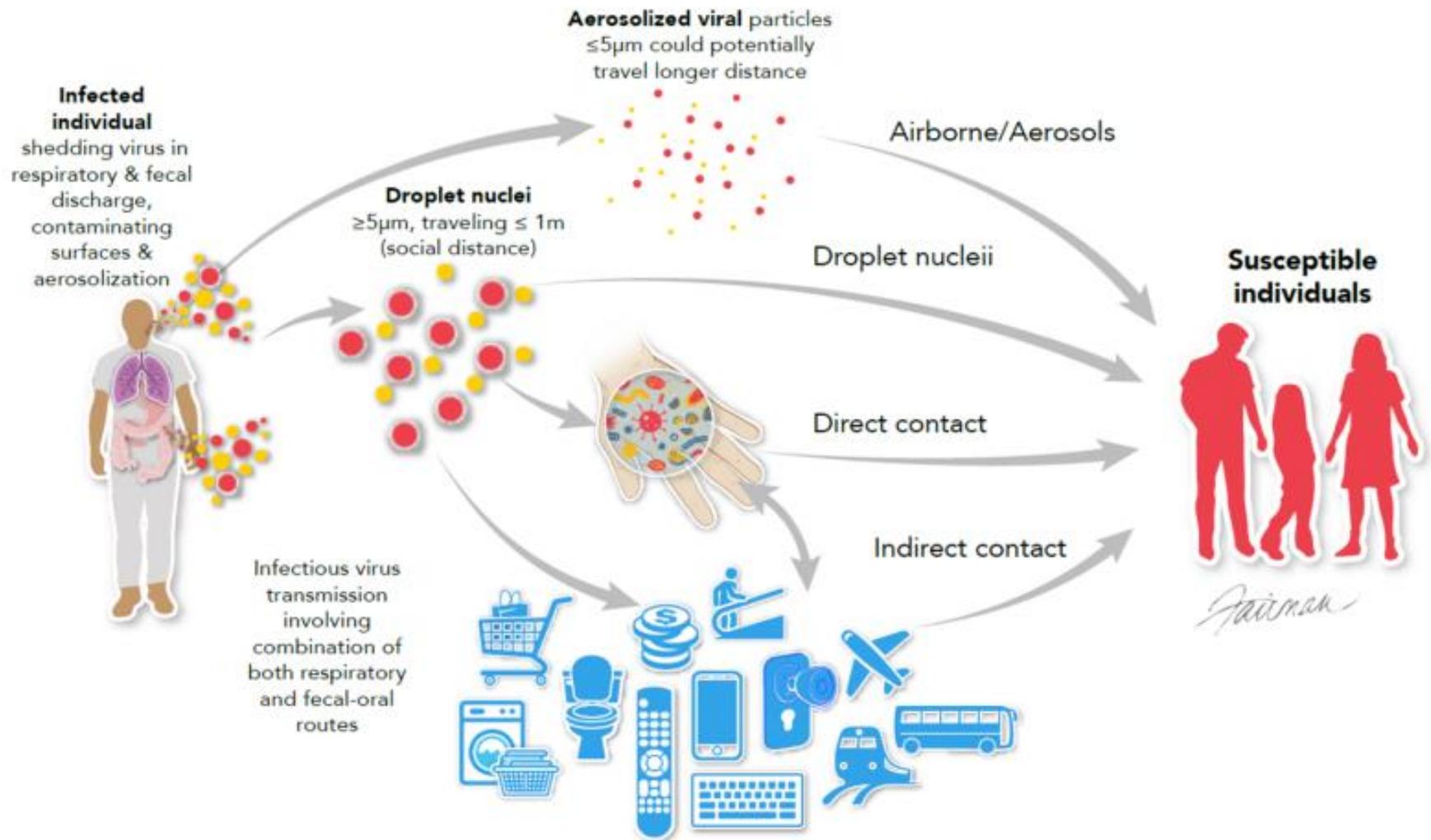
Quanto resiste il virus

Superfici	Particelle virali infettanti rilevate fino a	Particelle virali infettanti scomparse dopo
Carta da stampa e carta velina	30 minuti	3 ore
Tessuto	1 giorno	2 giorni
Legno	1 giorno	2 giorni
Banconote	2 giorni	4 giorni
Vetro	2 giorni	4 giorni
Plastica	4 giorni	7 giorni
Acciaio inox	4 giorni	7 giorni
Mascherine chirurgiche strato interno	4 giorni	7 giorni
Mascherine chirurgiche strato esterno	7 giorni	non determinato

Fonte: Ministero della Salute

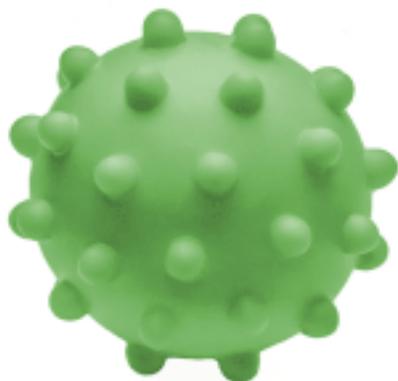
L'Ego-Hub

In che situazione ci troviamo



Alcuni modi per distruggere il virus

FOUR WAYS TO DESTROY CORONAVIRUS



THE ANATOMY OF THE VIRUS

Coronaviruses are a group of viruses. The specific coronavirus that causes COVID-19 is called SARS-CoV-2.



SARS-CoV-2 is a new virus, so there's currently no treatment for it. By cleaning hands and surfaces we can stop it spreading.

1 SOAP AND WATER



✓ HANDS ✓ HARD SURFACES

SOAP MOLECULES



WASH HANDS FOR A MINIMUM OF 20 SECONDS

HOW DOES IT DESTROY THE VIRUS?

Soap molecules dissolve the fatty outside layer of the virus. Any type of soap is effective, so it doesn't matter what type you use.

2 ALCOHOL HAND SANITISER



✓ HANDS ✓ HARD SURFACES



ETHANOL ISOPROPANOL

MIN. 60% ALCOHOL (HANDS) OR 70% (SURFACES)

HOW DOES IT DESTROY THE VIRUS?

Alcohol molecules dissolve the fatty outside layer of the virus and damage the structures of virus proteins.

3 BLEACH SOLUTION



✗ HANDS ✓ HARD SURFACES

NaClO

SODIUM HYPOCHLORITE

Cl₂

Don't mix bleach with other cleaners. This can generate toxic chlorine gas.

MINIMUM CONCENTRATION OF 0.1% HYPOCHLORITE

HOW DOES IT DESTROY THE VIRUS?

Bleach oxidises and destroys virus proteins and genetic material. It should be left on surfaces for at least 10 minutes.

4 HYDROGEN PEROXIDE



✗ HANDS ✓ HARD SURFACES

H₂O₂

HYDROGEN PEROXIDE

Don't mix peroxide with vinegar. This makes corrosive peracetic acid.

MINIMUM CONCENTRATION OF 0.5% PEROXIDE

HOW DOES IT DESTROY THE VIRUS?

Peroxide oxidises and destroys virus proteins and genetic material. It should be left on surfaces for at least 10 minutes.



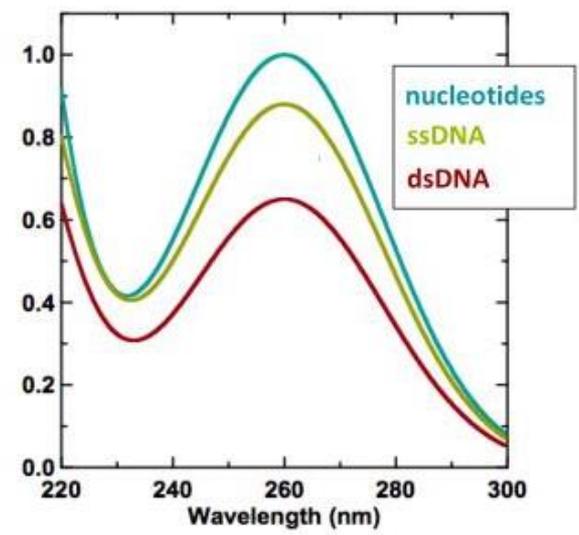
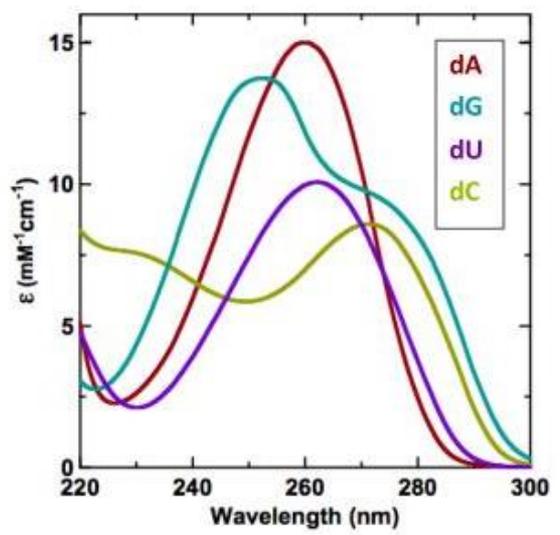
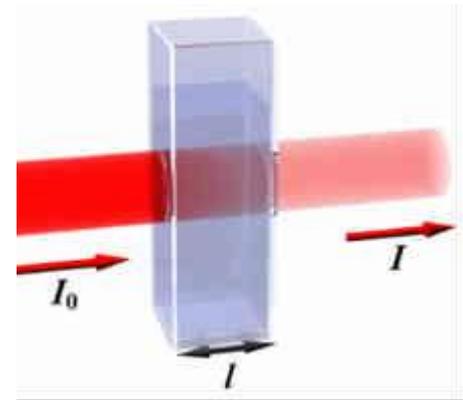
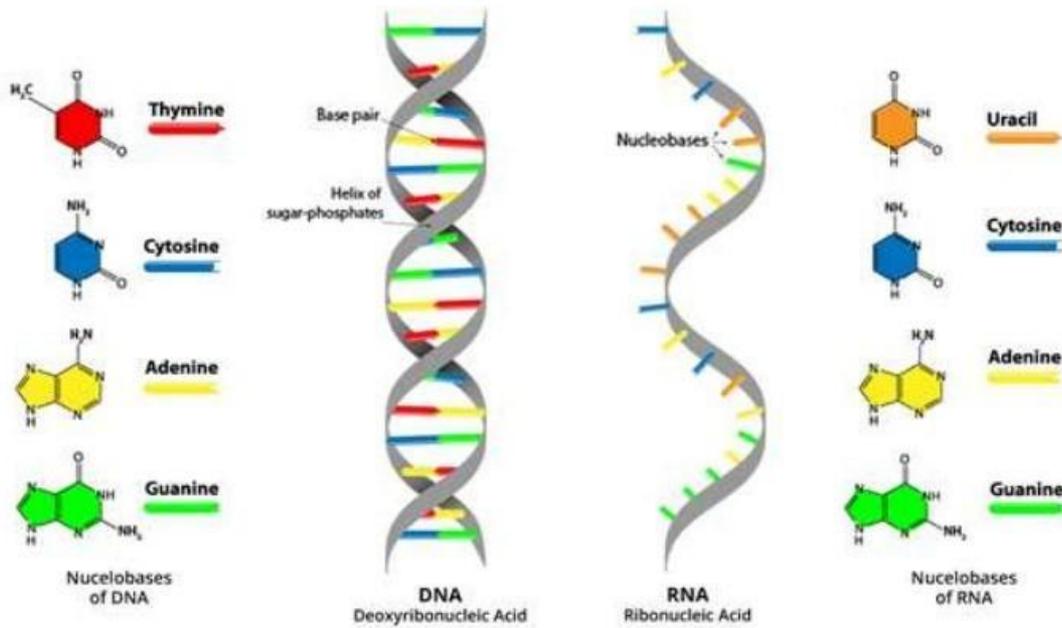
The History of Ultraviolet Germicidal Irradiation for Air Disinfection

Figure 1. Overview of selected key events in the history of UVGI air disinfection

NICHOLAS G. REED^a

1877	Downes and Blunt ^a discover the ability of sunlight to prevent microbial growth. It is later shown that the ability of light to inactivate microorganisms is dependent on the dose (intensity × time) and wavelength of radiation and the sensitivity of the specific type of microorganism.
1930	Gates ^b publishes the first analytical bactericidal action spectrum with peak effectiveness at 265 nm, very near the 254 nm output of low-pressure Hg germicidal lamps.
1933	Wells ^c presents the concept of airborne infection via “droplet nuclei”—evaporated droplets containing infectious organisms that can remain suspended in the air for extended durations.
1935	Wells and Fair ^d demonstrate the ability of UVGI to efficiently inactivate airborne microorganisms and prove the concept of infection via the airborne route.
1937	Wells et al. ^e use upper-room UVGI to prevent the epidemic spread of measles in suburban Philadelphia day schools where infection outside the school is unlikely.
1940s to 1950s	Several studies ^{f,g} are unable to reproduce Wells et al.’s success in using UVGI to prevent the spread of measles in schoolchildren, contributing to the disillusionment with and abandonment of UVGI for air disinfection. These failures have since been attributed to infections occurring outside the irradiated schools.
1956–1962	Riley ^h exposes guinea pigs to air originating from an occupied TB ward and proves that TB is spread via the airborne route. A group of guinea pigs receiving infected air via a UVGI irradiated duct were not infected, while a group receiving air via a non-irradiated duct were infected.
1969–1972	Riley and colleagues ^{i,j} conduct model room studies evaluating the use of upper-room UVGI to reduce the concentration of aerosolized test organisms in the lower room. They also show that air mixing between the upper and lower room is imperative for effective disinfection and confirm that UVGI is less effective at high humidity.
1974–1975	Riley et al. ^m determine virulent tubercle bacilli and BCG to be equally susceptible to UVGI and measure the disappearance rate of aerosolized BCG in a model room with and without upper-room UVGI. Upper-room UVGI is shown to be highly effective in disinfecting the lower room, quantitatively demonstrating the potential of upper-room UVGI to reduce TB infection.
1985–1992	After decades of decline, there is an unexpected rise in TB in the United States, leading to a renewed interest in UVGI for air disinfection. ^{n,o}
1990s to present	New in-depth efforts are undertaken, aimed toward quantitatively examining UVGI efficacy and safety and providing guidance for the proper use of UVGI.

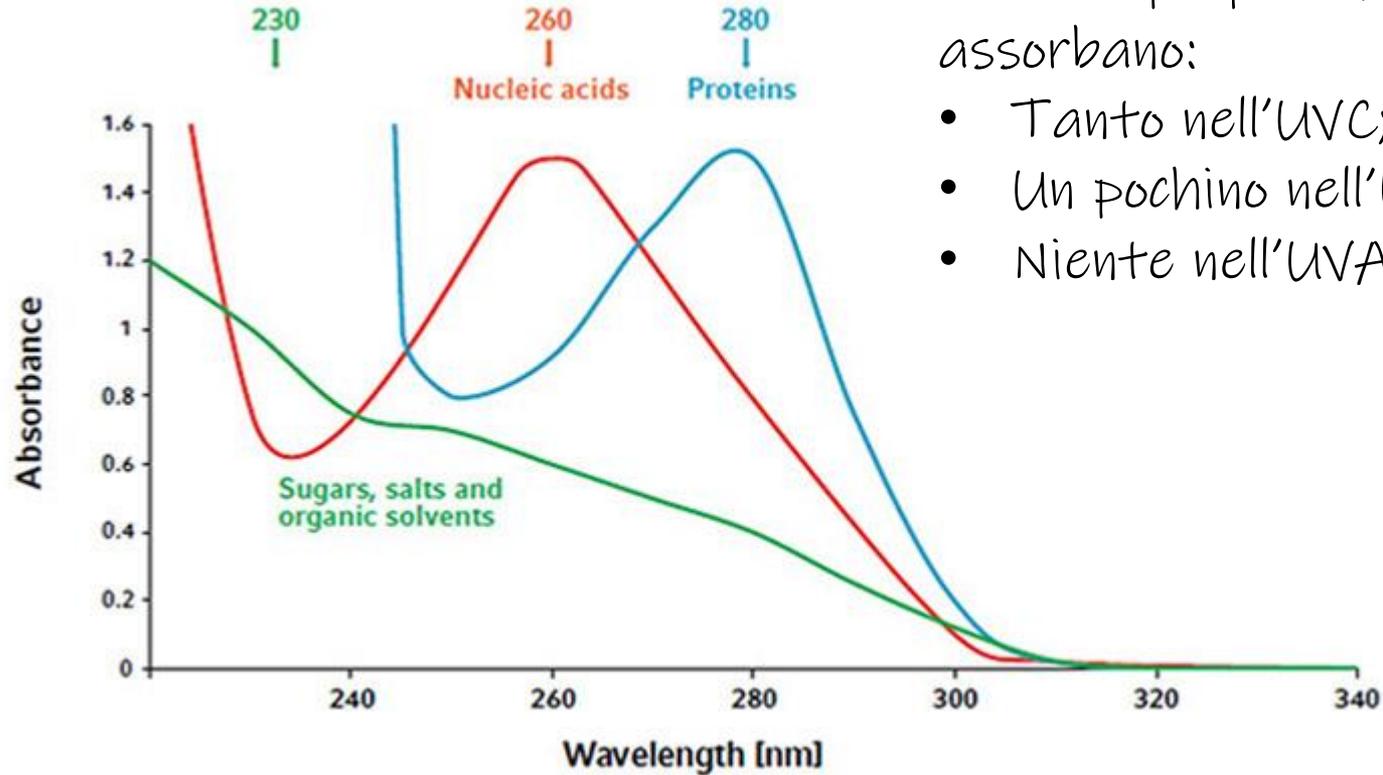
Assorbimento acidi nucleici



SARS-CoV-2 è un virus RNA.

Sembra proprio che assorbano nell'UV!

Assorbimento biomolecole



Sembra proprio che assorbano:

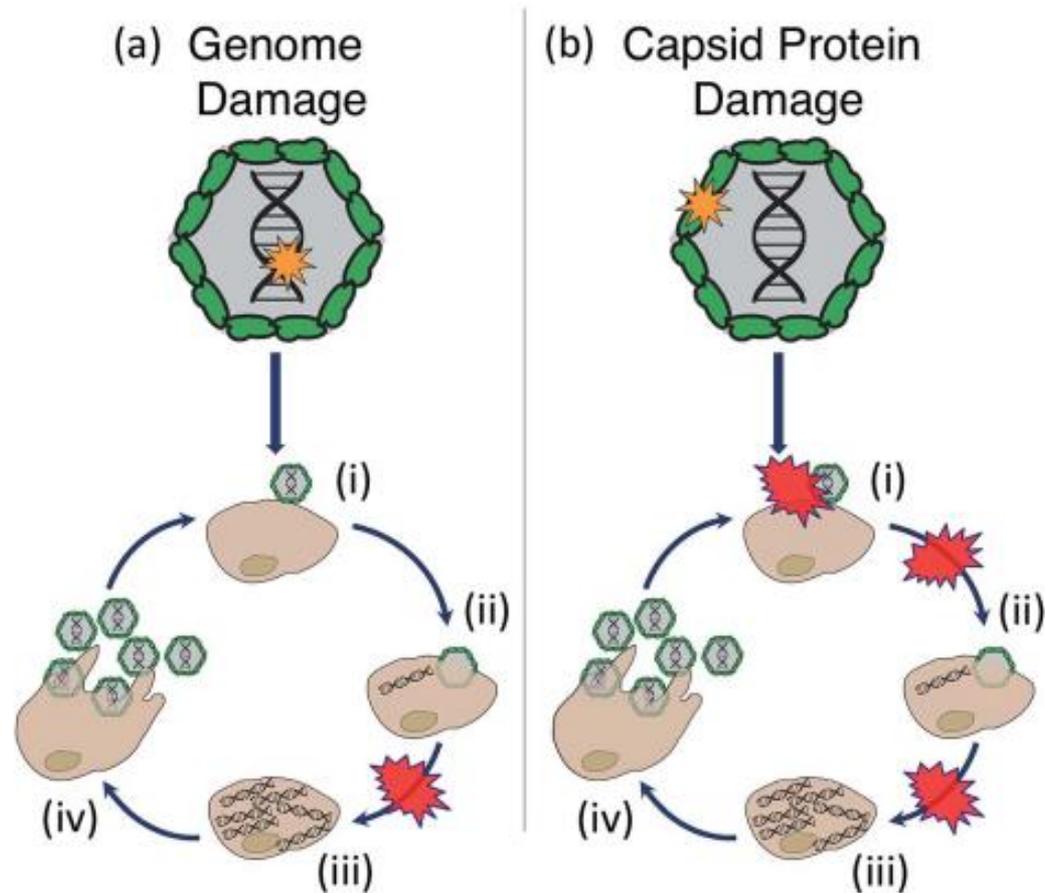
- Tanto nell'UVC;
- Un pochino nell'UVB;
- Niente nell'UVA.

UVC

UVB

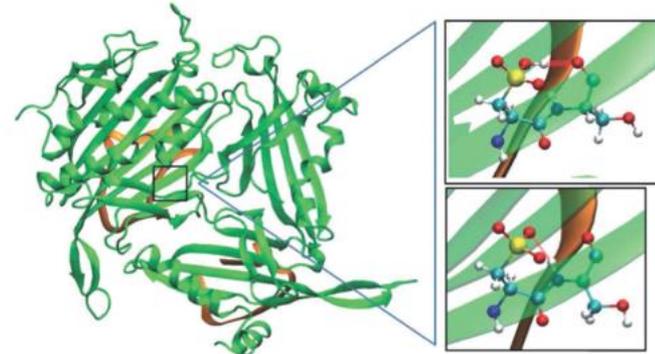
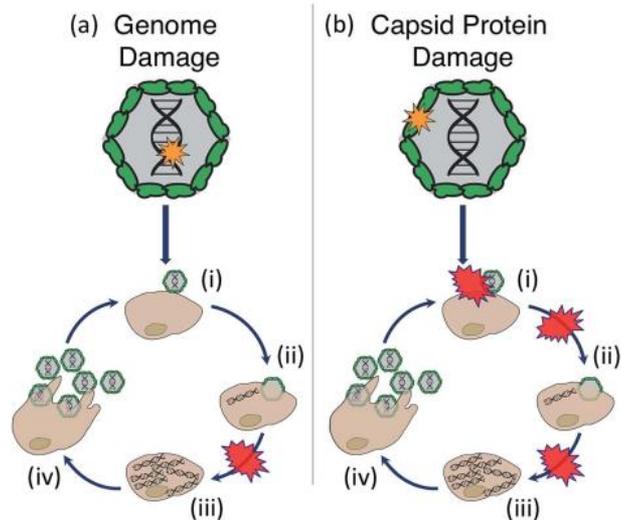
UVA

Assorbendo la radiazione UV...

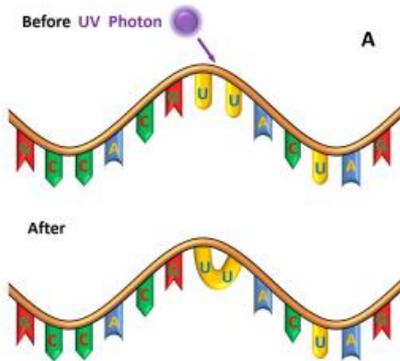


- Danneggiamento irreversibilmente il genoma: il virus non sa più replicare;
- Danneggiamento le proteine del capsid e quindi il virus «scoppia» e comunque non entra nella cellula;
- Questo succede sostanzialmente con la radiazione UV-C e sono **meccanismi endogeni**.

Assorbendo la radiazione UV...



ChemBioChem 2012, 13, 837–845



A

Theor Chem Acc (2011) 128:705–711

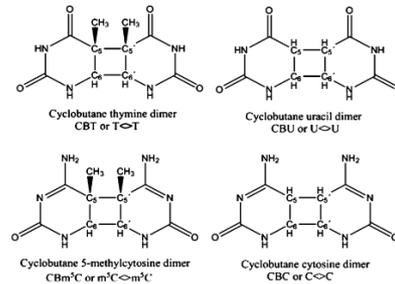
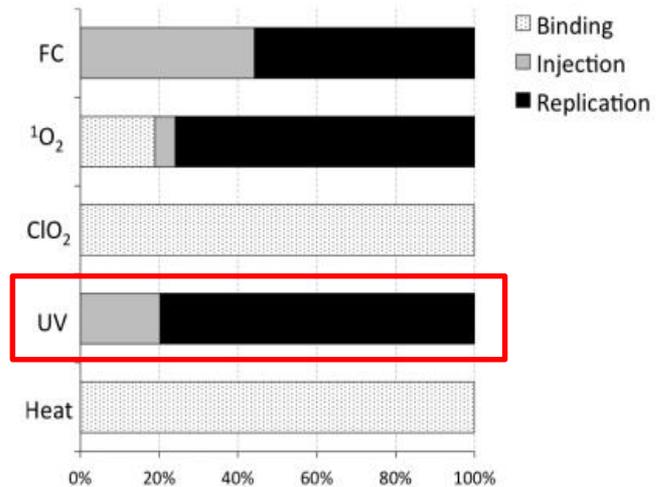


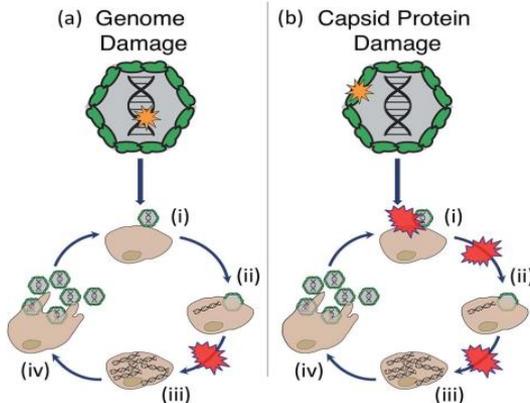
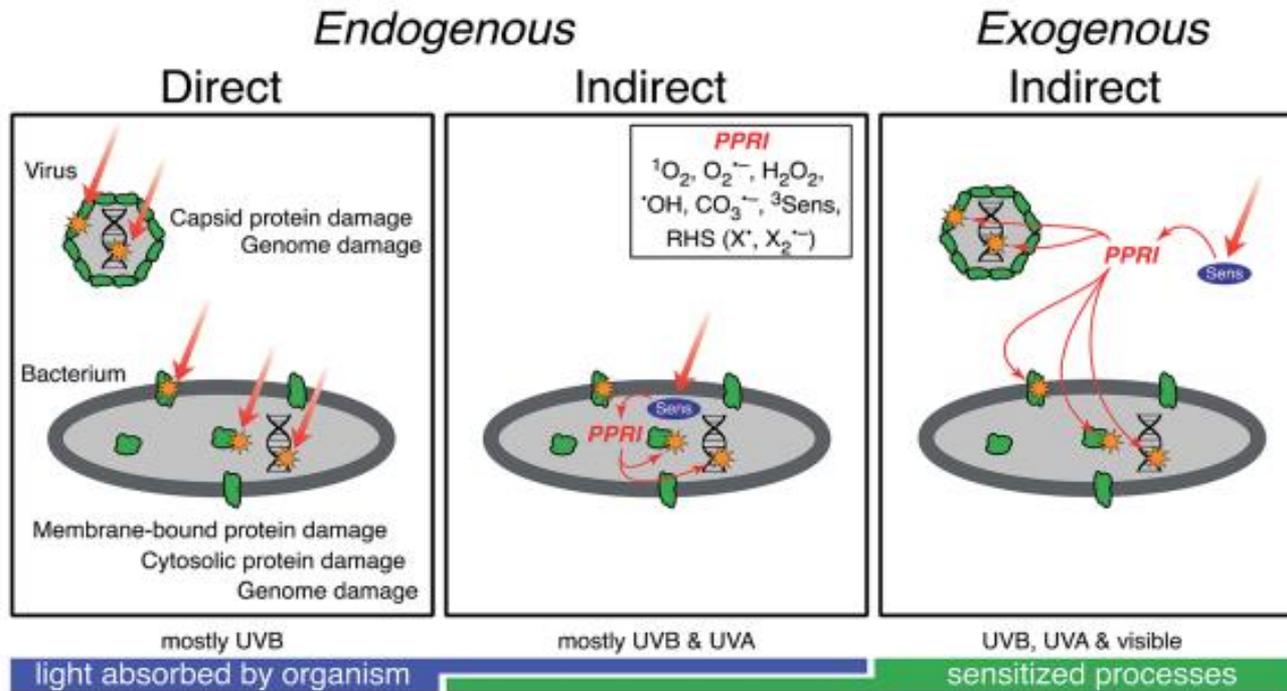
Figure 1: A) Scheme of UV-RNA-damaging mechanism emission spectrum of a low-pressure mercury va

GMS Hygiene and Infection Control 2020, Vol. 15, ISSN 2196-5226



L'UV ha soprattutto un effetto nel bloccare la replicazione (genoma danneggiato)

Assorbendo la radiazione UV...



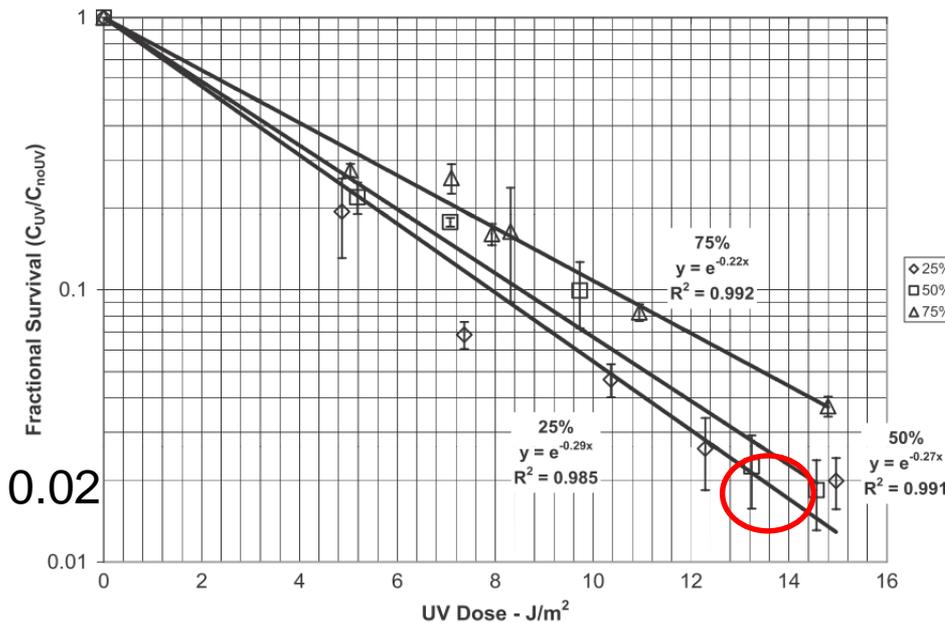
- Nell'ambiente possono esserci anche delle sostanze che se illuminate riescono a reagire in modo tale da «rovinare» il virus.
- Si tratta di meccanismi esogeni...difficili in aria, più probabili in acqua.
- Questi meccanismi possono attivarsi anche con UV-B e UV-A.

Quanti UVC "servono"?

- Le lampade UV emettono un'intensità (irradianza) espressa solitamente in mW/cm^2 , oppure W/m^2 ;
- Il virus verrà illuminato per un certo tempo;
- La dose UVC è la quantità importante ed è il prodotto delle due quantità:

$$\text{Dose} = \text{Irradianza} \times \text{Tempo di esposizione}$$

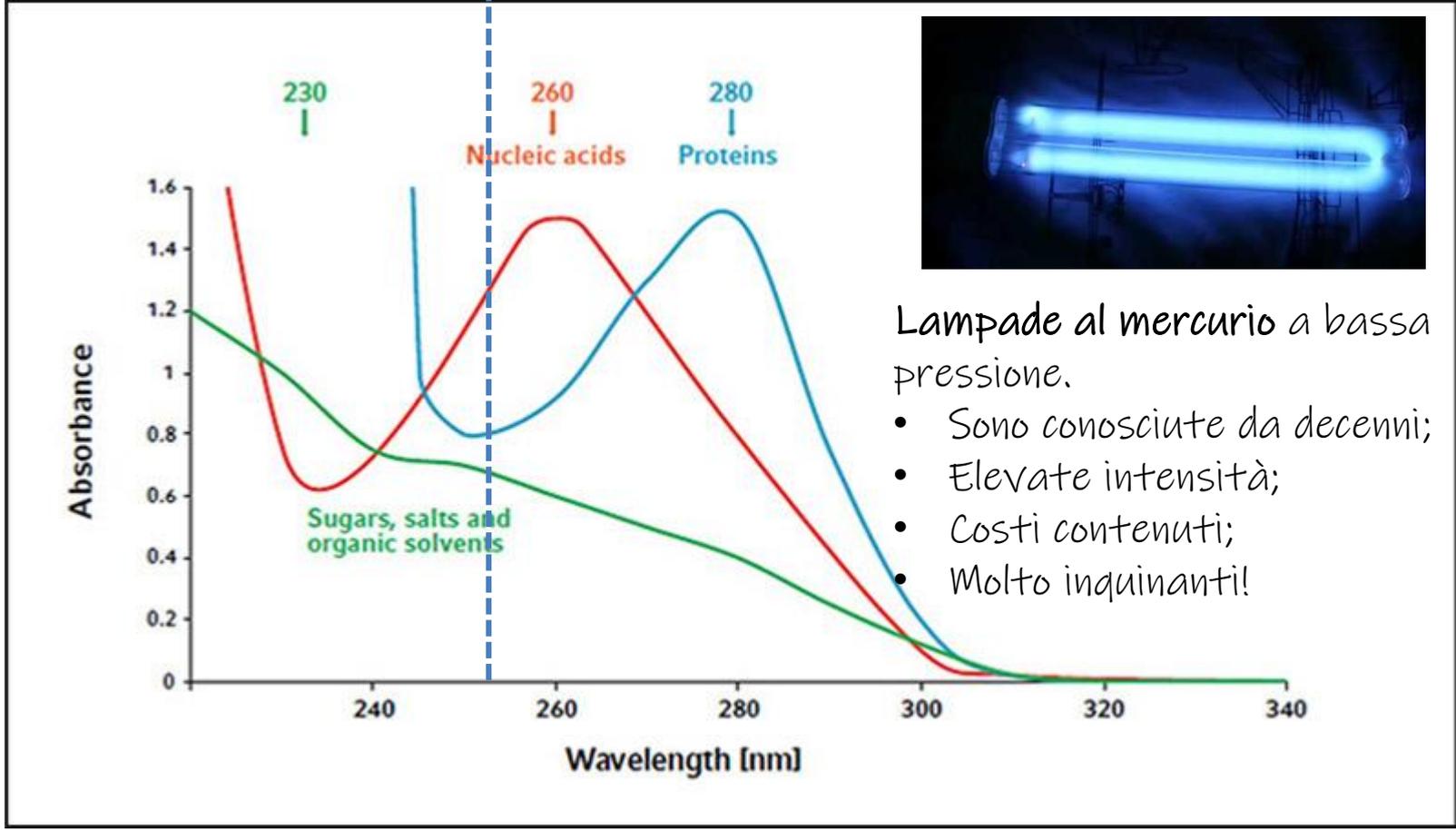
- La dose si misura in mJ/cm^2 oppure in J/m^2



Questo è l'esempio del virus dell'influenza. Bastano 14 J/m^2 di UVC a 254 nm perché rimanga attivo solo il 2% (0.02) del virus. Ne abbiamo eliminato il 98%; Con una lampada al mercurio «normale» bastano pochi secondi.

Le sorgenti di radiazione UVC

254 nm



Lampade al mercurio a bassa pressione.

- Sono conosciute da decenni;
- Elevate intensità;
- Costi contenuti;
- Molto inquinanti!



Ultra Violet Germicidal Irradiation (UVGI)

Dosi UVC a 254 nm

Organisms:	Energy Dosage of Ultraviolet radiation (UV dose) in $\mu\text{Ws}/\text{cm}^2$ needed for kill factor	
	90% (1 log reduction)	99% (2 log reduction)
Bacteria		
Bacillus anthracis - Anthrax	4,520	8,700
Bacillus anthracis spores - Anthrax spores	24,320	46,200
Bacillus magaterium sp. (spores)	2,730	5,200
Bacillus magaterium sp. (veg.)	1,300	2,500
Bacillus paratyphus	3,200	6,100
Bacillus subtilis spores	11,600	22,000
Bacillus subtilis	5,800	11,000
Clostridium tetani	13,000	22,000
Corynebacterium diphtheriae	3,370	6,510
Ebertelia typhosa	2,140	4,100
Escherichia coli	3,000	6,600
Leptospiracanicola - infectious Jaundice	3,150	6,000
Micrococcus candidus	6,050	12,300
Micrococcus sphaeroides	1,000	15,400
Mycobacterium tuberculosis	6,200	10,000
Neisseria catarrhalis	4,400	8,500
Phytomonas tumefaciens	4,400	8,000
Proteus vulgaris	3,000	6,600
Pseudomonas aeruginosa	5,500	10,500
Pseudomonas fluorescens	3,500	6,600
Salmonella enteritidis	4,000	7,600
Salmonella paratyphi - Enteric fever	3,200	6,100
Salmonella typhosa - Typhoid fever	2,150	4,100
Salmonella typhimurium	8,000	15,200
Sarcina lutea	19,700	26,400
Serratia marcescens	2,420	6,160
Shigella dysenteriae - Dysentery	2,200	4,200
Shigella flexneri - Dysentery	1,700	3,400
Shigella paradysenteriae	1,680	3,400
Spirillum rubrum	4,400	6,160
Staphylococcus albus	1,840	5,720
Staphylococcus aureus	2,600	6,600
Staphylococcus hemolyticus	2,160	5,500
Staphylococcus lactis	6,150	8,800
Streptococcus viridans	2,000	3,800
Vibrio comma - Cholera	3,375	6,500

Molds	90%	99%
Aspergillus flavus	60,000	99,000
Aspergillus glaucus	44,000	88,000
Aspergillus niger	132,000	330,000
Mucor racemosus A	17,000	35,200
Mucor racemosus B	17,000	35,200
Oospora lactis	5,000	11,000
Penicillium expansum	13,000	22,000
Penicillium roqueforti	13,000	26,400
Penicillium digitatum	44,000	88,000
Rhisopus nigricans	111,000	220,000
Protozoa	90%	99%
Chlorella Vulgaris	13,000	22,000
Nematode Eggs	45,000	92,000
Paramecium	11,000	20,000
Virus	90%	99%
Bacteriophage - E. Coli	2,600	6,600
Infectious Hepatitis	5,800	8,000
Influenza	3,400	6,600
Poliovirus - Poliomyelitis	3,150	6,600
Tobacco mosaic	240,000	440,000
Yeast	90%	99%
Brewers yeast	3,300	6,600
Common yeast cake	6,000	13,200
Saccharomyces carevisiae	6,000	13,200
Saccharomyces ellipsoideus	6,000	13,200
Saccharomyces spores	8,000	17,600

<https://www.americanairandwater.com/uv-facts/uv-dosage.htm>

Dosi UVC a 254 nm

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Bacillus anthracis - Anthrax

4,520

8,700

Bacillus anthracis spores - Anthrax spores

24,320

46,200

Phytomonas tumefaciens	4,400	8,000
Proteus vulgaris	3,000	6,600
Pseudomonas aeruginosa	5,500	10,500
Pseudomonas fluorescens	3,500	6,600
Salmonella enteritidis	4,000	7,600
Salmonella paratyphi - Enteric fever	3,200	6,100
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Poliovirus - Poliomyelitis	3,150	6,600
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Saccharomyces spores	8,000	17,600

<https://www.americanairandwater.com/uv-facts/uv-dosage.htm>

E per il SARS-CoV-2?

Table 1: Summary of Ultraviolet Studies on Coronaviruses

Microbe	D ₉₀ Dose J/m ²	UV k m ² /J	Base Pairs kb	Source
Coronavirus	6.6	0.35120	30741	Walker 2007 ^a
Berne virus (Coronaviridae)	7.2	0.32100	28480	Weiss 1986
SARS-CoV-2 (Italy-INMI1)	12.3	0.18670	29811	Bianco 2020
Murine Coronavirus (MHV)	15.0	0.15351	31335	Hirano 1978
SARS Coronavirus (Frankfurt 1)	16.4	0.14040	29903	Eickmann 2020
Canine Coronavirus (CCV)	28.5	0.08079	29278	Saknimit 1988 ^b
Murine Coronavirus (MHV)	28.5	0.08079	31335	Saknimit 1988 ^b
SARS Coronavirus (CoV-P9)	40.0	0.05750	29829	Duan 2003 ^c
SARS-CoV-2 (SARS-CoV-2/Hu/DP/Kng/19-027)	41.7	0.05524	29811	Inagaki 2020
Murine Coronavirus (MHV)	103.0	0.02240	31335	Liu 2003
SARS Coronavirus (Hanoi)	133.9	0.01720	29751	Kariwa 2004 ^d
SARS Coronavirus (Urbani)	2410	0.00096	29751	Darnell 2004
Average	237	0.00972	including all studies	
Average excluding outliers	47	0.04943	excluding Walker, Weiss & Darnell	
Average for SARS-CoV-2	27	0.08528	two studies, 90% inactivation	

^a (Jingwen 2020)

^b (estimated)

^c (mean estimate)

^d (at 3 logs)

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E per il SARS-CoV-2?

Table 1: Summary of Ultraviolet Studies on Coronaviruses

Microbe	D ₉₀ Dose J/m ²	UV k m ² /J	Base Pairs kb	Source
Coronavirus	6.6	0.35120	30741	Walker 2007 ^a
Berne virus (Coronaviridae)	7.2	0.32100	28480	Weiss 1986
SARS-CoV-2 (Italy-INMI1)	12.3	0.18670	29811	Bianco 2020
Murine Coronavirus (MHV)	15.0	0.15351	31335	Hirano 1978
SARS Coronavirus (Frankfurt 1)	16.4	0.14040	29903	Eickmann 2020
Canine Coronavirus (CCV)	28.5	0.08079	29278	Saknimit 1988 ^b
Murine Coronavirus (MHV)	28.5	0.08079	31335	Saknimit 1988 ^b
SARS Coronavirus (CoV-P9)	40.0	0.05750	29829	Duan 2003 ^c
SARS-CoV-2 (SARS-CoV-2/Hu/DP/Kng/19-027)	41.7	0.05524	29811	Inagaki 2020
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Per il SARS diverse pubblicazioni riportano dosi tra 16.6 J/m² e i 2400 J/m² !!

C'è qualcosa che non va...

E per il SARS-CoV-2?

Volevamo fare un esperimento il più «pulito» possibile che simuli l'effetto della radiazione UV sul virus.

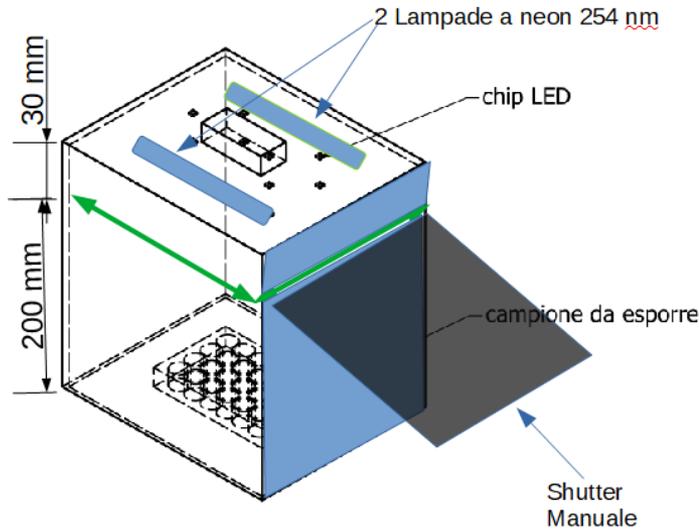


Virus in aerosol

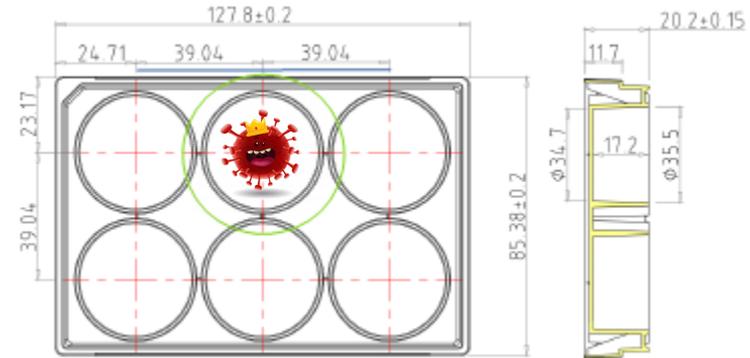


Il virus sospeso in un liquido di cui conosciamo la composizione.

L'esperimento UV

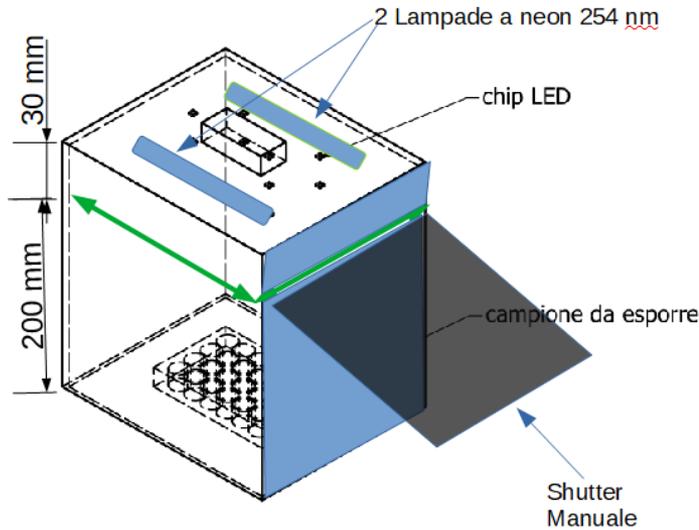


Ci siamo dotati di lampade UV
e le abbiamo tarate con spettroradiometro.

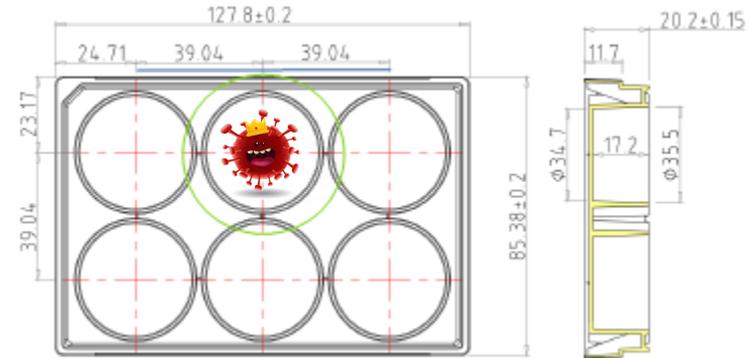


Definito le condizioni di misura;
Scelto le cellule da infettare;
Scelti i tempi di esposizione (dosi) e di
valutazione della replicazione virale.

L'esperimento UV



Ci siamo dotati di lampade UV
e le abbiamo tarate con spettroradiometro.



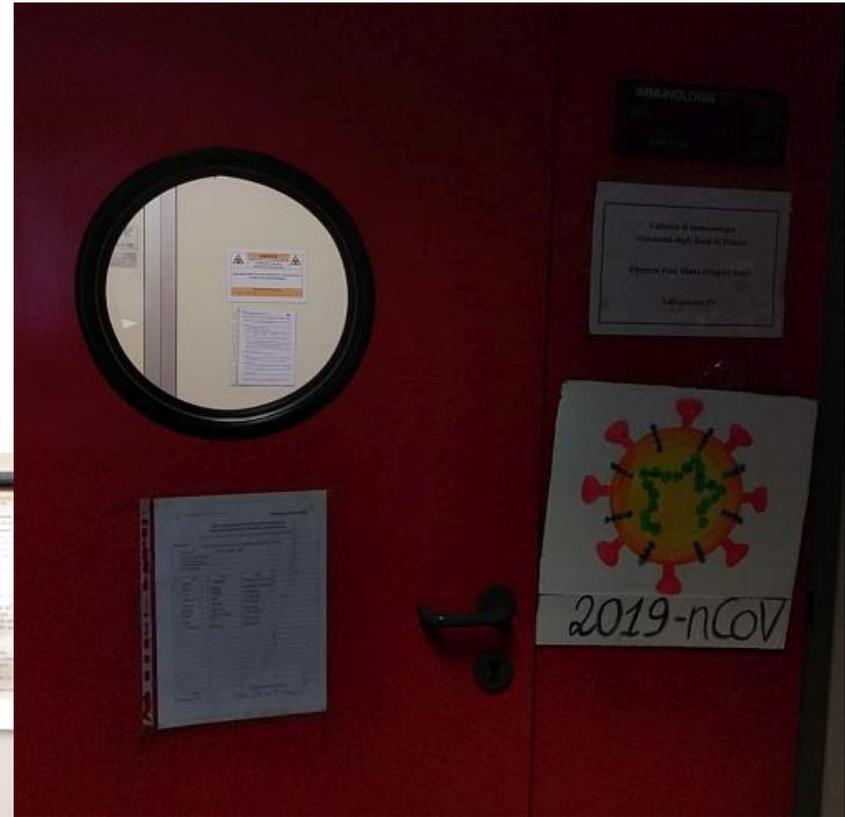
Definito le condizioni di misura;
Scelto le cellule da infettare;
Scelti i tempi di esposizione (dosi) e di
valutazione della replicazione virale.

Tre concentrazioni di virus:

- Bassa concentrazione (superficie contaminata)
- Concentrazione media (goccioline di saliva)
- Alta concentrazione media (tampone di una persona molto malata)

Prof. Biasin, Prof. Trabattoni, Prof. Clerici, Università degli studi di Milano
Dott. Lualdi, Dott. Cavalleri, Istituta Nazionale dei Tumori, Milano

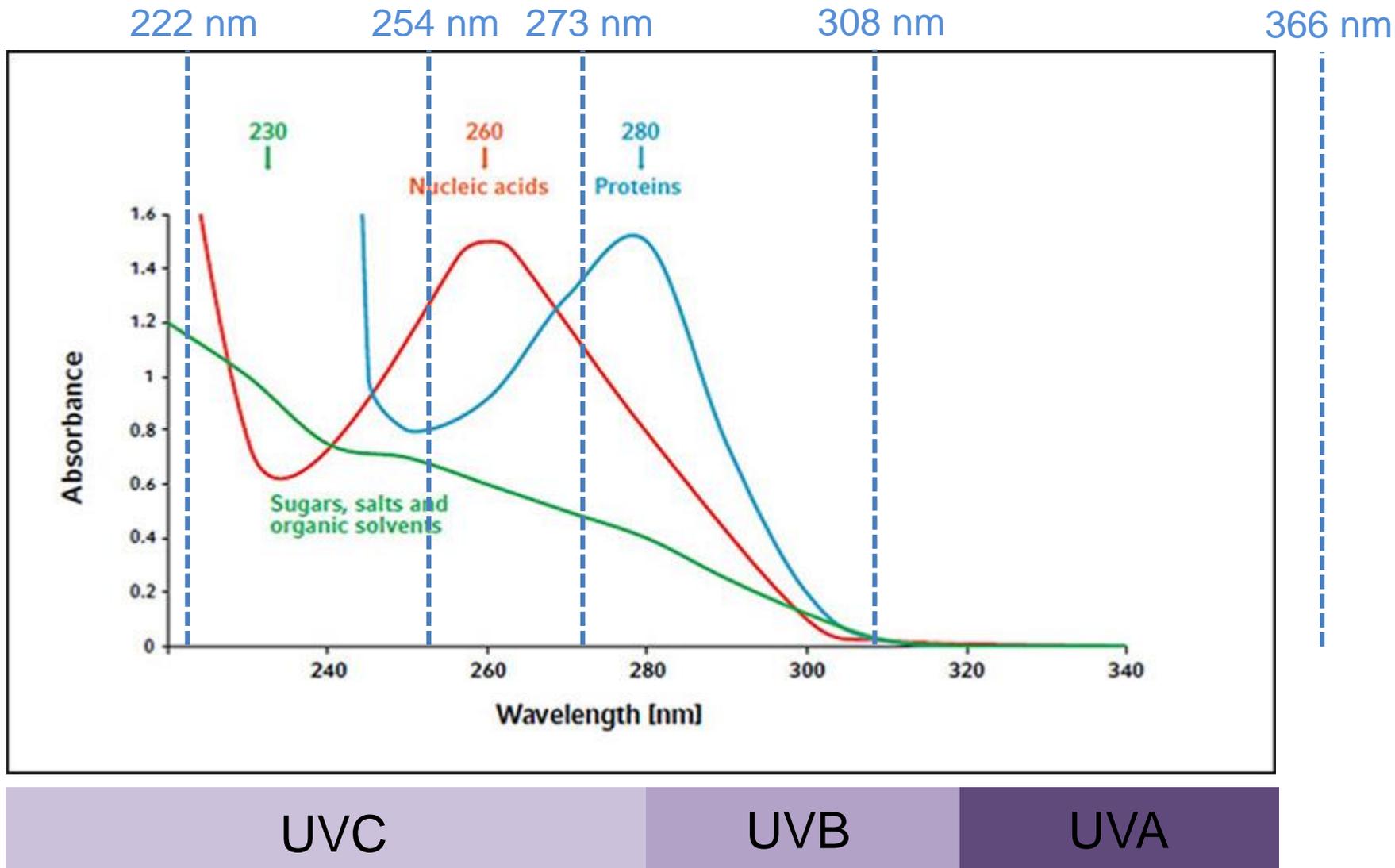
L'esperimento UV



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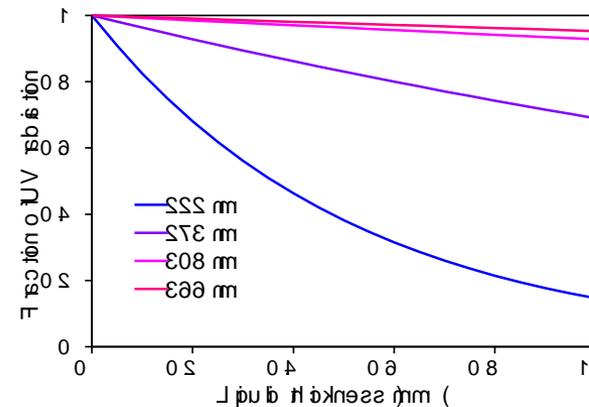
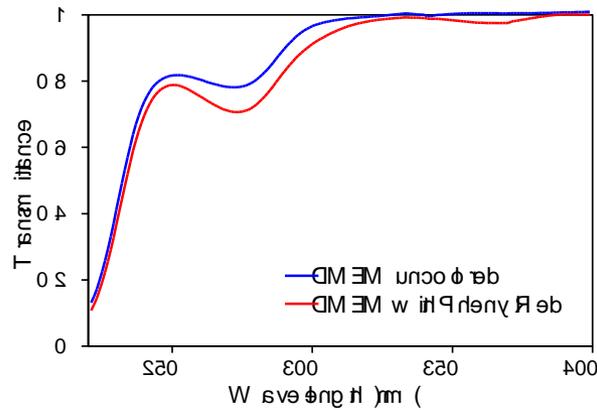
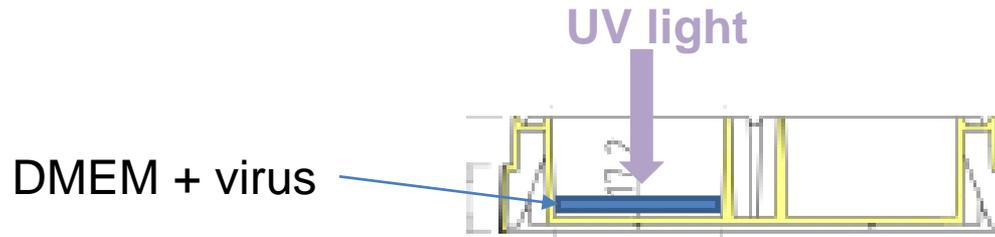
Prof. Biasin, Prof. Trabattoni, Prof. Clerici, Università degli studi di Milano
Dott. Lualdi, Dott. Cavalleri, Istituta Nazionale dei Tumori, Milano

Quali lunghezze d'onda?



Ultra Violet Germicidal Irradiation (UVGI)

Assorbimento del mezzo



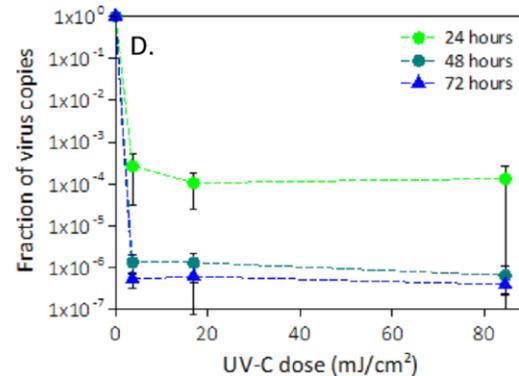
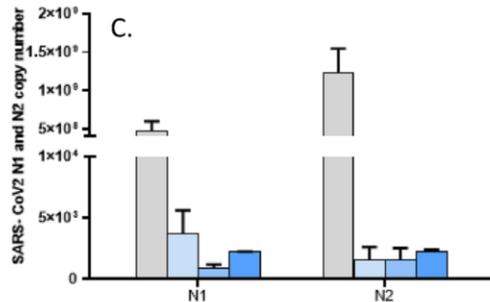
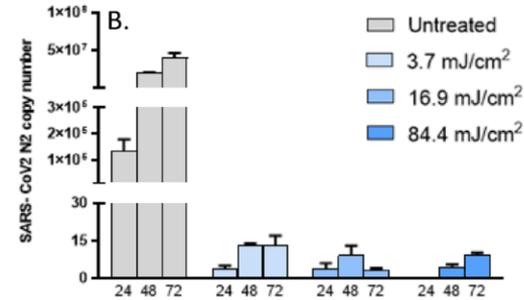
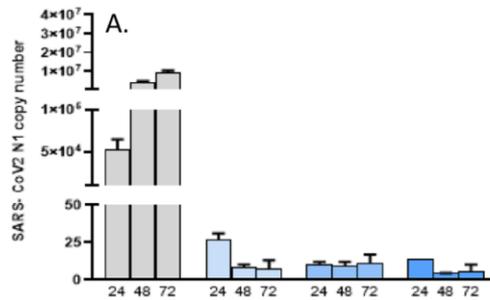
Vediamo come il DMEM ha uno spettro di assorbimento che dipende fortemente dalla lunghezza d'onda.

Bisogna tenerne conto per la valutazione della effettiva dose fornita al virus.

Risultati a 254 nm

Sci. Rep. 2021

Concentrazione media, 5 MOI (goccioline di saliva)

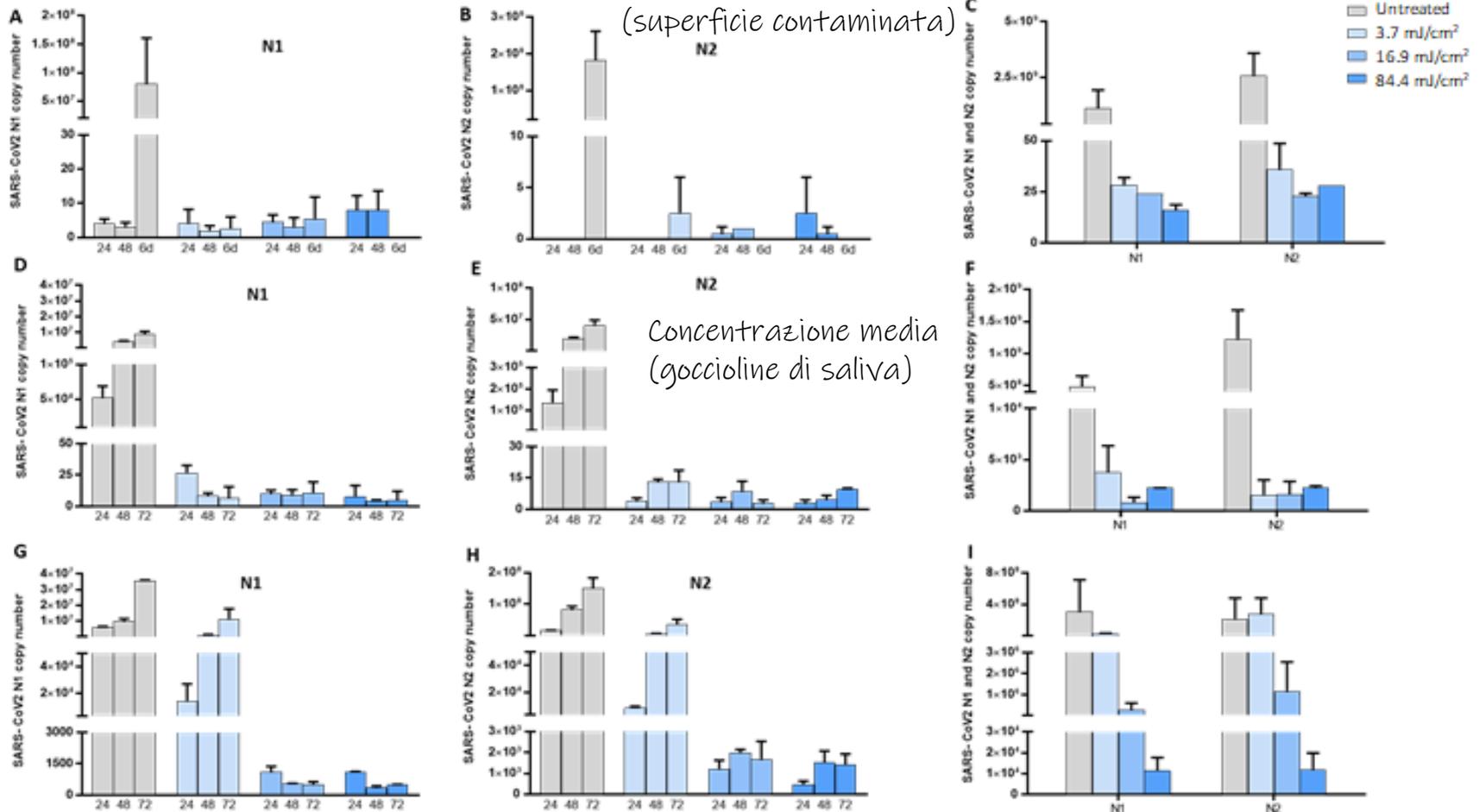


Bastano 3.7 mJ/cm² di UVC a 254 nm per disattivare completamente il virus!

Risultati a 254 nm

Sci. Rep. 2021

Bassa concentrazione
(superficie contaminata)



Alta concentrazione media (tampone di una persona molto malata)

Se la concentrazione di virus è molto alta serve una dose maggiore per la completa disattivazione.

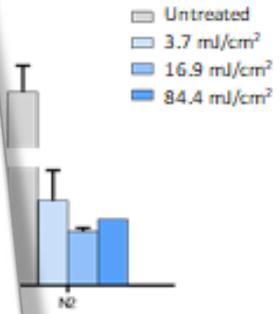
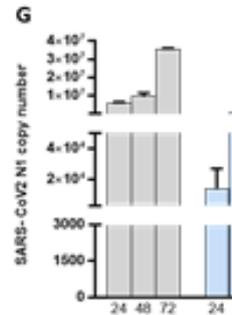
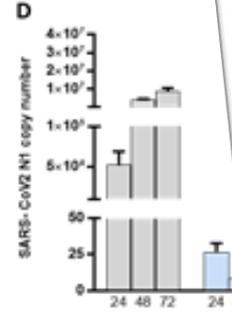
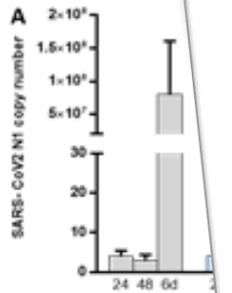
UV-C irradiation is highly effective in inactivating and inhibiting SARS-CoV-2 replication

Comments (4)

ID Andrea Bianco, ID Mara Biasin, ID Giovanni Pareschi, ID Adalberto Cavaliere,
 ID Claudia Cavatorta, ID Claudio Fenizia, ID Paola Galli, ID Luigi Lessio,
 ID Manuela Lualdi, ID Edoardo Redaelli, ID Irma Saulle, ID Daria Trabattoni,
 ID Alessio Zanutta, ID Mario Clerici

doi: <https://doi.org/10.1101/2020.06.05.20123463>

This article is a preprint and has not been peer-reviewed [what does this mean?]. It reports new medical research that has yet to be evaluated and so should not be used to guide clinical practice.



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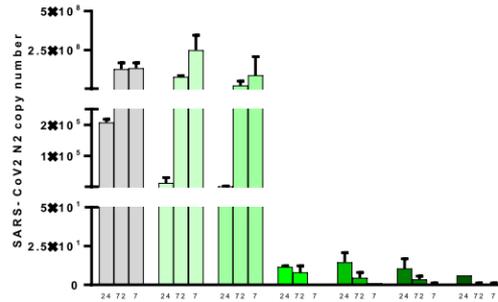
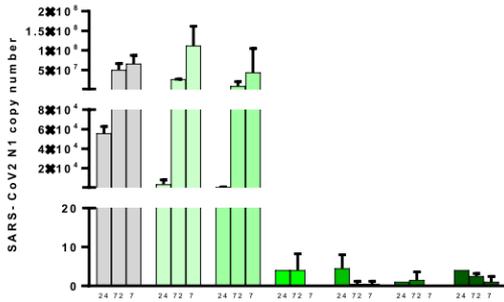
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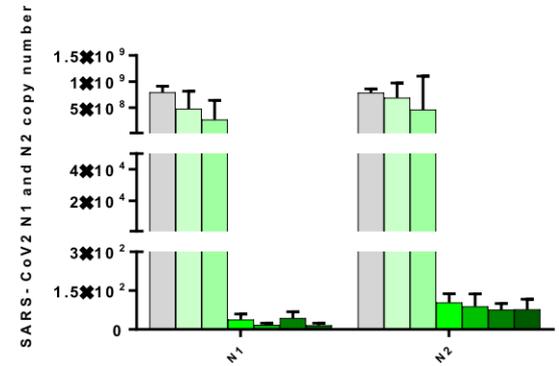
Alta cov
Se la concent
completa disa

Risultati a 273 nm

Concentrazione media (goccioline di saliva)



Cell culture supernatants

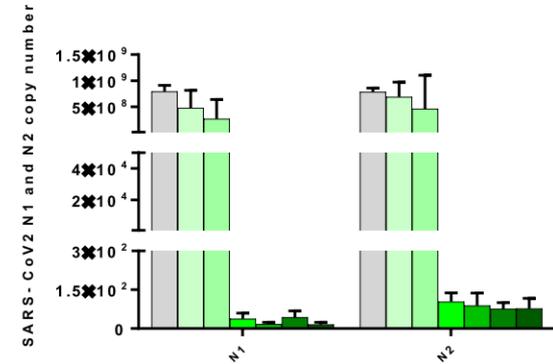
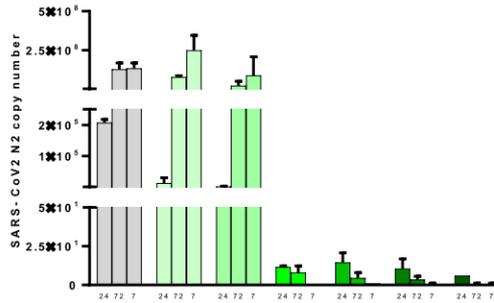
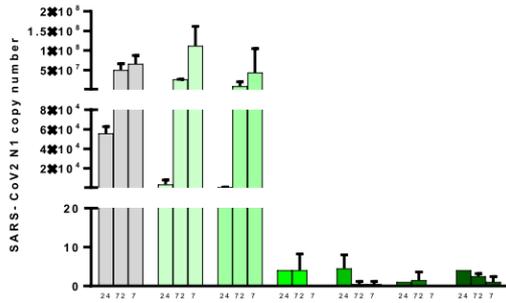


Intracellular (7 days)

Le dosi necessarie per la completa inibizione sono identiche a quelle a 254 nm... niente di sorprendente!

Risultati a 273 nm

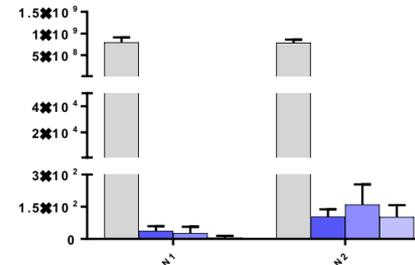
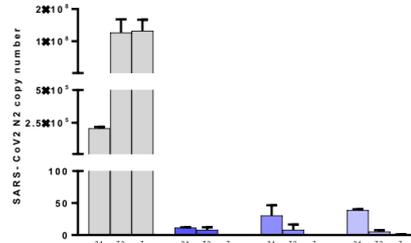
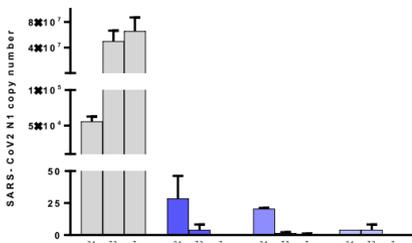
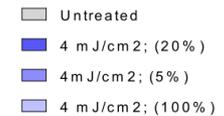
Concentrazione media (goccioline di saliva)



Cell culture supernatants

Intracellular (7 days)

L'inibizione è indipendentemente da come la dose viene somministrata!

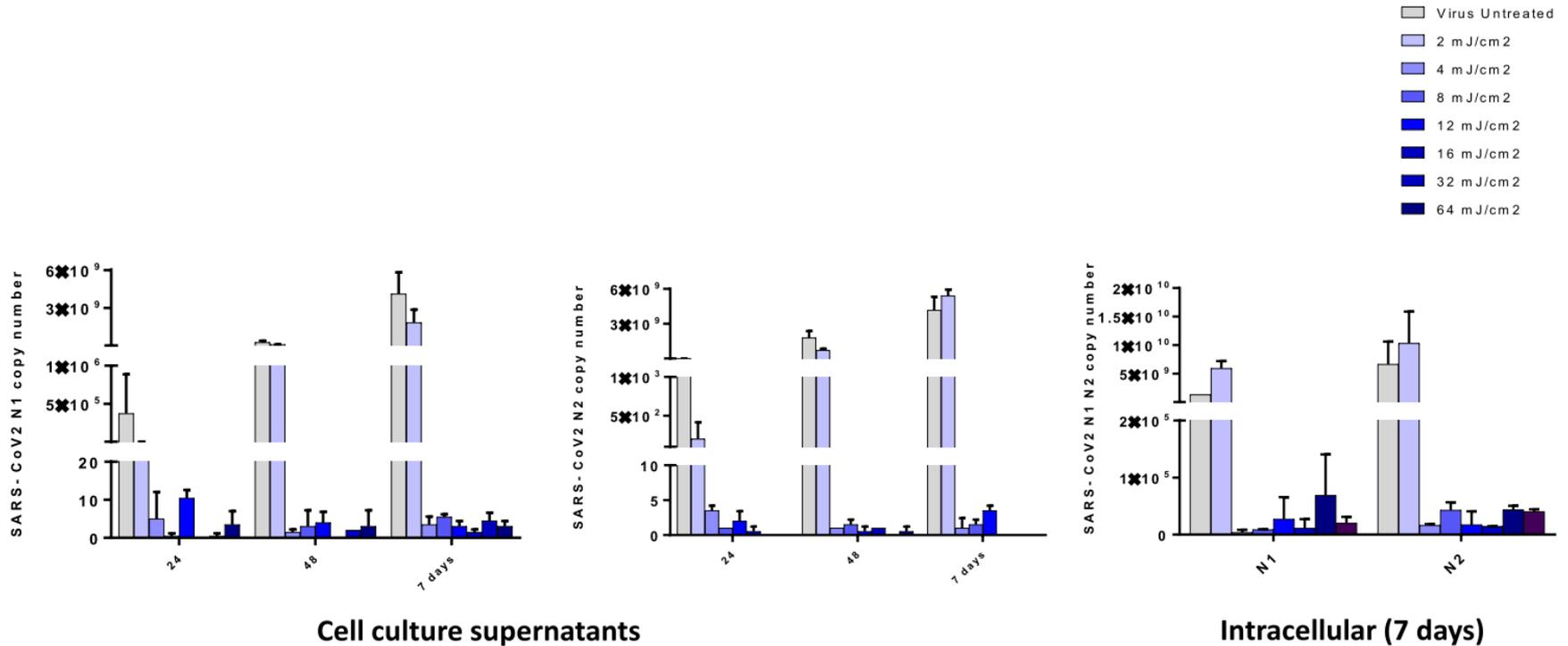


Cell culture supernatants

Intracellular (7 days)

Risultati a 222 nm

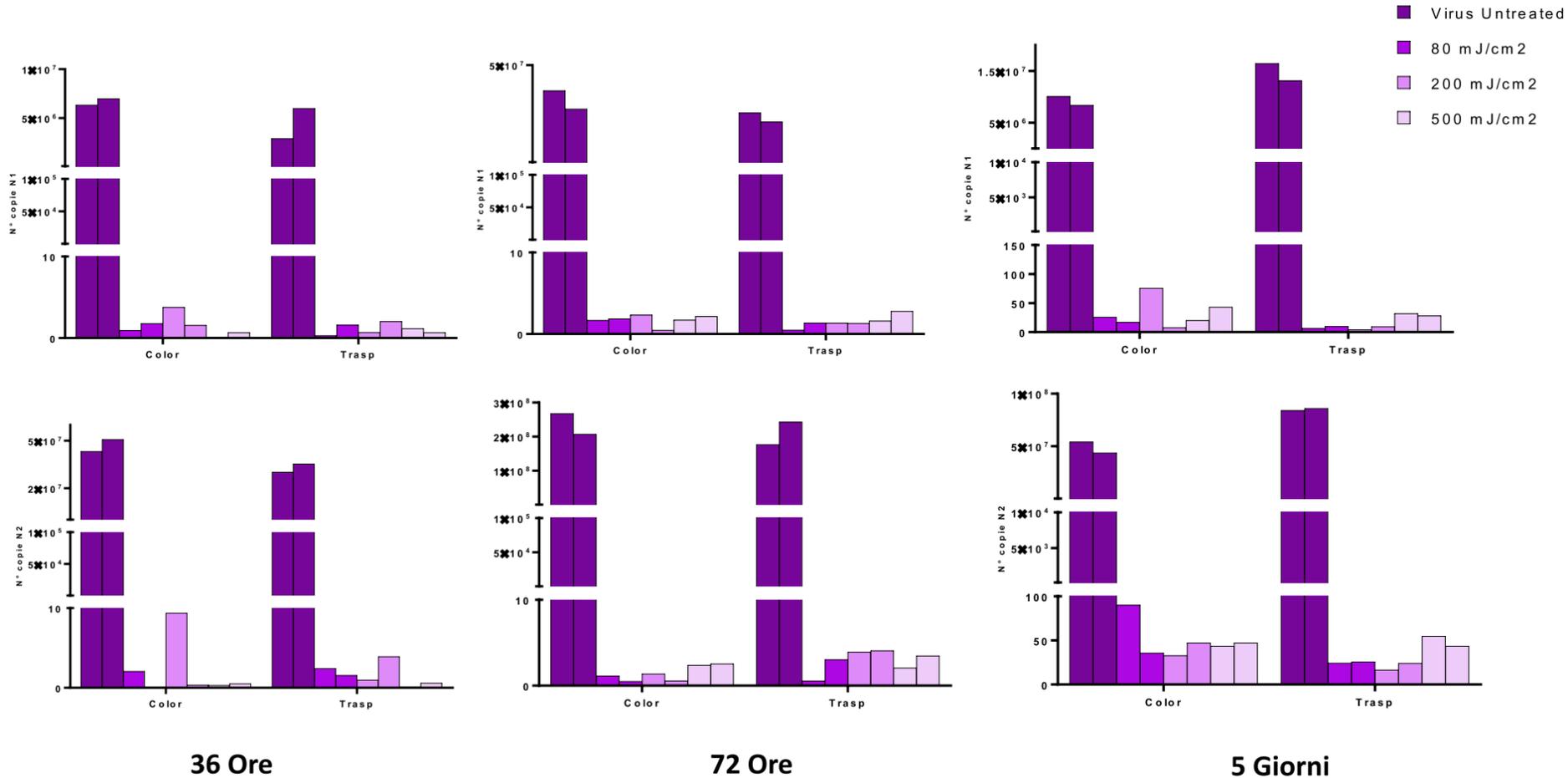
Concentrazione media (goccioline di saliva)



Anche nel far UVC le dosi sono comparabili con le altre lunghezze d'onda UVC

Risultati a 308 nm

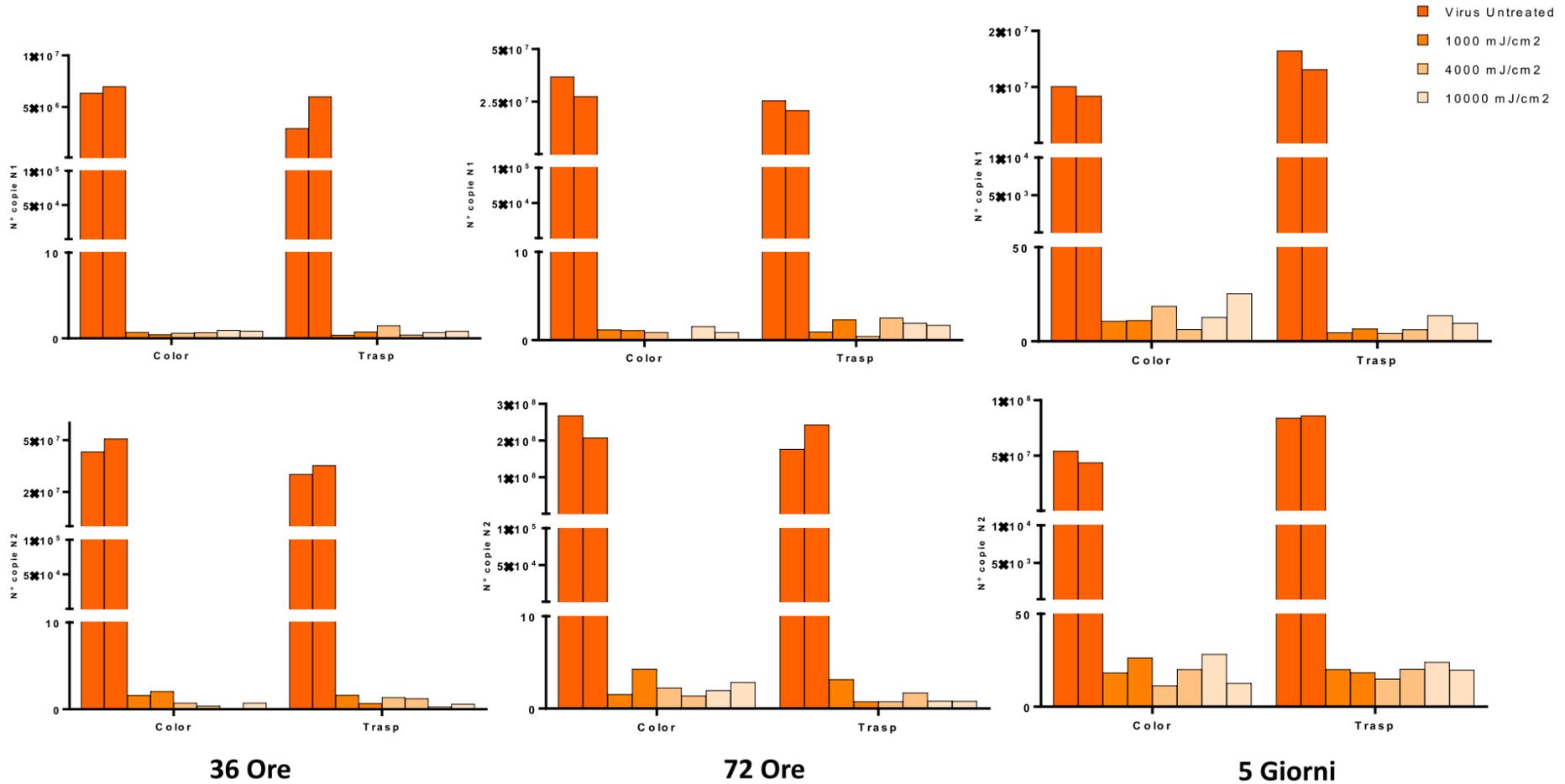
Concentrazione media (goccioline di saliva)



Per l'UVB abbiamo almeno un fattore 50 di efficacia in meno...servono 200 mJ/cm² per la completa inibizione.

Risultati a 366 nm

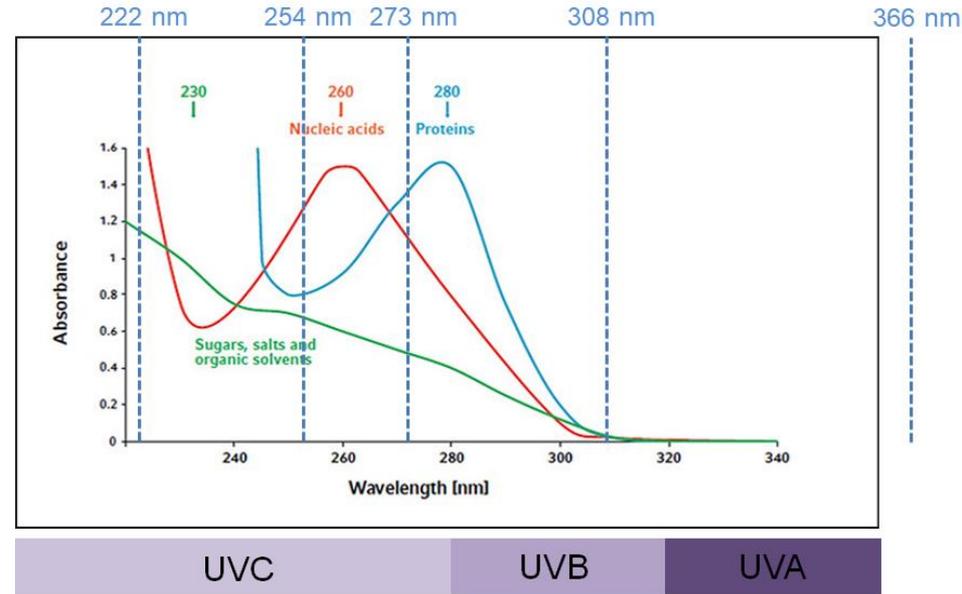
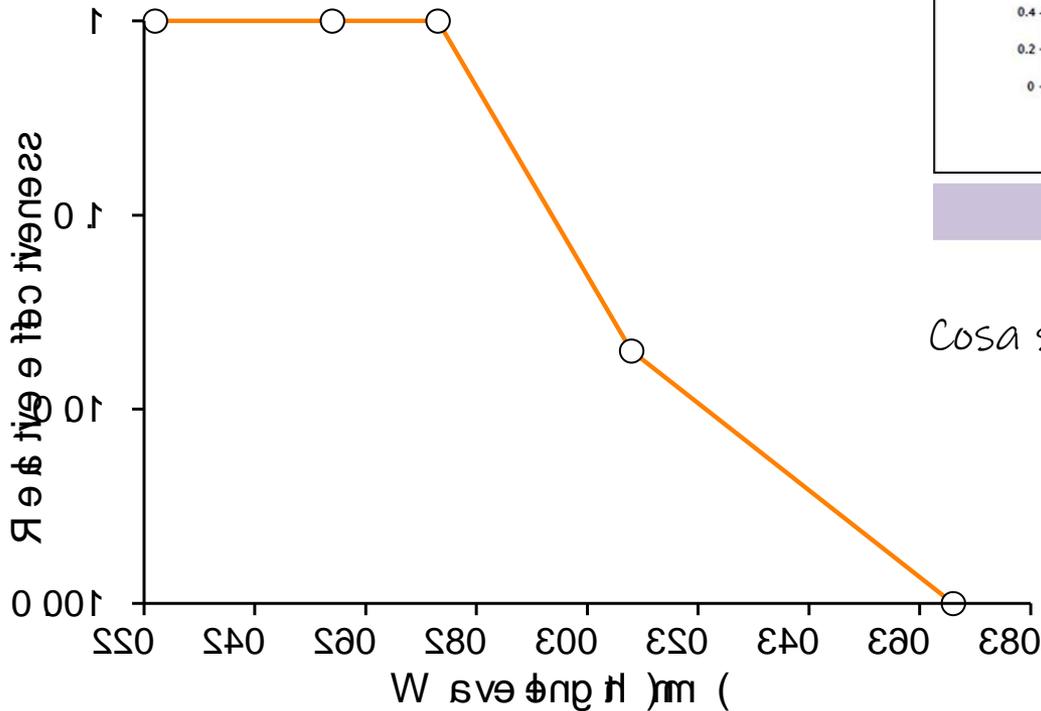
Concentrazione media (goccioline di saliva)



Per l'UVA abbiamo almeno un fattore 1000 di efficacia in meno...servono 4000 mJ/cm² per la completa inibizione.

Action spectrum...

Vediamo che è inaspettatamente buona l'efficacia...guardando l'assorbimento)



Cosa succede più in su (andando nel visibile)?

Cosa abbiamo imparato che

- E' bello e coinvolgente lavorare con persone con una visione «del mondo» completamente diversa dalla nostra!
- E' cruciale mettere a punto degli esperimenti in cui si hanno tutti i parametri sotto controllo;
- E' opportuno fare esperimenti utili sia a guidare la progettazione di dispositivi di disinfezione sia a capire cosa succede a livello microbiologico;
- Il SARS-CoV-2 è (fortunatamente) un virus molto suscettibile alla radiazione UVC;
- Che l'efficacia della radiazione UVB ed UVA non è poi così male come si potrebbe pensare dagli spettri di assorbimento;