


# UVC e TFD na inativação viral

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CEPOF
CENTRO DE PESQUISAS EM ÓPTICA E FOTÔNICA

# Microrganismos e células – comparação dos tamanhos

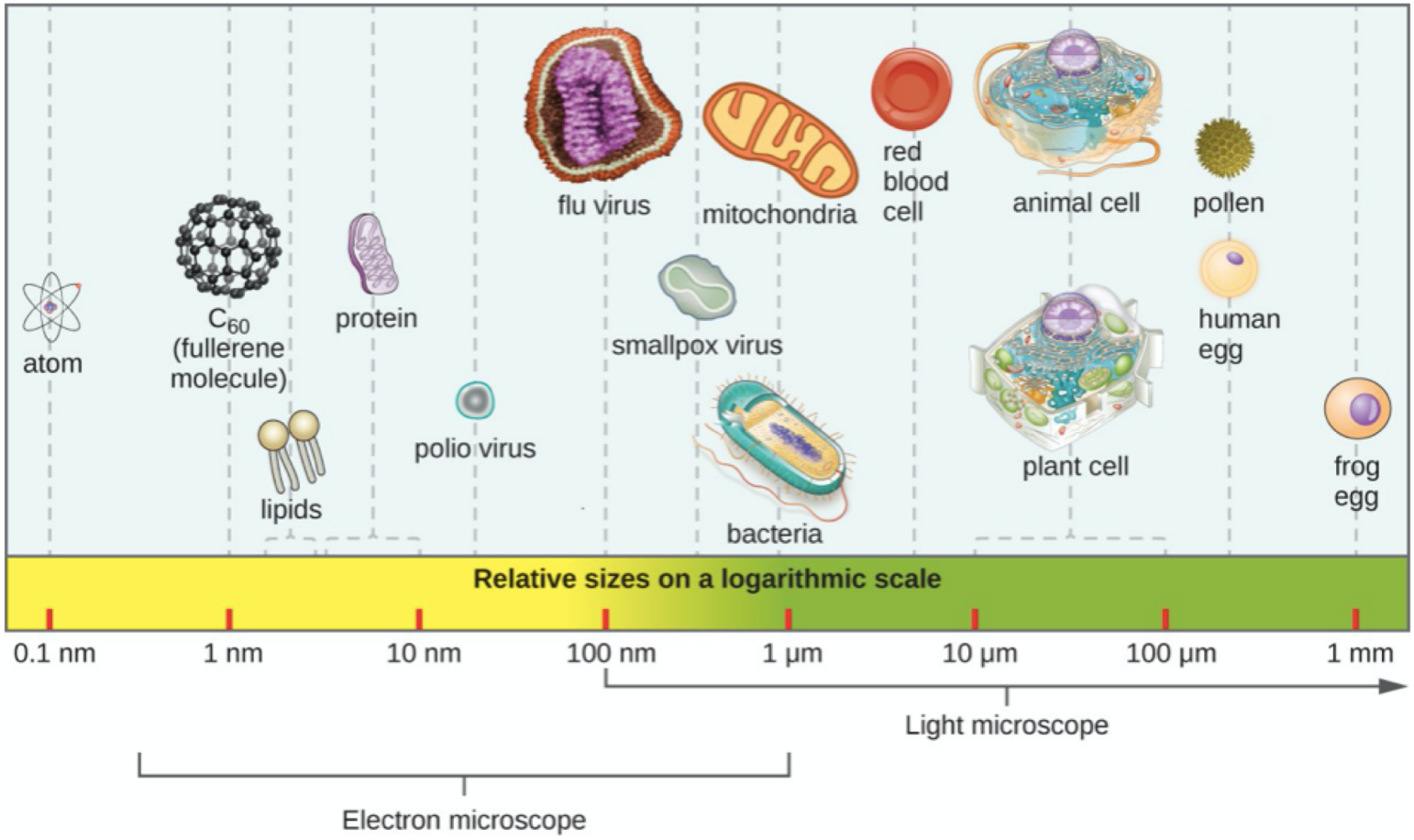


Figure 1. The relative sizes of various microscopic and nonmicroscopic objects. Note that a typical virus measures about 100 nm, 10 times smaller than a typical bacterium (~1 µm), which is at least 10 times smaller than a typical plant or animal cell (~10–100 µm). An object must measure about 100 µm to be visible without a microscope.

https://courses.lumenlearning.com/microbiology/chapter/types-of-microorganisms/

# Controle microbiano por UVC – diferentes MOs

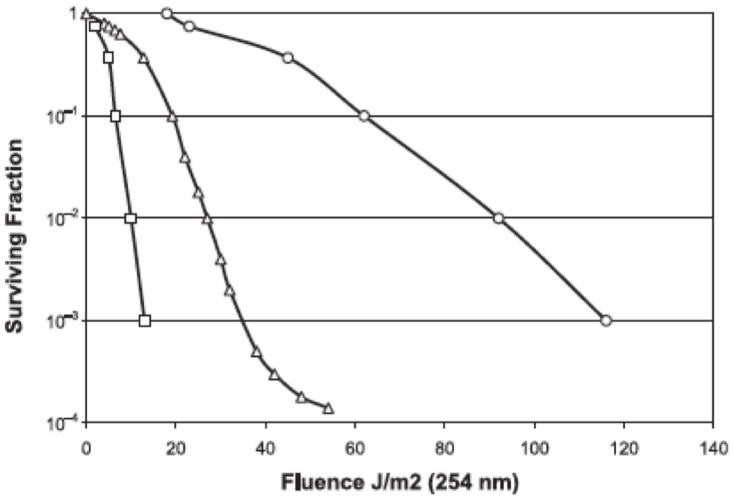


**Figure 2.** Differential UVC (254nm) sensitivity of a wild strain and mutants of *E. coli*. The curves represent the survival of a radiation proficient (B/r, circles) and deficient (Bs, squares) mutant compared to the sensitivity of a wild-type (wt, triangles) *E. coli* (10).

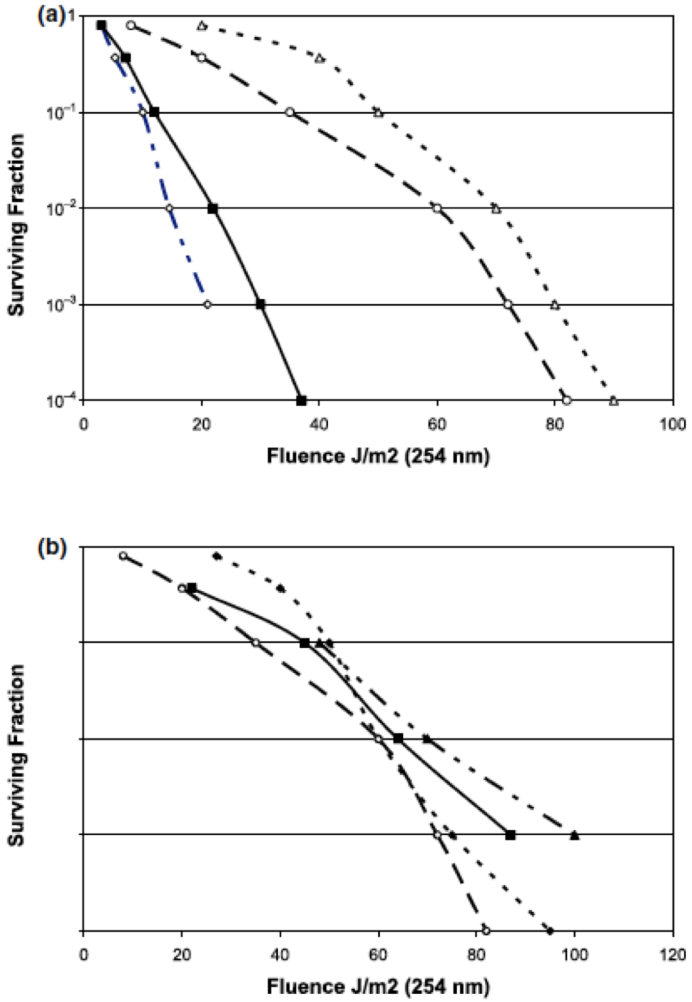


**Figure 3.** UVC (254 nm) sensitivity of several vegetative bacteria relevant to human health. The graph represents that bacterial cell fraction surviving after irradiation at the fluence indicated on the *x*-axis. Data shown is for: (a) *Vibrio cholerae* (diamonds [31]), *Yersinia enterocolitica* (squares [30]), *Salmonella typhi* (triangles [32]); and (b) vegetative cells of *Bacillus anthracis* Sterne (squares [38]), *Shigella sonnei* (diamonds [27]), and *Burkholderia pseudomallei* (triangles [39]). *E. coli* (wt [28]) is included in both panels (circles) for comparison.

TP Coohill and JL Sagripanti. Photochem Photobiol 84 (2008)

COVID-19 Map - Johns Hopkin

coronavirus.jhu.edu/map.html

JOHNS HOPKINS UNIVERSITY & MEDICINE | CORONAVIRUS RESOURCE CENTER

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Tracking Home | Critical Trends | Global Map | U.S. Map | Data in Motion

# COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU)

**Global Cases**
**55.079.810**

**Cases by Country/Region/Sovereignty**

- 11.205.485 US
- 8.874.290 India
- 5.876.464 Brazil
- 2.041.293 France
- 1.954.912 Russia
- 1.496.864 Spain
- 1.394.299 United Kingdom
- 1.318.384 Argentina
- 1.205.881 Italy
- 1.205.217 Colombia
- 1.009.396 Mexico

Admin0

Last Updated at (M/D/YYYY)
11/17/2020 6:26 AM


Cumulative Cases | Active Cases | Incidence Rate | Case-Fatality Ratio | Testing Rate

191
countries/regions

*Lancet Inf Dis* Article: Here. Mobile Version: Here. Data sources: Full list. Downloadable database: GitHub, Feature Layer.
Lead by JHU CSSE. Technical Support: Esri Living Atlas team and JHU APL. Financial Support: JHU, NSF, Bloomberg Philanthropies and Stavros Niarchos Foundation. Resource support: Slack.

**Global Deaths**
**1.328.167**

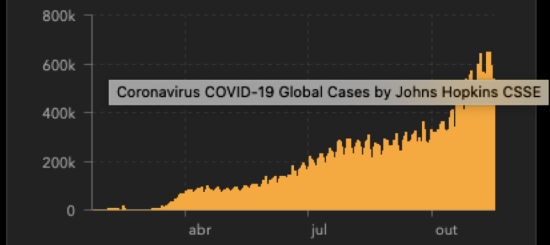
- 247.220 deaths US
- 166.014 deaths Brazil
- 130.519 deaths India
- 98.861 deaths Mexico
- 52.240 deaths United Kingdom
- 45.733 deaths

Global Deaths

**US State Level**
**Deaths, Recovered**

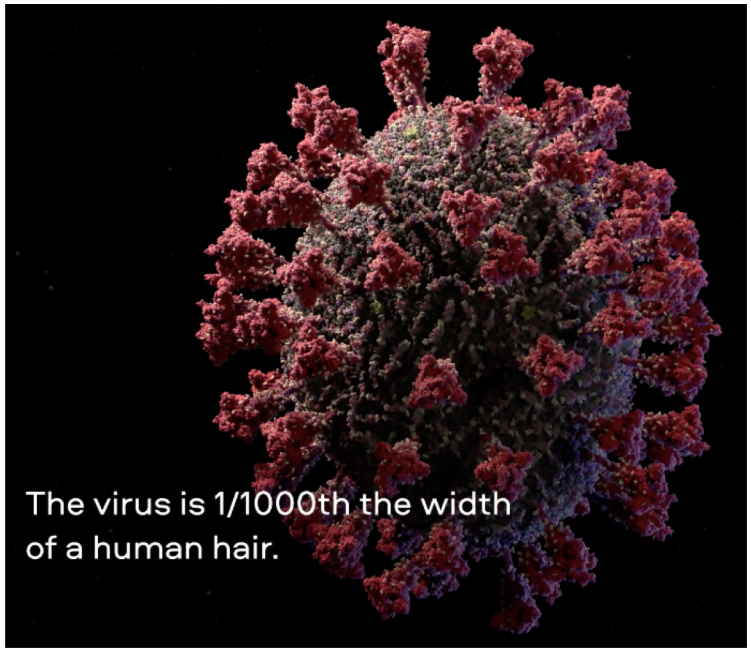
- 34.054 deaths, 81.908 recovered New York US
- 20.032 deaths, 875.521 recovered Texas US
- 18.306 deaths, recovered California US
- 17.559 deaths, recovered Florida US
- 16.580 deaths, 39.643 recovered

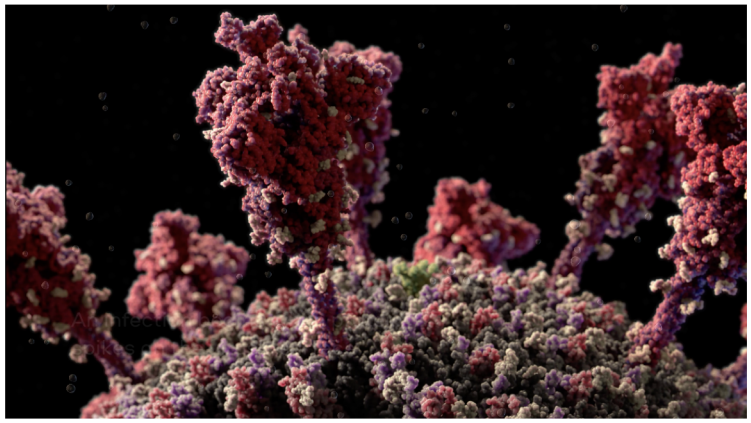
US Deaths, Rec...

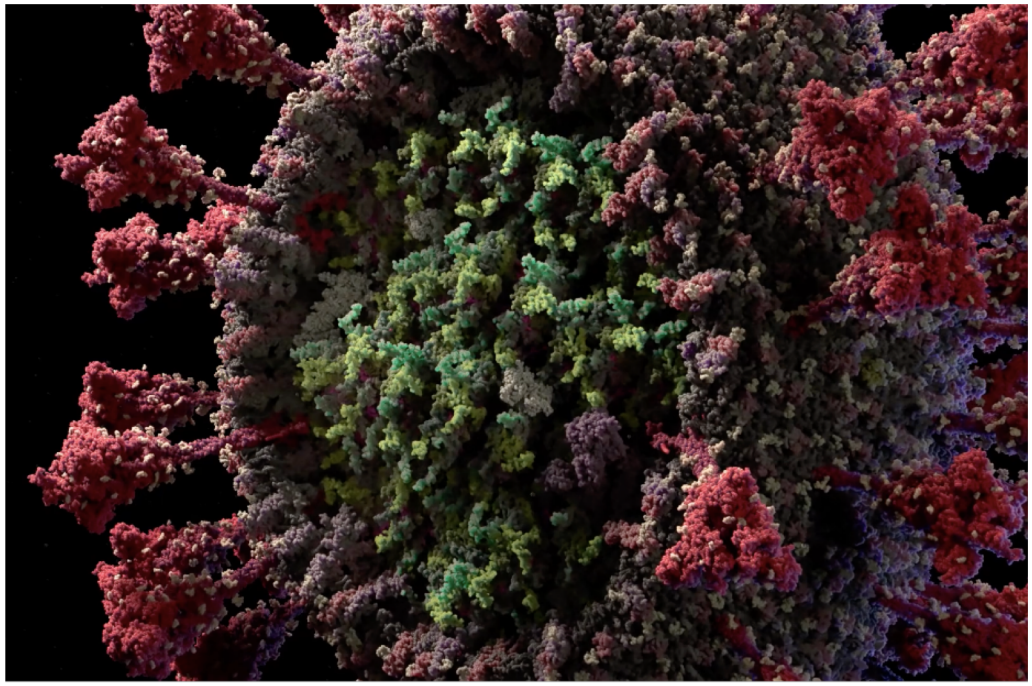



Daily Cases

# Estrutura do SARS-Cov-2



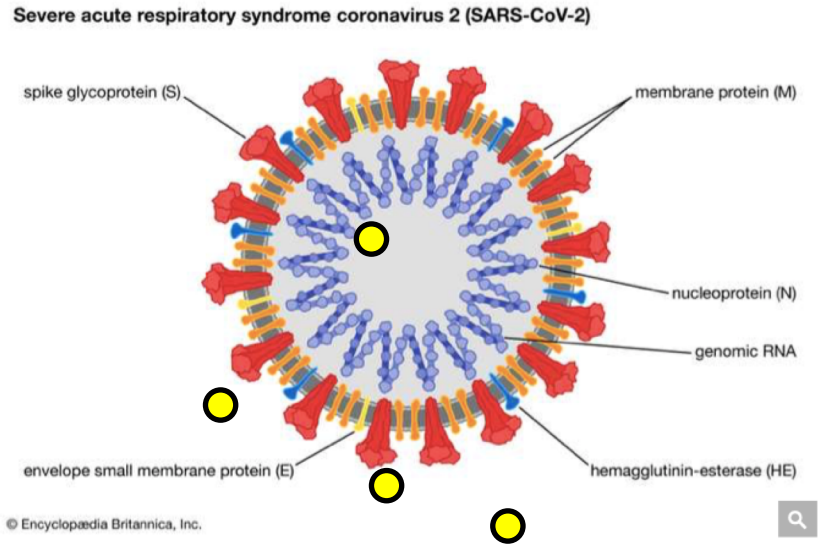





Coronavírus
RNA fita simples (26 a 32 kb)
~ 120 nm

https://visual-science.com/projects/sars-cov-2/animation/

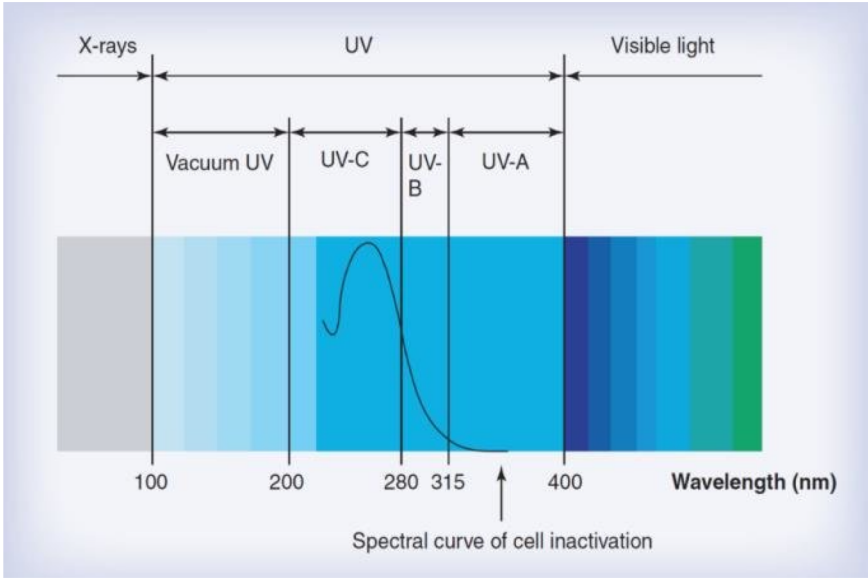
# Ação germicida da radiação UVC - vírus




**SARS-CoV-2**

The coronavirus SARS-CoV-2, the cause of the COVID-19 pandemic.

*Encyclopædia Britannica, Inc./Patrick O'Neill Riley*




T Dai et al. Exp Rev (2012)

Energia de dissociação

N-H = 3,2 eV

C-C = 3,6-3,9 eV

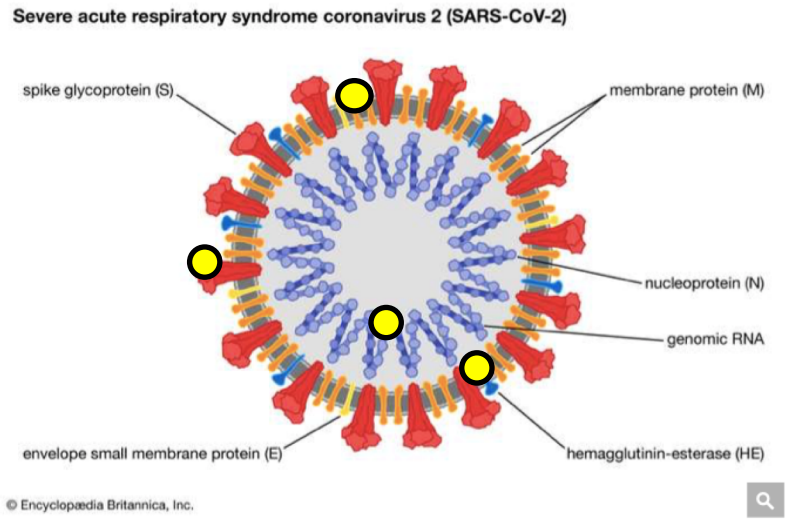
C-H = 3,5 eV

X-H em aa (intervalo): 3,32 a 5,49 eV

$E_{254nm}$ = 4.88 eV

BN Moore and RR Julian. Phys Chem Chem Phys 14 (2012)

# Dano da radiação UVC em RNA-vírus envelopados

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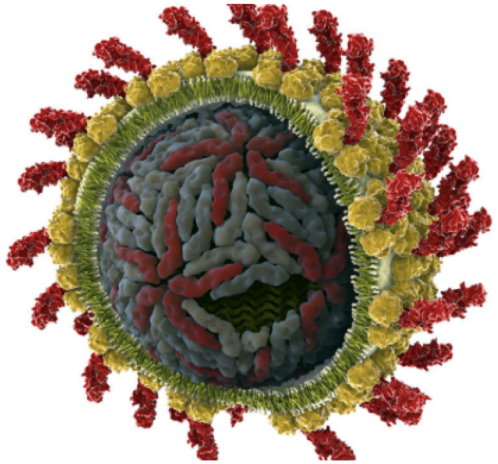


https://www.sciencemag.org/news/2017/04/new-report-halves-number-people-infected-hepatitis-c-worldwide

Vírus da Hepatite C
Flavivírus
55 a 65 nm
RNA fita simples (9,6 kb)

## Danos induzidos no RNA

- Oxidação, clorinação, nitração e alquilação
- Fotoprodutos - EROs

# Inativação do vírus de hepatite C em perfusato de órgãos para transplante

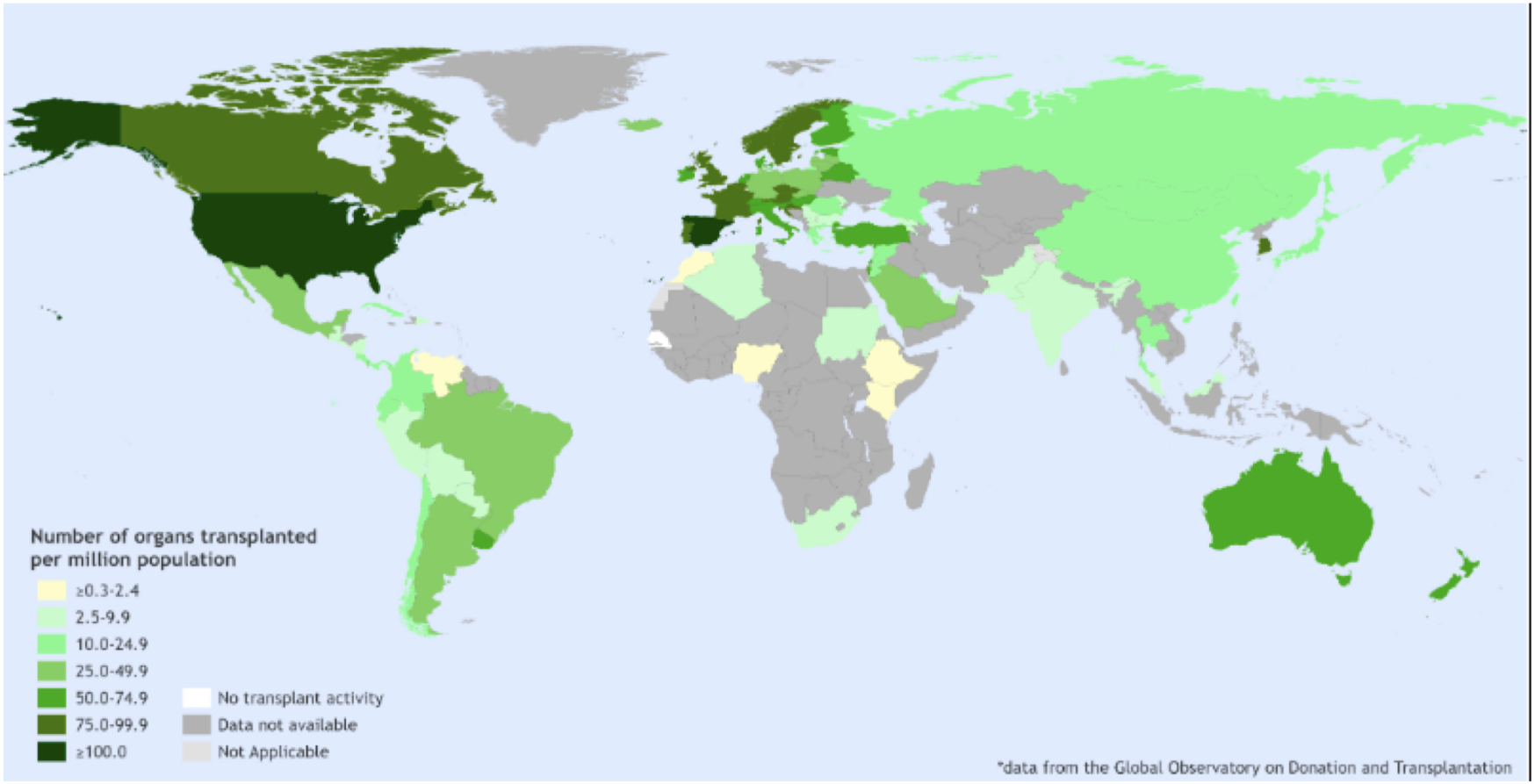
Pulmão

(University Health Network - Prof. Marcelo Cypel)

Fígado e rim

FMUSP (Profs. Luiz A Carneiro D Albuquerque, Flávio H Ferreira Galvão e Silvano Raia)

# Solid organ transplants data (Global Observatory on Donation and Transplantation)




146 840 transplants reported in 2018

# Organ Donation Statistics

How many people are waiting for a transplant? Who receives organs, and what organs are most needed? Scroll down to explore data from the Organ Procurement and Transplantation Network.

## Statistics at a Glance

**109,000+**
**Number of men, women, and children on the national transplant waiting list as of September 2020.**

**39,718**
**transplants were performed in 2019.**

**17**
**people die each day waiting for an organ transplant.**

### We All Need to Register. Here's Why:

**90%**
**of U.S. adults support organ donation**

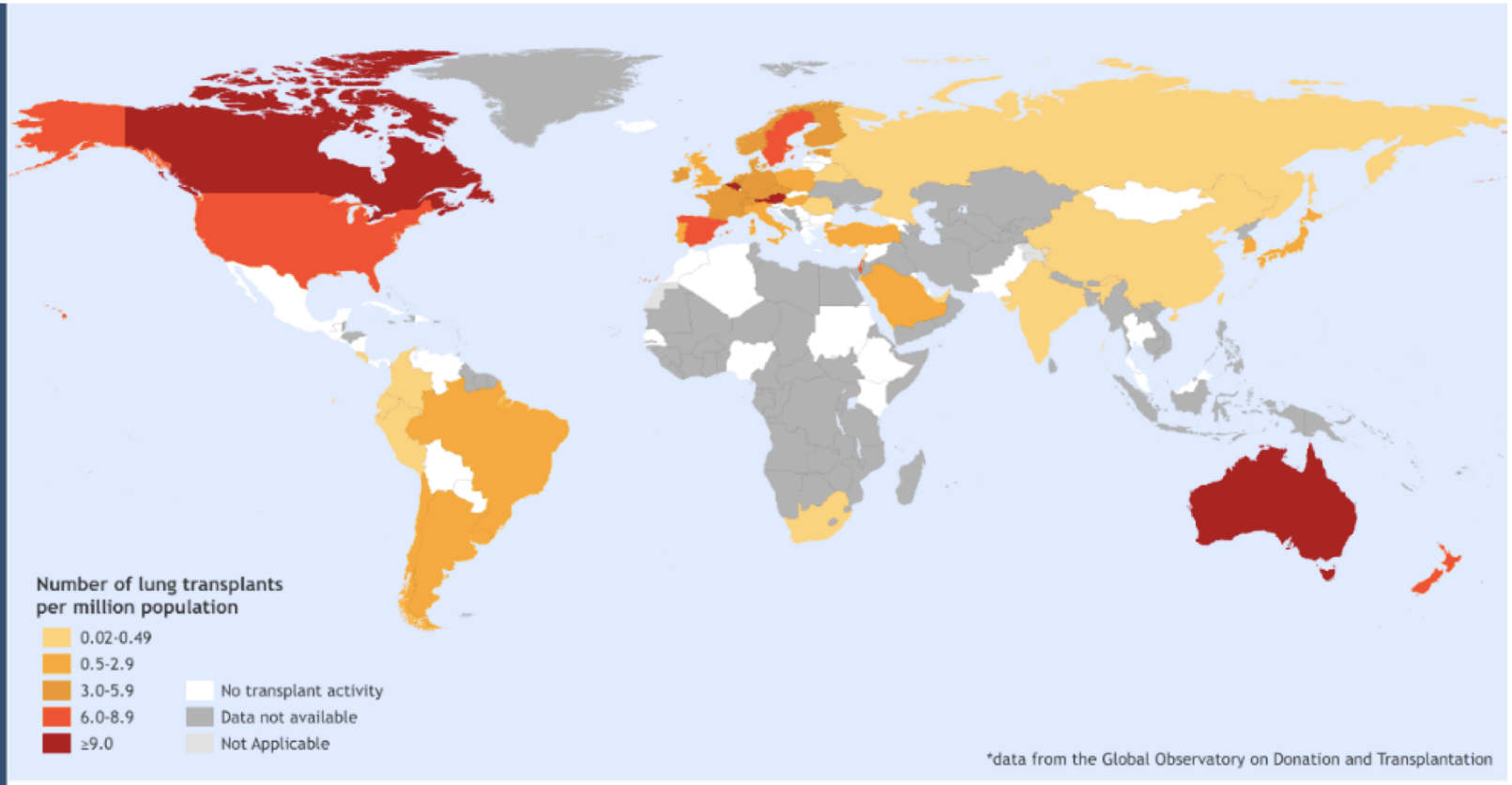
*but only*

**60%**
**are actually signed up as donors.**

**every 9 minutes**
**another person is added to the transplant waiting list.**

**only 3 in 1,000**
**people die in a way that allows for organ donation.**

**Lung transplantation activities 2018***




The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: Global Observatory on Donation and Transplantation
Map Production: Information Evidence and Research (IER) World Health Organization

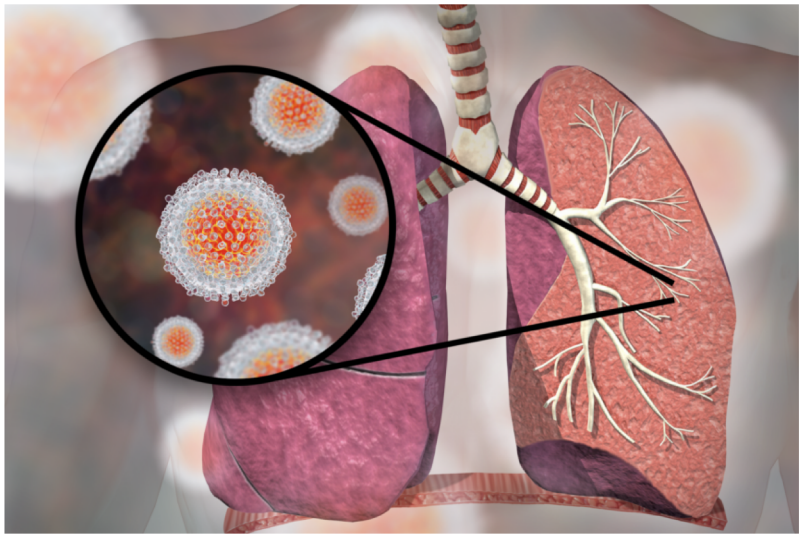
World Health Organization



6 475 lung transplants reported in 2018

6% increase vs 2017

# HCV+ organs for HCV- patients

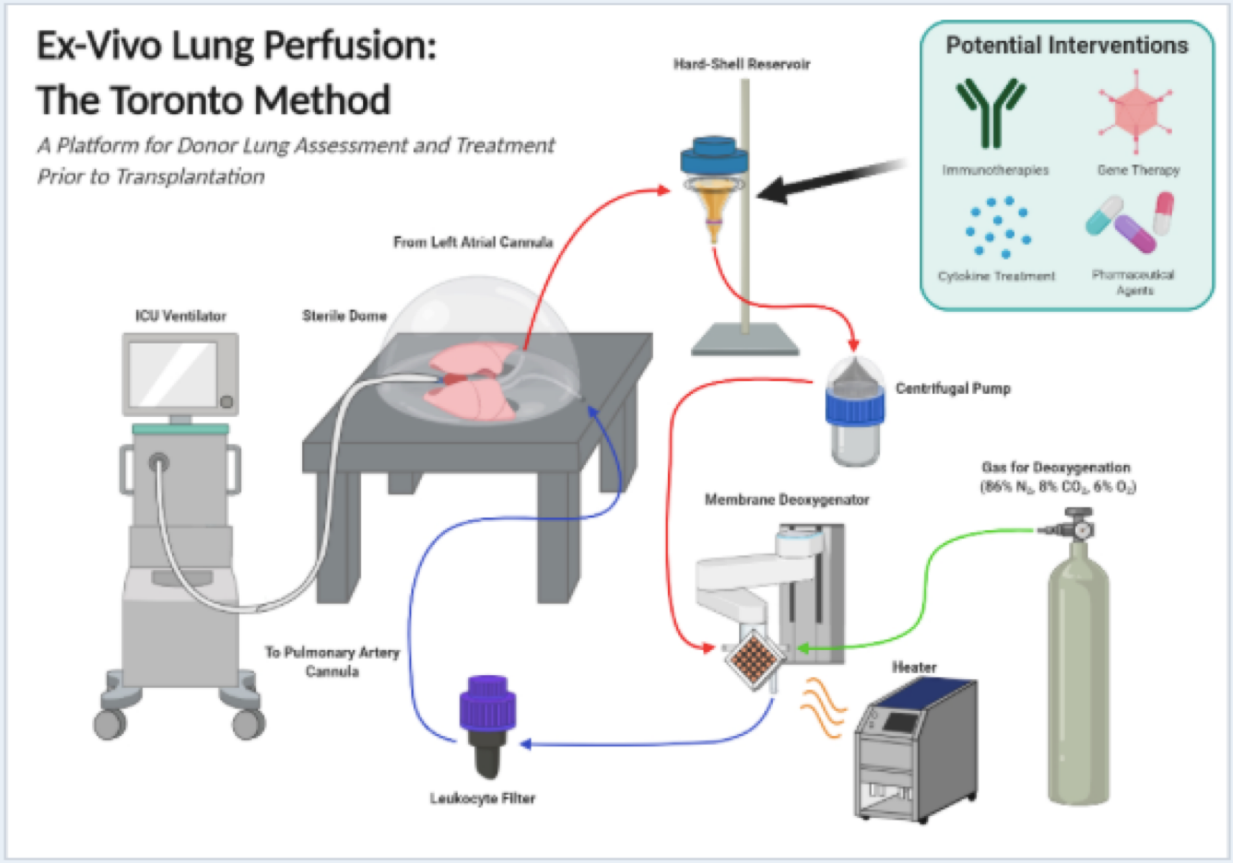


In the US, 1000 patients die yearly while waiting for heart or lung transplantation.

Direct-acting antivirals (DAAs) for HCV treatment "has raised the possibility of substantially increasing the donor organ pool by enabling the transplantation of hearts and lungs from HCV-infected donors into recipients who do not have HCV infection."

"In our trial, hearts and lungs from HCV-infected donors were transplanted safely with excellent graft function at 6 and 12 months," the authors concluded. "However, longer-term data are needed to fully define the risk-benefit profile."

A Wooley et al. New Engl J Med 2019



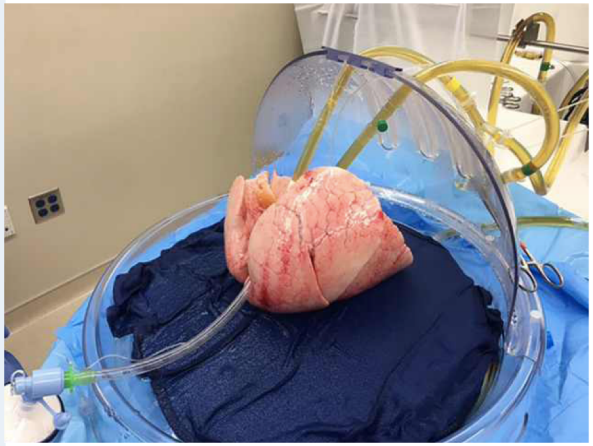

Ex-Vivo Lung Perfusion: The Toronto Method

Copy link Vote

Terrance Ku

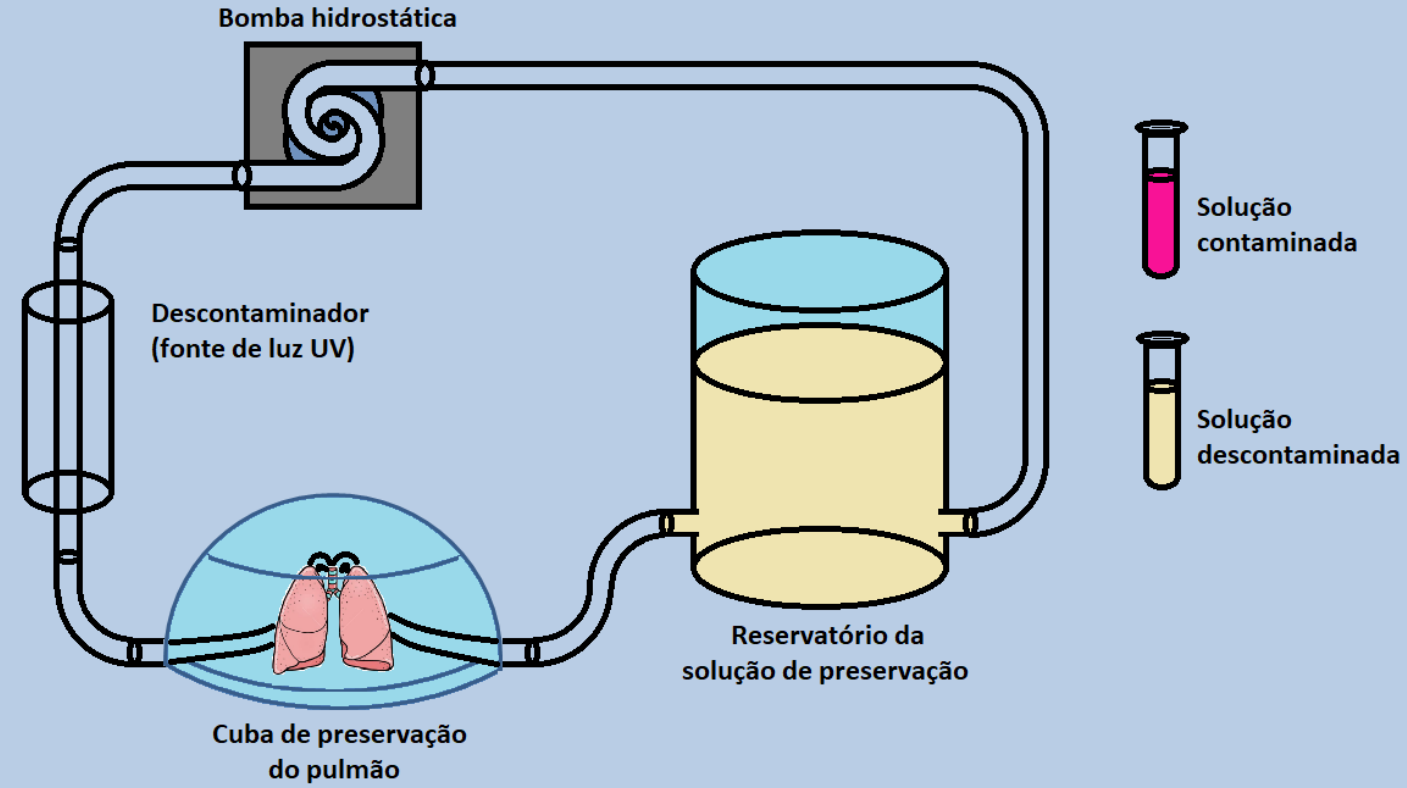
### Description

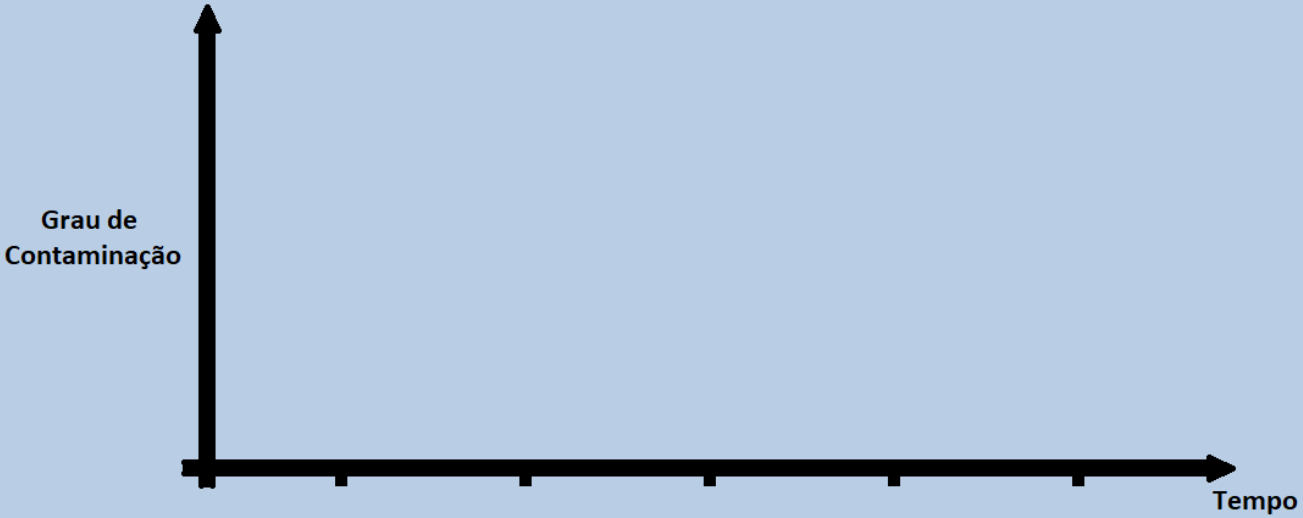
Illustrated is the Toronto method of ex-vivo lung perfusion (EVLP). The donor lung is cannulated on the pulmonary artery and the atrial cuff. Oxygenated perfusate exits from the atrial cannula into the reservoir. The centrifugal pump then propels the perfusate through a deoxygenator and heat exchanger. Lastly, the perfusate returns through a leukocyte filter into the pulmonary cannula. EVLP serves as a platform to assess and potentially treat grafts with therapeutics prior to transplantation.



G Makdisi et al. ATM 5 (2017)

M Cypel, A Neyrinck, TN Machuca. Intensive Care Med 45 (2019)

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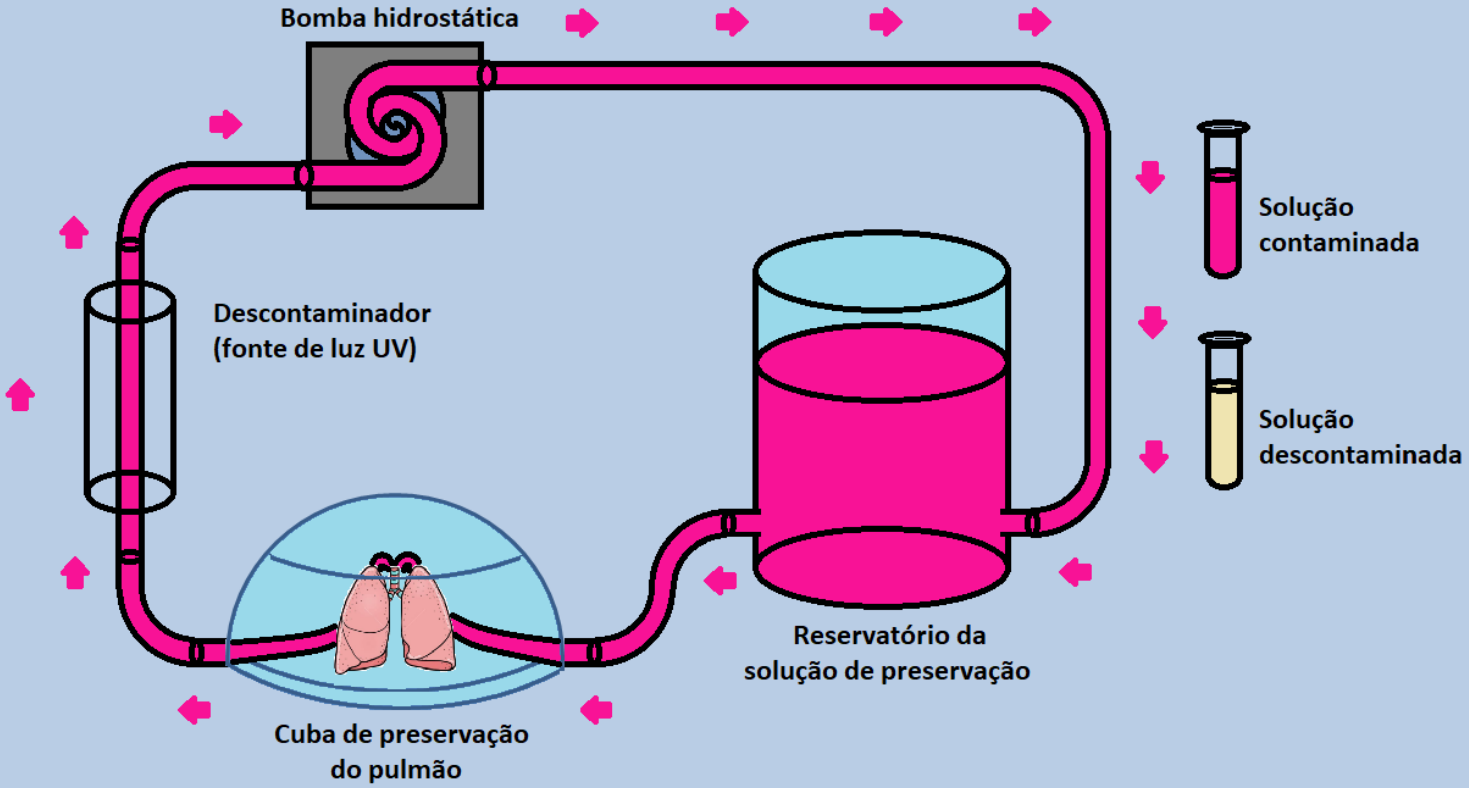


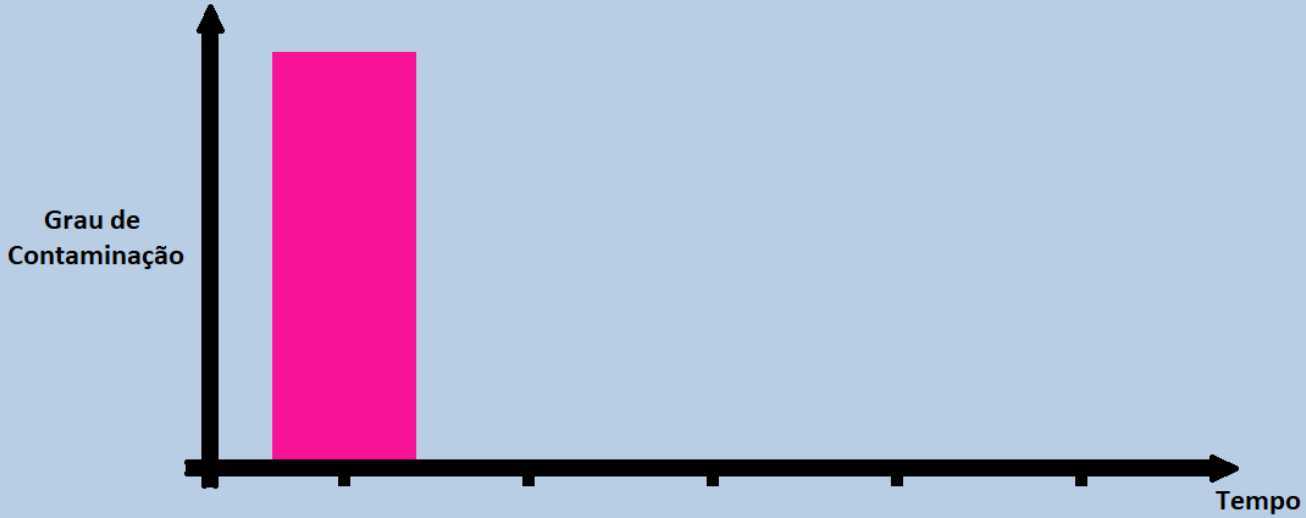


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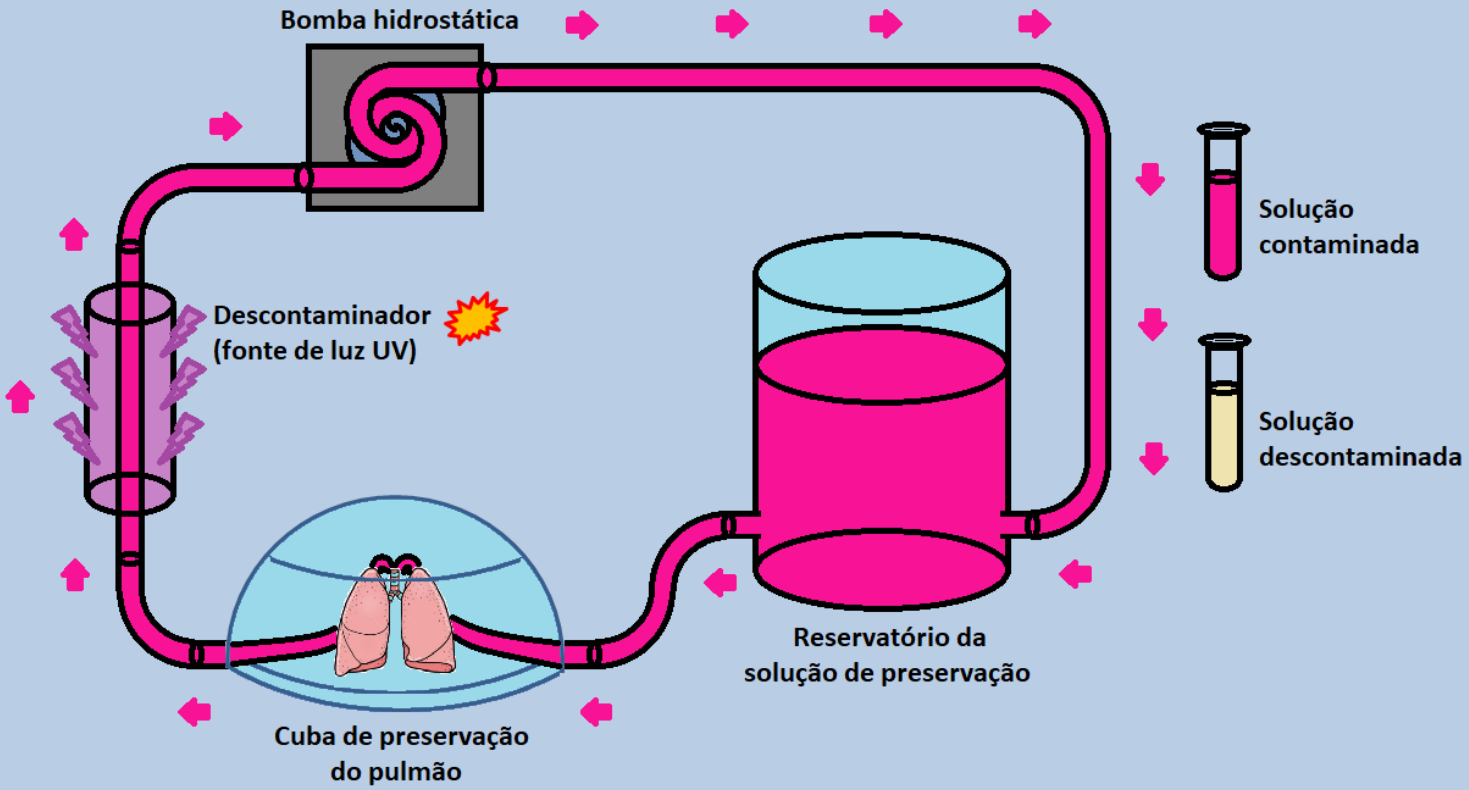


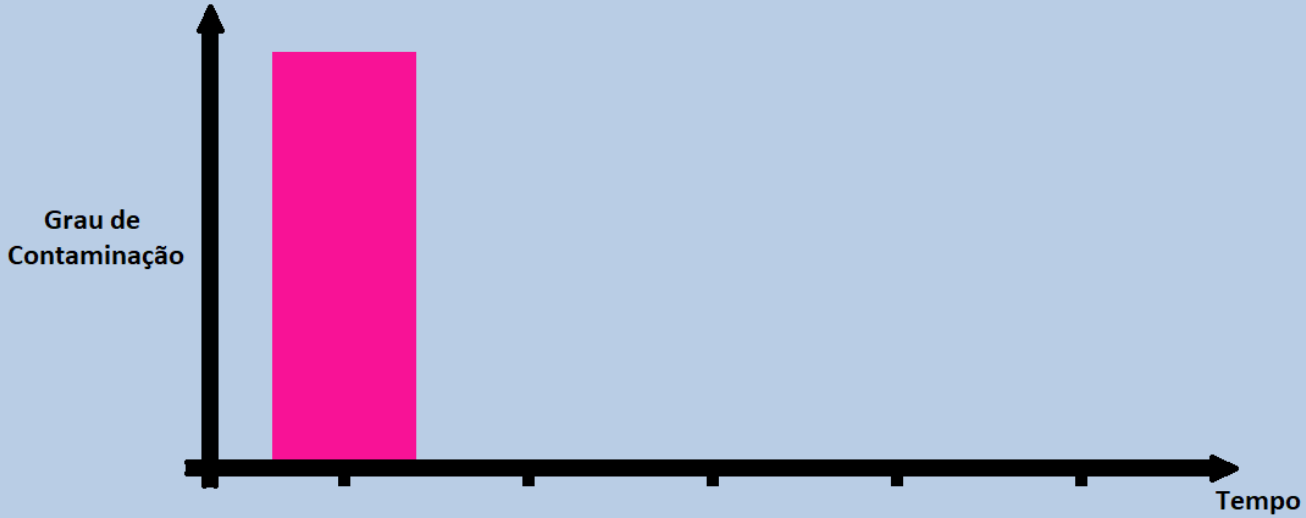


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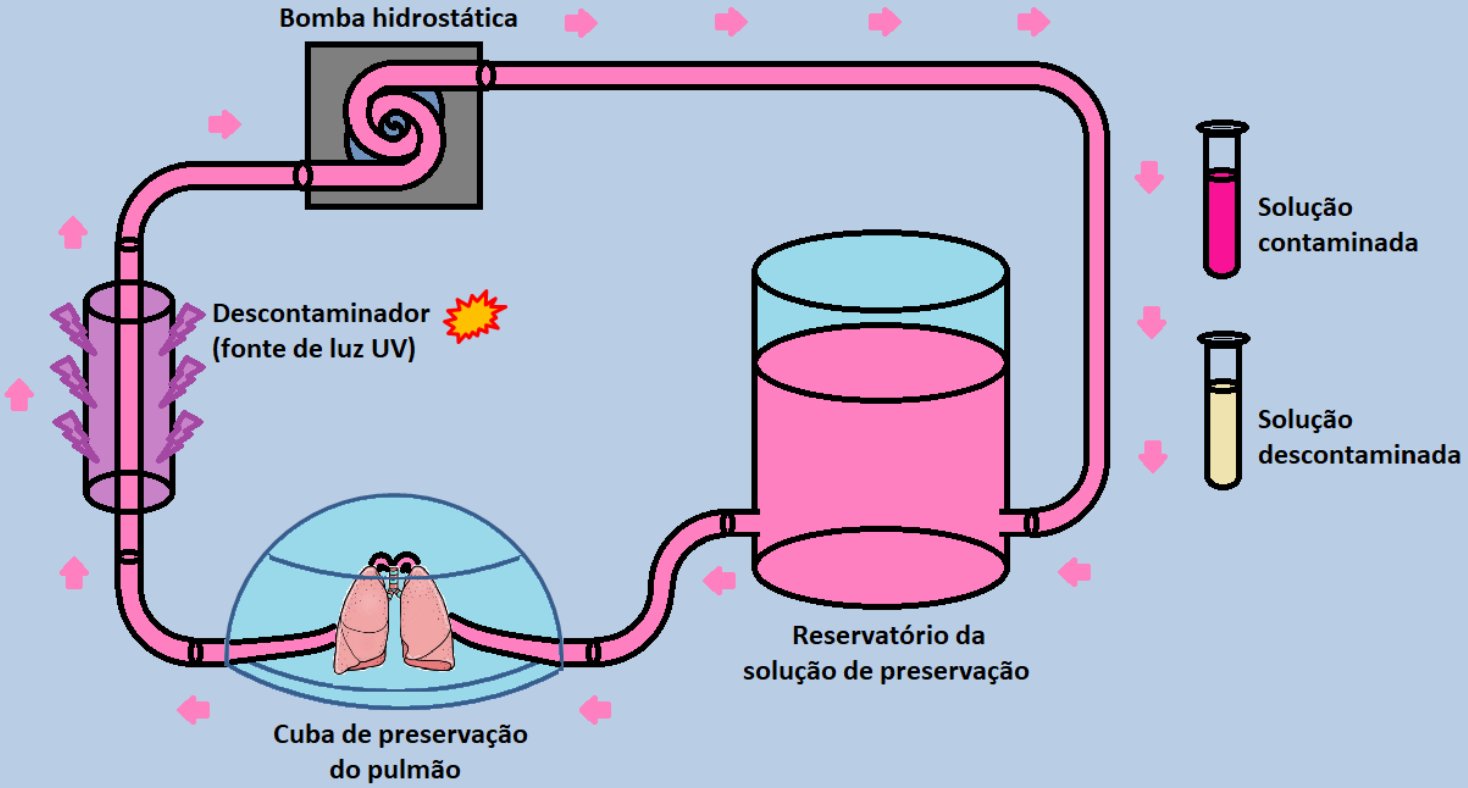


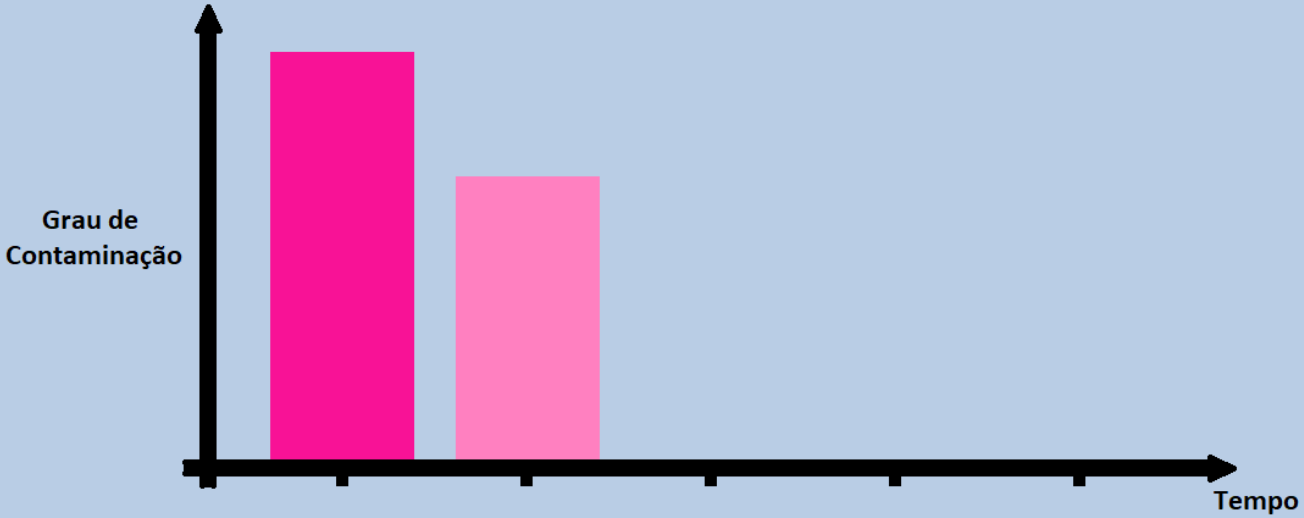


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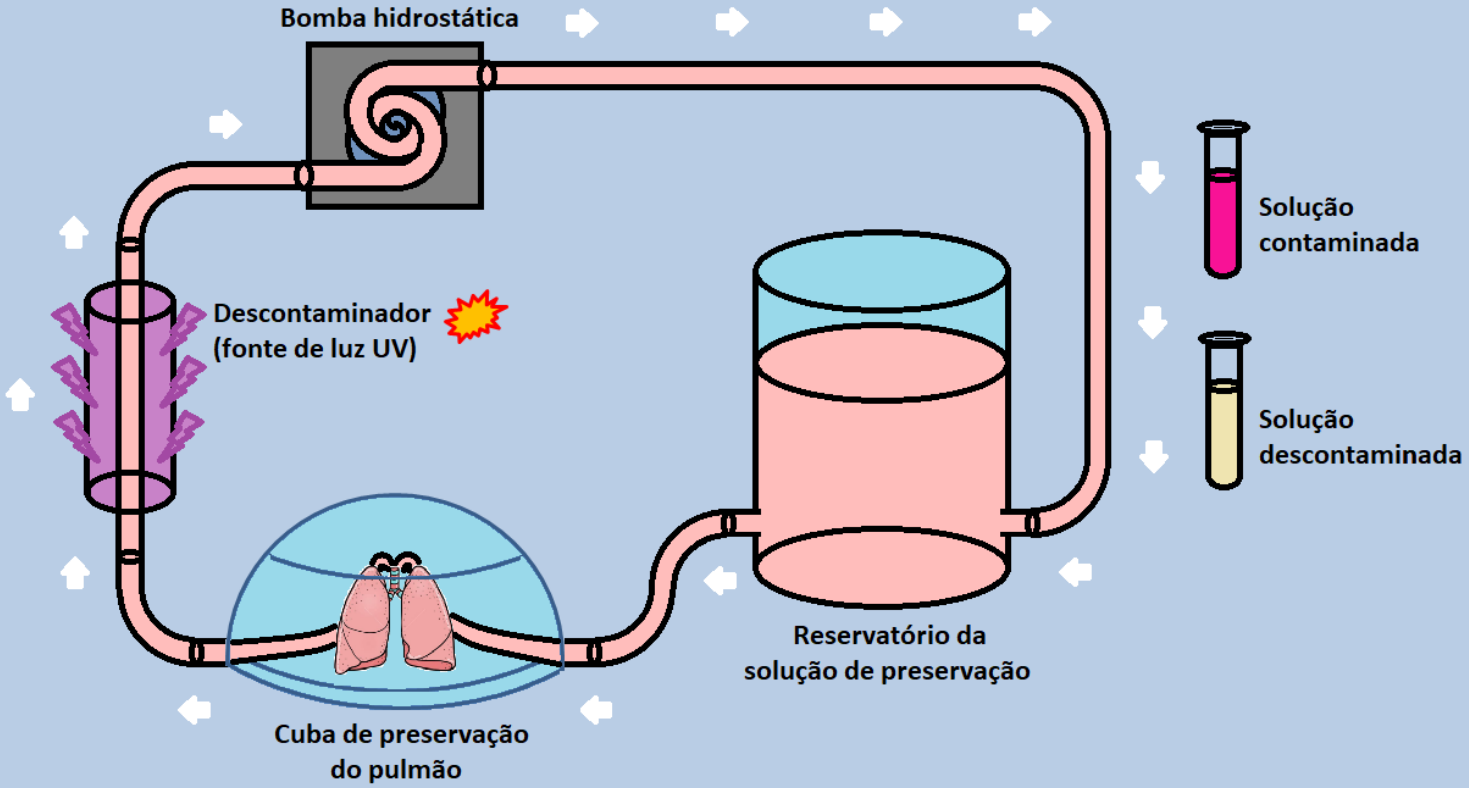


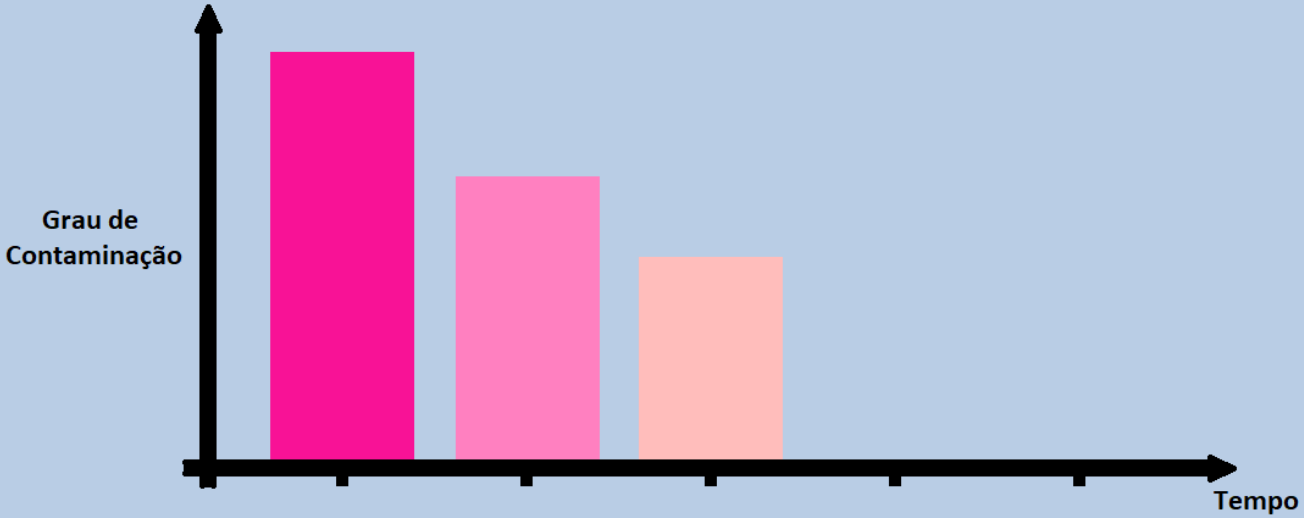


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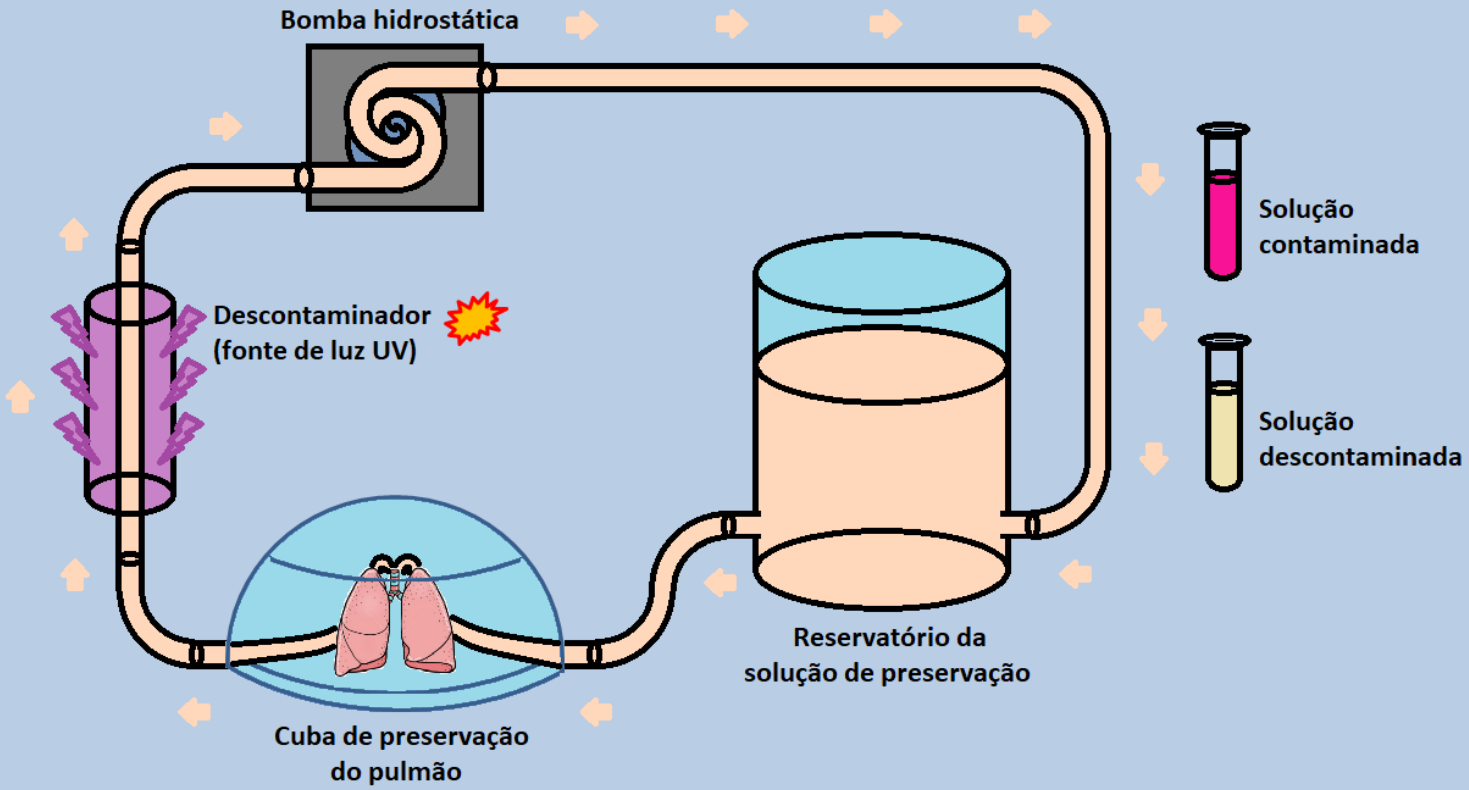


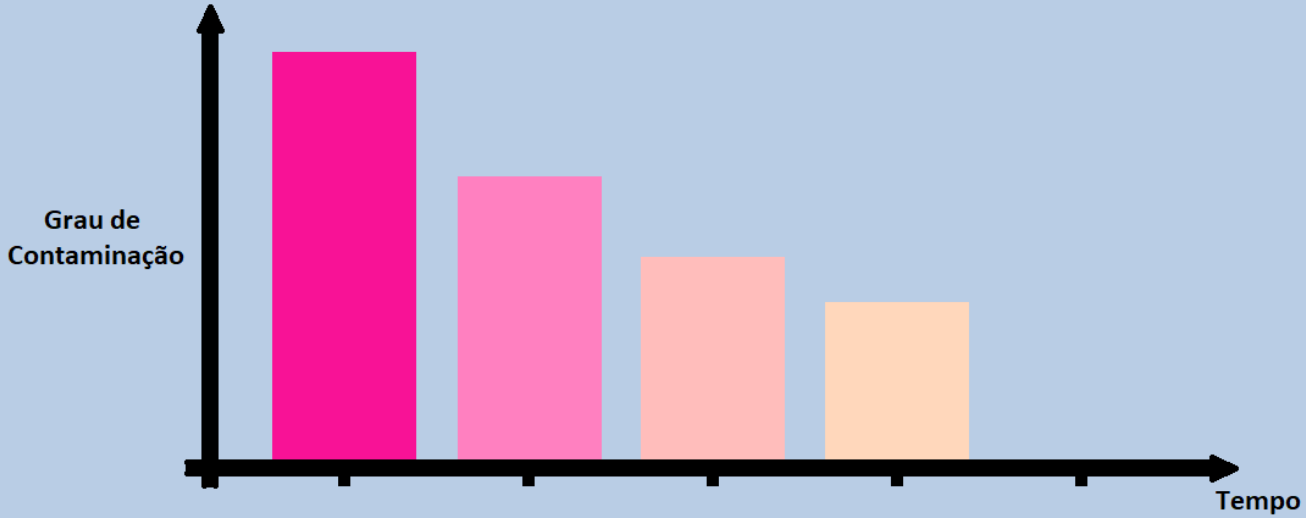


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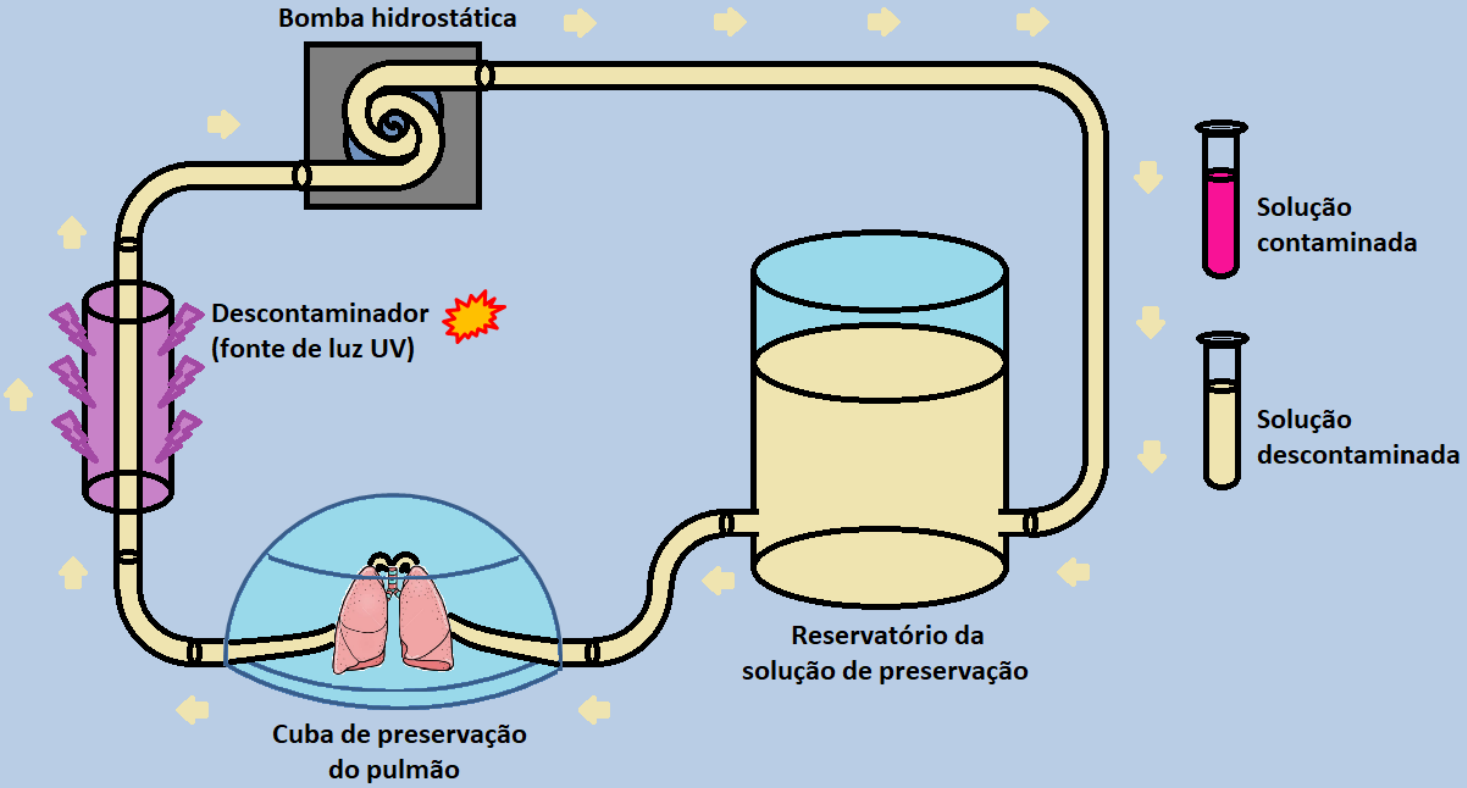


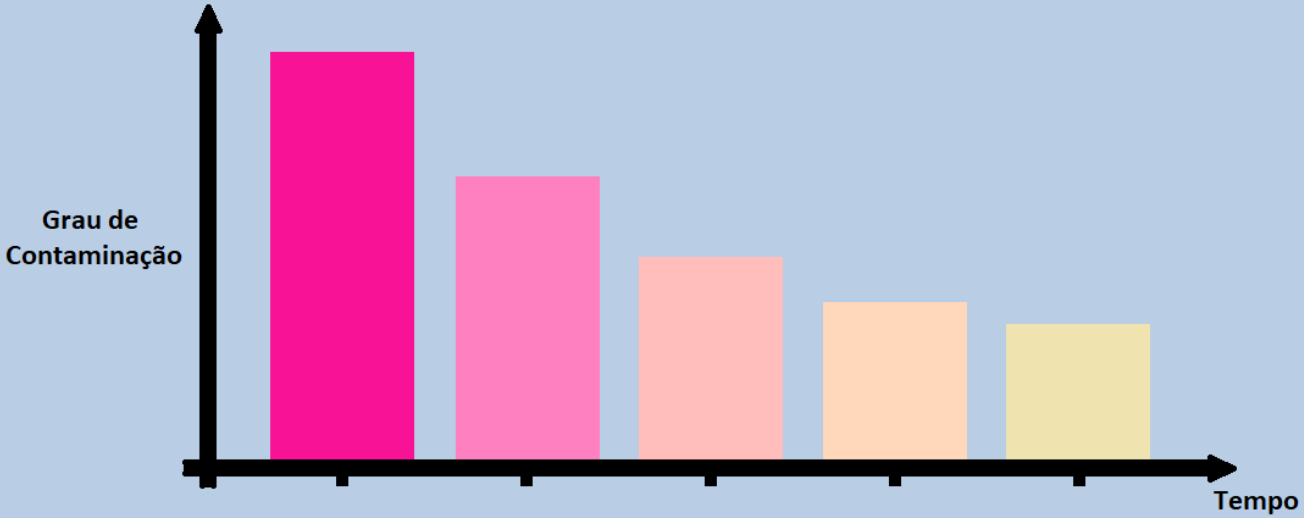


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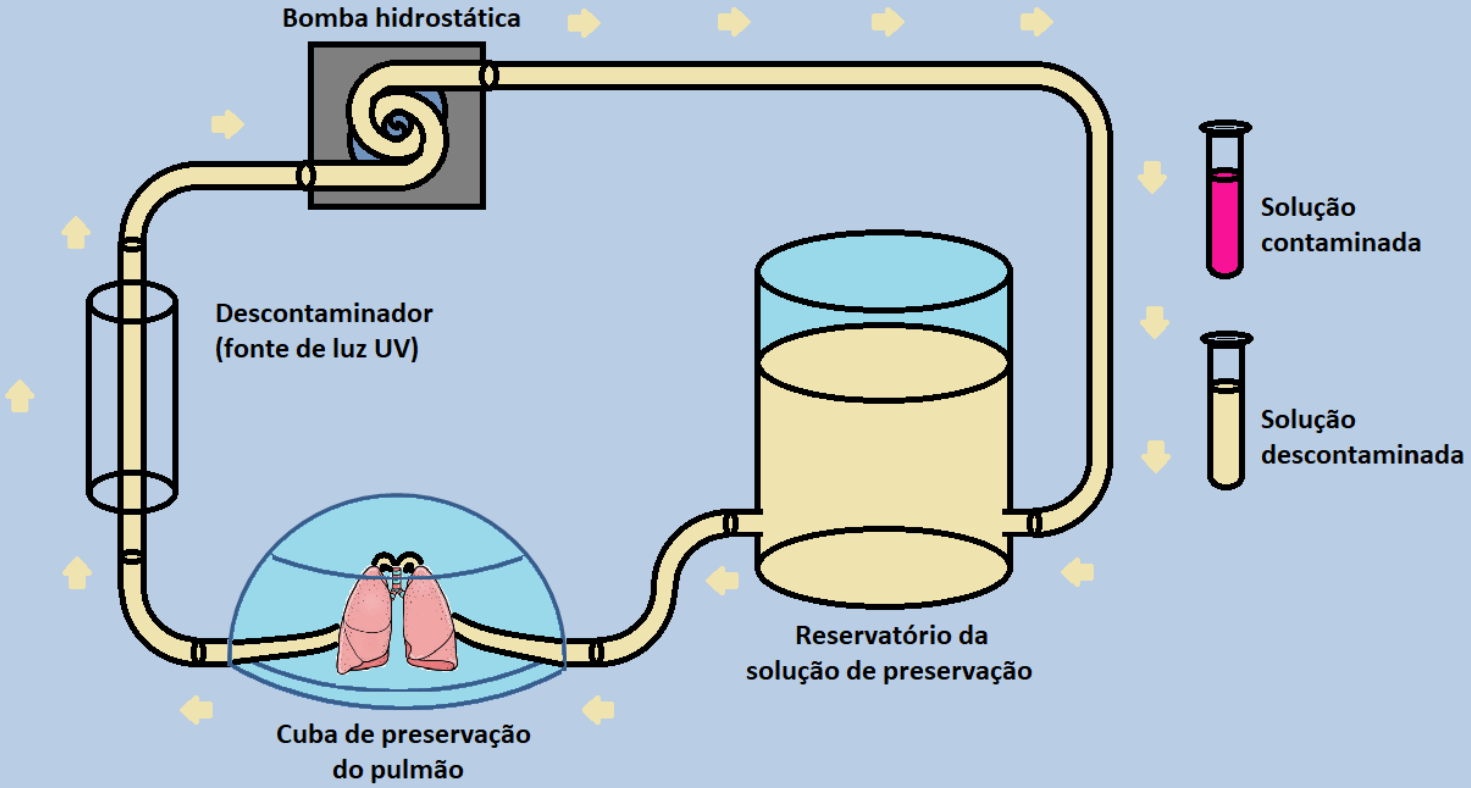


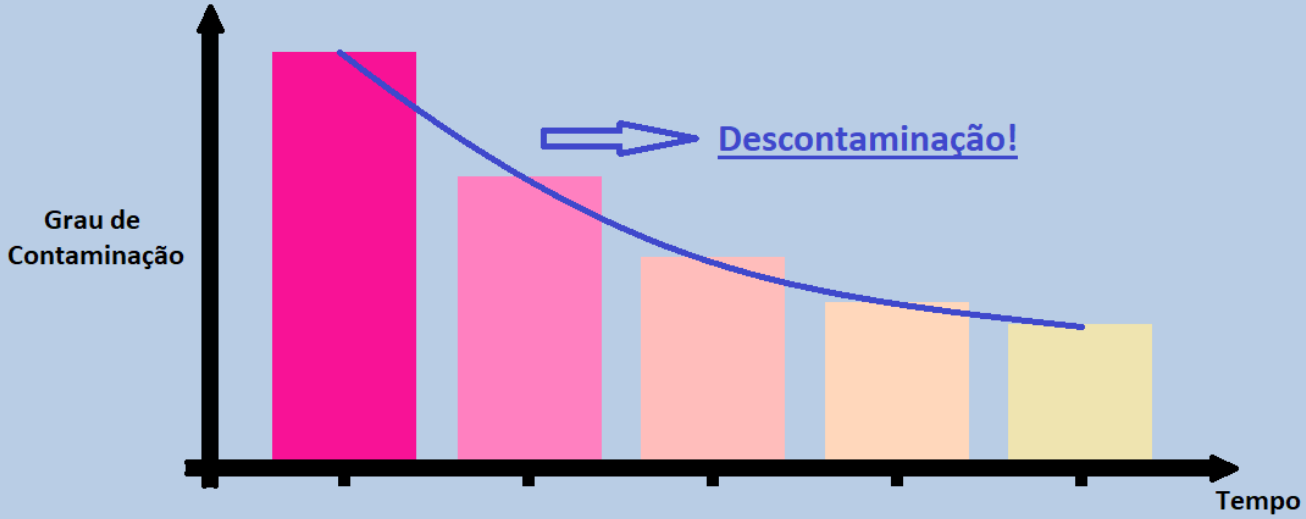


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# PDT and UVC – lung graft decontamination

nature COMMUNICATIONS

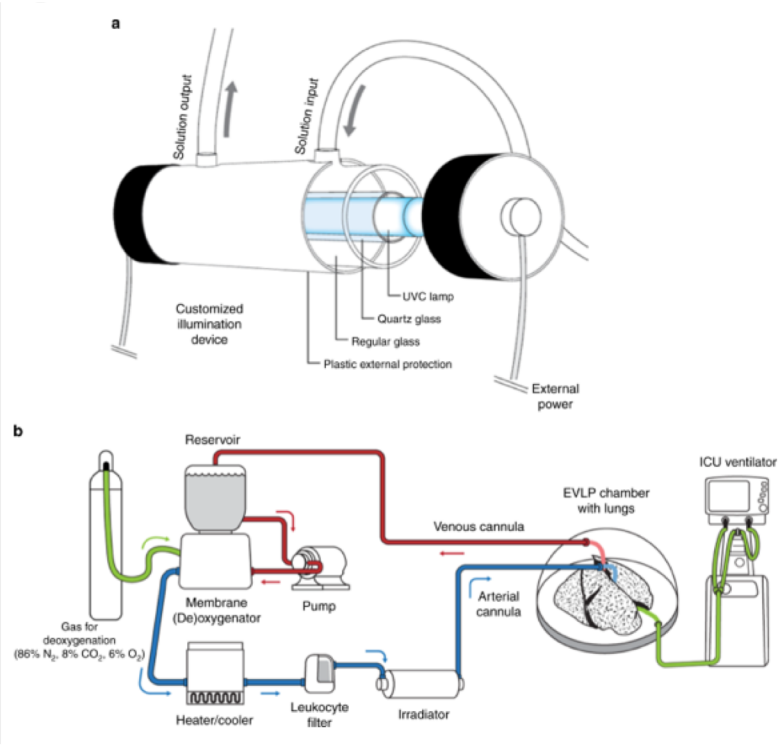
ARTICLE

https://doi.org/10.1038/s41467-018-08261-z OPEN

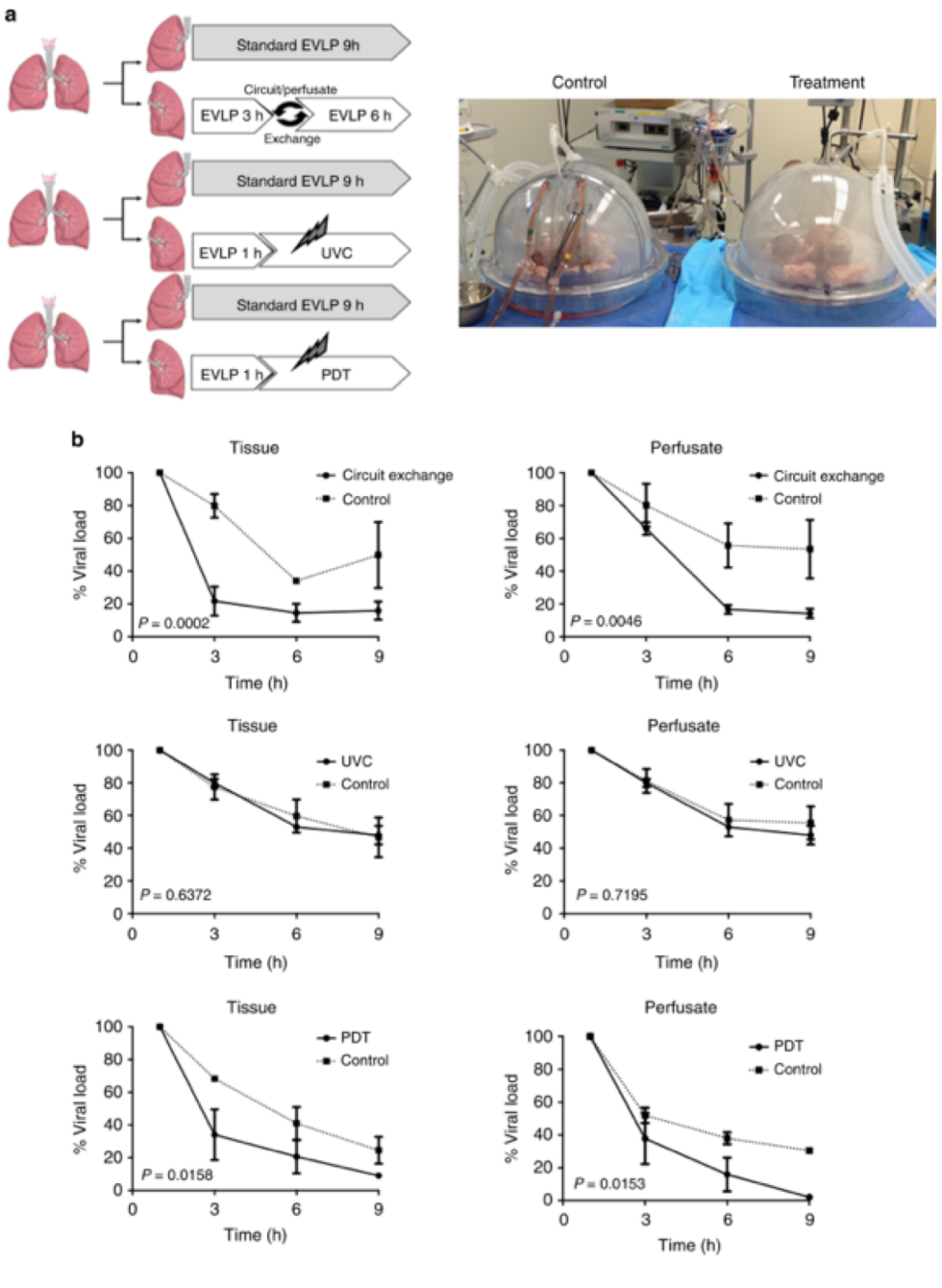
## Inactivating hepatitis C virus in donor lungs using light therapies during normothermic ex vivo lung perfusion

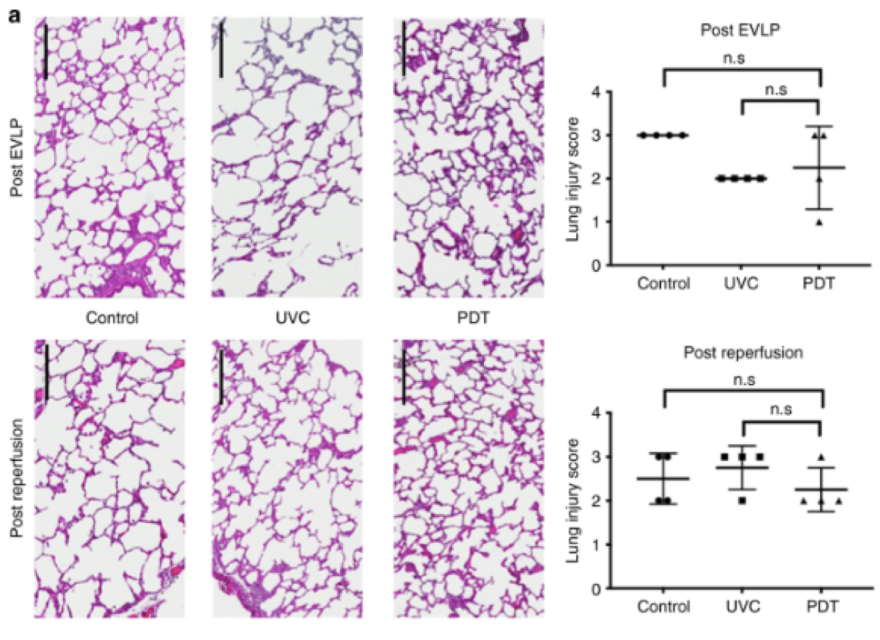
Marcos Galasso[1], Jordan J. Feld[2], Yui Watanabe[1], Mauricio Pipkin[1], Cara Summers[1], Aadil Ali[1], Robert Qaqish[1], Manyin Chen[1], Rafaela V.P. Ribeiro[1], Khaled Ramadan[1], Layla Pires[1], Vanderlei S. Bagnato[3], Cristina Kurachi[3], Vera Cherepanov[2], Gray Moonen[1], Anajara Gazzalle[1], Thomas K. Waddell[1], Mingyao Liu[1], Shaf Keshavjee[1], Brian C. Wilson[4], Atul Humar[5] & Marcelo Cypel[1,5]

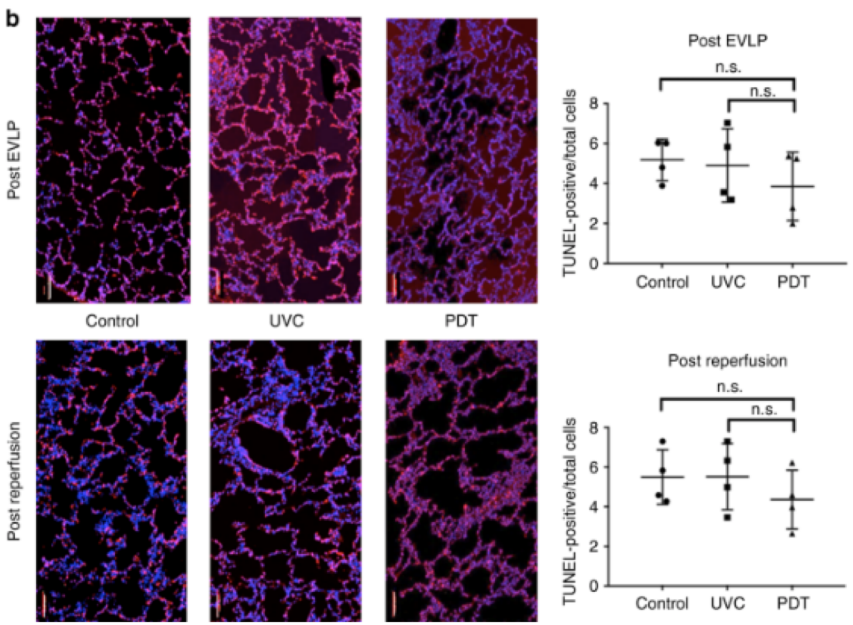
Availability of organs is a limiting factor for lung transplantation, leading to substantial mortality rates on the wait list. Use of organs from donors with transmissible viral infections, such as hepatitis C virus (HCV), would increase organ donation, but these organs are generally not offered for transplantation due to a high risk of transmission. Here, we develop a method for treatment of HCV-infected human donor lungs that prevents HCV transmission. Physical viral clearance in combination with germicidal light-based therapies during normothermic ex-vivo Lung Perfusion (EVLP), a method for assessment and treatment of injured donor lungs, inactivates HCV virus in a short period of time. Such treatment is shown to be safe using a large animal EVLP-to-lung transplantation model. This strategy of treating viral infection in a donor organ during preservation could significantly increase the availability of organs for transplantation and encourages further clinical development.



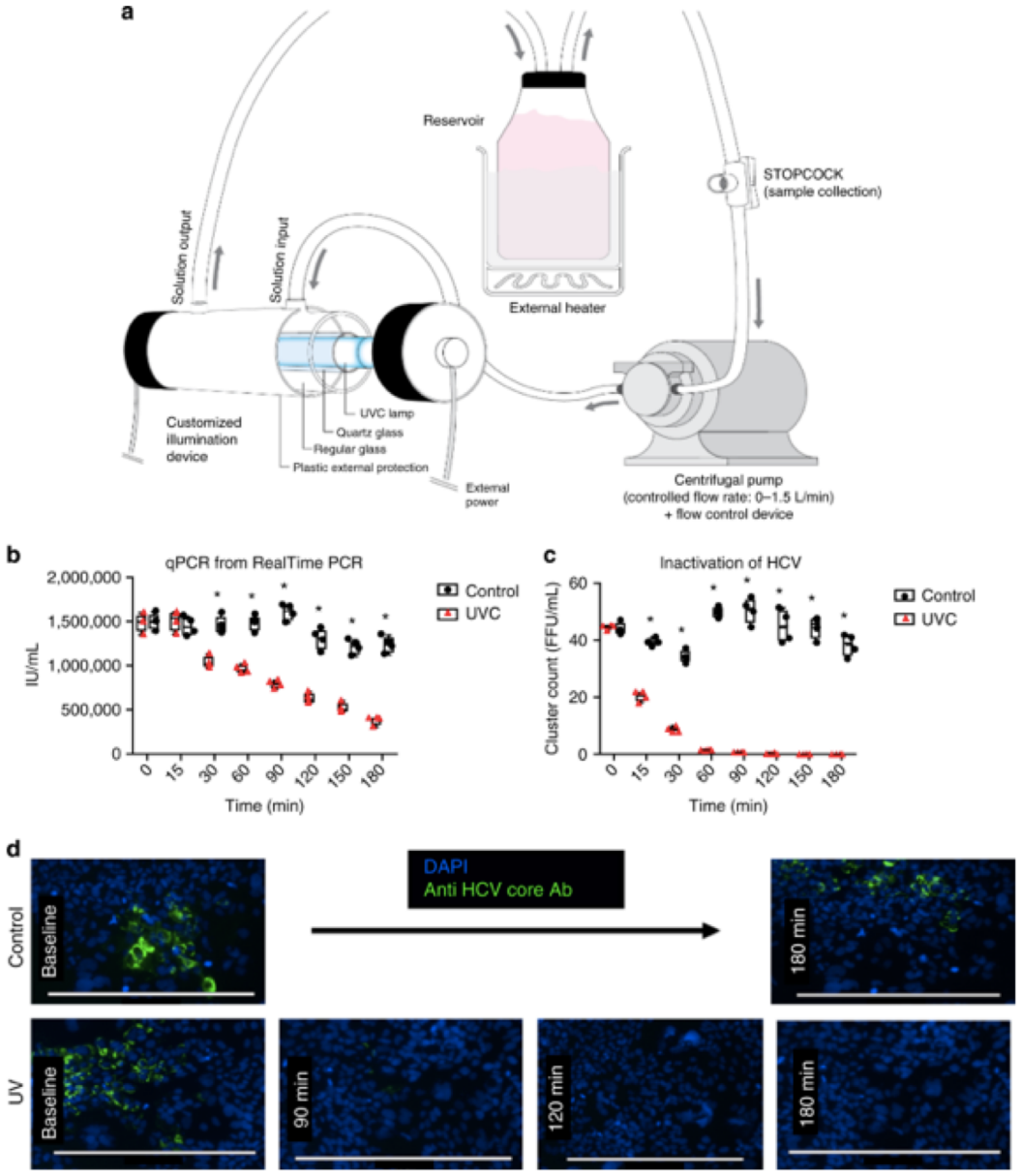

M Galasso et al., *Nat Commun*, 2019

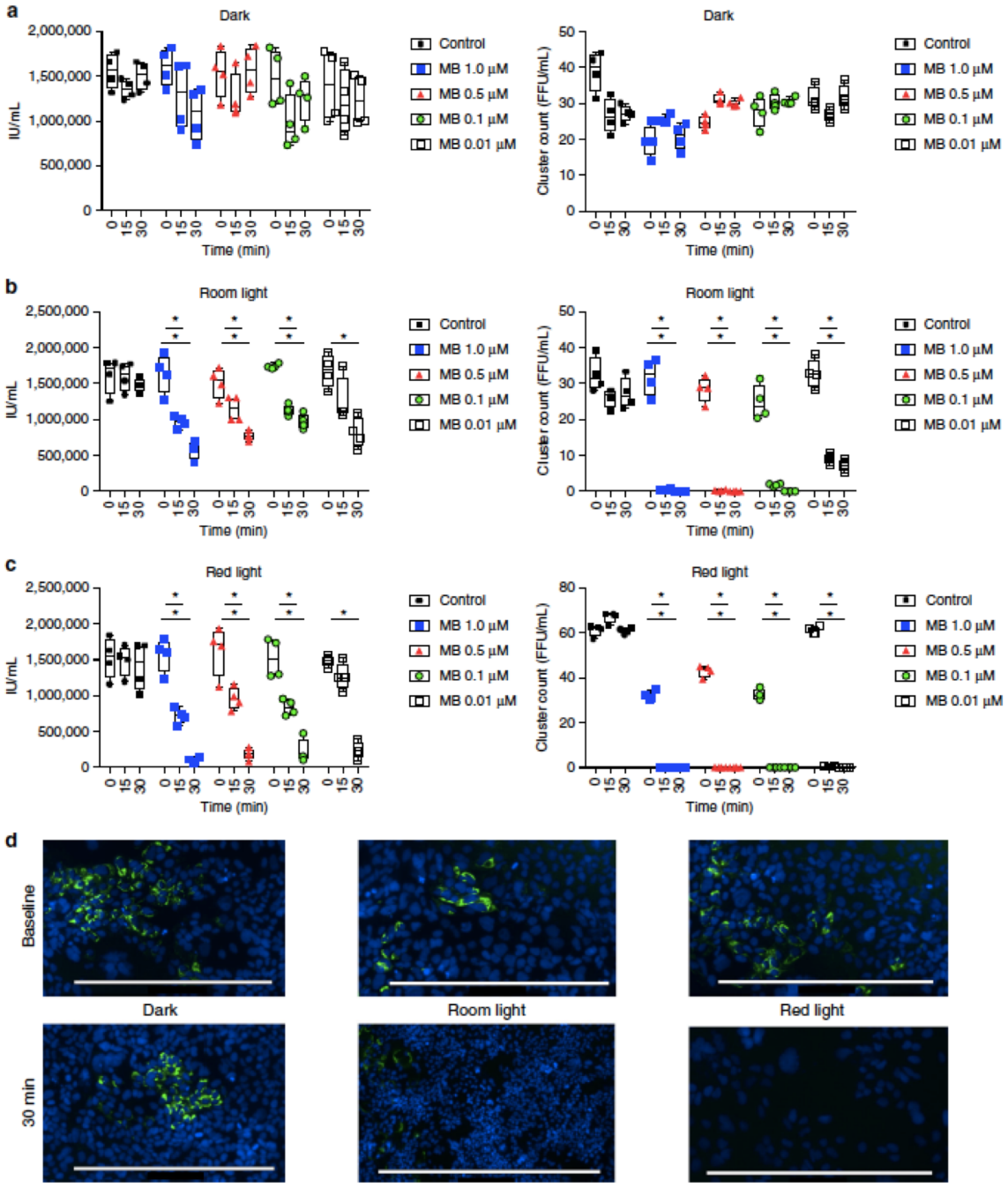

a
Standard EVLP 9h
Circuit/perfusate
EVLP 3 h
EVLP 6 h
Exchange
Standard EVLP 9 h
EVLP 1 h
UVC
Standard EVLP 9 h
EVLP 1 h
PDT
Control
Treatment
b
Tissue
Circuit exchange
Control
% Viral load
P = 0.0002
Time (h)
Perfusate
Circuit exchange
Control
% Viral load
P = 0.0046
Time (h)
Tissue
UVC
Control
% Viral load
P = 0.6372
Time (h)
Perfusate
UVC
Control
% Viral load
P = 0.7195
Time (h)
Tissue
PDT
Control
% Viral load
P = 0.0158
Time (h)
Perfusate
PDT
Control
% Viral load
P = 0.0153
Time (h)




Pre-clinical large animal safety studies using EVLP/LbT treatments. **a** Lung injury score after transplantation, scale bar = 100 $\mu$m. **b** Cell death assessment (TUNEL) after transplantation, scale bar = 400um (N.S. after one-way ANOVA statistical analysis). MB: methylene blue; CIT: cold ischemia time; PDT: photodynamic therapy; Ultraviolet C (UVC) irradiation; EVLP: ex vivo lung perfusion; LbT: Light based therapy. Error bar indicates standard deviation


a
Reservoir
STOPCOCK
(sample collection)
Solution output
Solution input
External heater
UVC lamp
Quartz glass
Regular glass
Plastic external protection
Customized
illumination
device
External
power
Centrifugal pump
(controlled flow rate: 0–1.5 L/min)
+ flow control device
b
qPCR from RealTime PCR
IU/mL
2,000,000
1,500,000
1,000,000
500,000
0
0 15 30 60 90 120 150 180
Time (min)
Control
UVC
c
Inactivation of HCV
Cluster count (FFU/mL)
60
40
20
0
0 15 30 60 90 120 150 180
Time (min)
Control
UVC
d
DAPI
Anti HCV core Ab
Control
Baseline
180 min
UV
Baseline
90 min
120 min
180 min


a
Dark
IU/mL
2,000,000
1,500,000
1,000,000
500,000
0
Time (min)
0 15 30
Control
MB 1.0 μM
MB 0.5 μM
MB 0.1 μM
MB 0.01 μM
Cluster count (FFU/mL)
50
40
30
20
10
b
Room light
2,500,000
c
Red light
80
60
d
Baseline
30 min
Dark
Room light
Red light

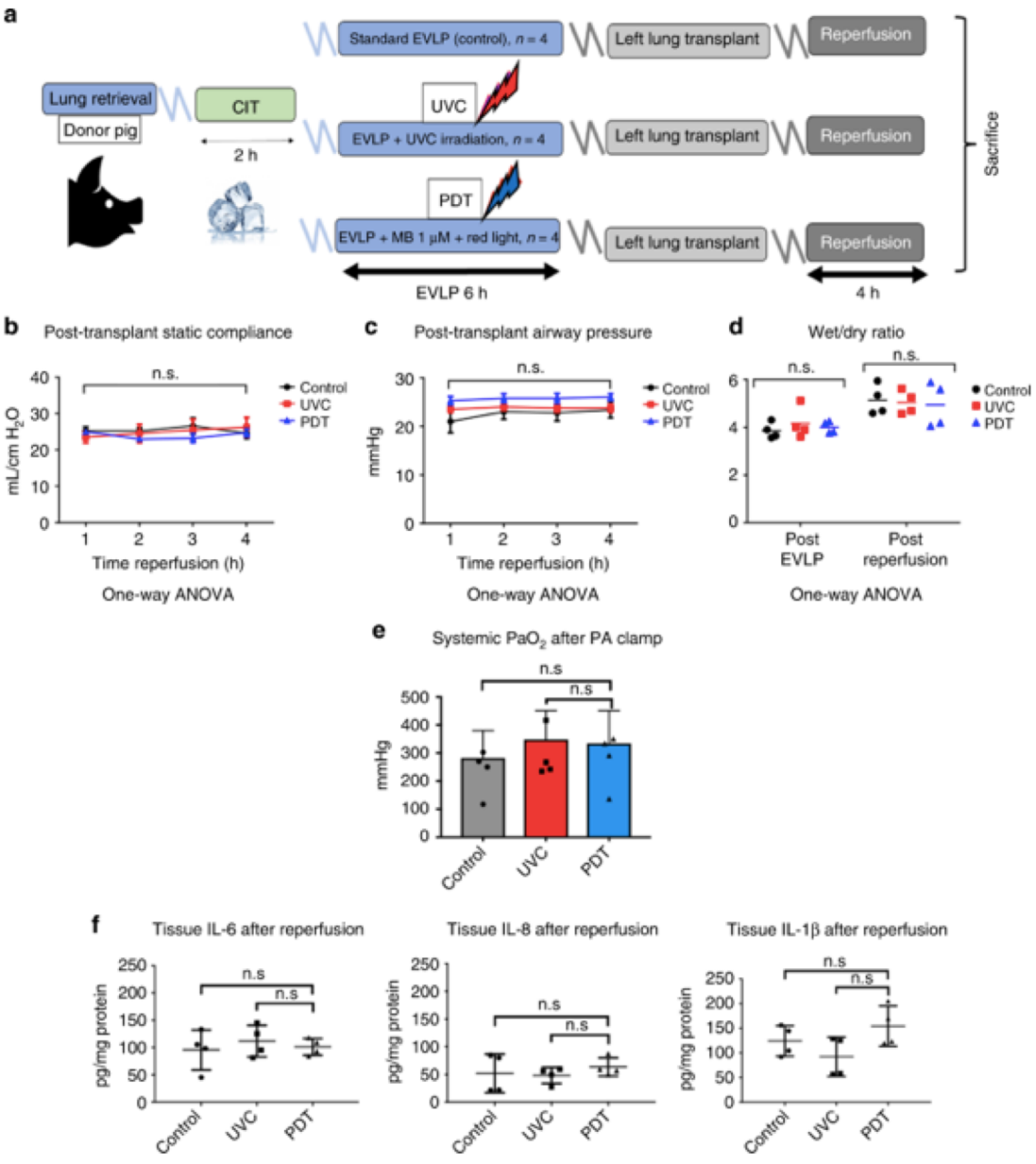
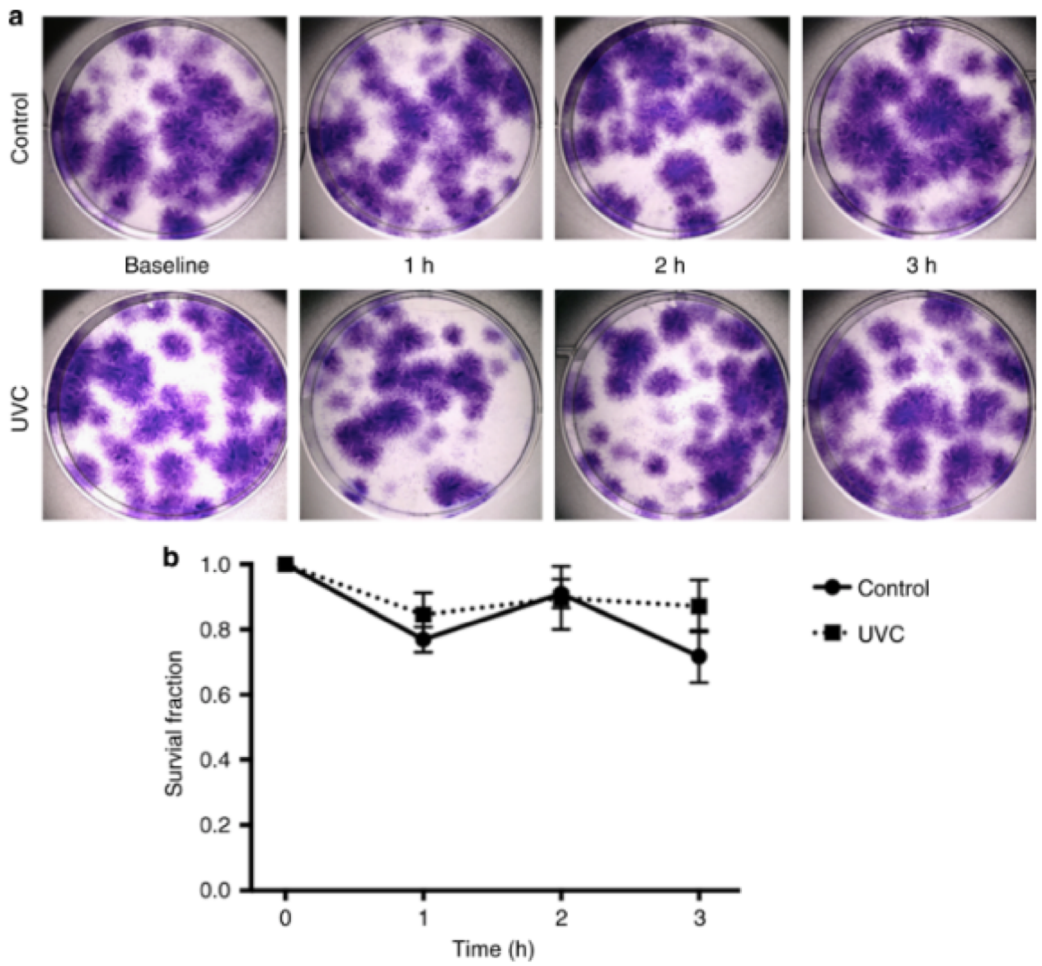



**Fig. 5** Pre-clinical large animal safety studies using EVLP/LbT treatments. **a** Schematic of a pre-clinical EVLP and lung transplantation model, designed to assess potential acute lung injury in donor lungs after LbT applied during EVLP ($n = 4$, each group): (1) Control (standard EVLP technique); (2) UVC (254 nm; 31 mW/cm²); (3) PDT, using 1µmol/L MB diluted in the perfusion solution associated with red light irradiation (660 nm; 20 mW/cm²). **b**–**e** Lung function parameters after left lung transplantation (N.S. after one-way ANOVA statistical analysis). **f** Graft inflammatory cytokine assessment in lung tissue after transplantation (N.S. after one-way ANOVA statistical analysis. Error bar indicate standard deviation)




Clonogenic cell assay performed in six-well plates, with clones produced by LL 24 ATCC ® CCL-151™ human fibroblasts. Steen was previously irradiated for 3 h while circulating in the mini-circuit and samples were taking hourly. **a** Cells were cultured for 12 days in a mixture of fresh Steen and media (control, upper images) and in a mixture of UVC-treated Steen and media (UVC, bottom images). No cytotoxic effect of the UVC was seen. **b** Survival fraction curves of LL 24 ATCC ® CCL-151™ human fibroblasts ($n = 3$ replicates). The survival curves

# Prevention of viral transmission during lung transplantation with hepatitis C-viraemic donors: an open-label, single-centre, pilot trial

*Marcelo Cypel*, Jordan J Feld*, Marcos Galasso, Rafaela V Pinto Ribeiro, Nikki Marks, Magdalena Kuczynski, Deepali Kumar, Ilona Bahinskaya, Vanderlei S Bagnato, Cristina Kurachi, Arthur S Slutsky, Jonathan C Yeung, Laura Donahoe, Marc de Perrot, Kazuhiro Yasufuku, Andrew Pierre, Matthew Binnie, Cecilia Chaparro, Tereza Martinu, Manyin Chen, Jussi Tikkanen, Chung-Wai Chow, Aman Sidhu, Thomas K Waddell, Shaf Keshavjee, Lianne G Singer, Atul Humar*

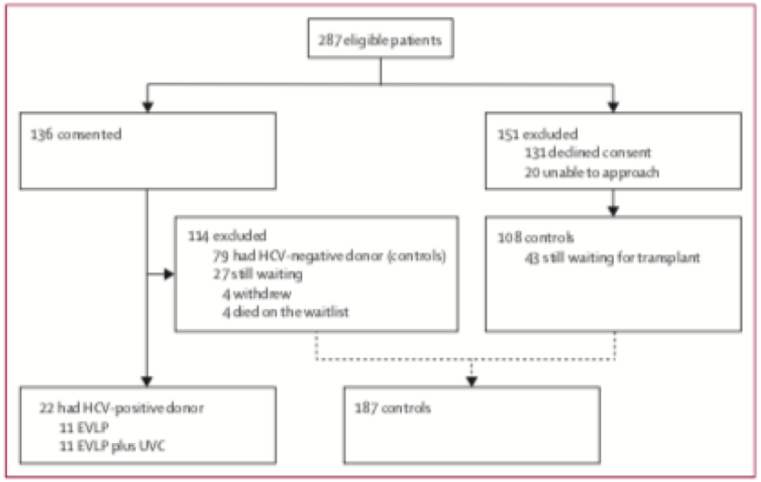



***Figure 1:*** **Trial profile**
EVLP=ex-vivo lung perfusion. HCV=hepatitis C virus. UVC=ultraviolet C.

| | HCV-negative donors (n=187) | HCV-positive donors (n=22) |
|---|---|---|
| Age, years | 52 (30–65) | 33 (29–37) |
| Donor from the USA | 11 (6%) | 16 (73%) |
| P/F, mm Hg | 424 (366–489) | 383 (315–495) |
| Donor was a smoker | 88 (47%) | 20 (90%) |
| Donor had a cardiac death | 50 (27%) | 2 (9%) |
| Ex-vivo lung perfusion | 65 (35%) | 22 (100%) |

Data are median (IQR) or n (%). P/F=partial pressure of oxygen/fraction of inspired oxygen.

***Table 1:*** **Donor characteristics**

| | HCV-negative recipients (n=187) | HCV-positive recipients (n=22) |
|---|---|---|
| Age, years | 60 (47–67) | 65 (60–68) |
| Reason for transplant | | |
| Interstitial lung disease | 108 (58%) | 13 (59%) |
| Chronic obstructive pulmonary disease | 47 (25%) | 7 (31%) |
| Cystic fibrosis | 24 (13%) | 2 (9%) |
| Status 2–3 | 125 (67%) | 11 (50%) |
| Positive crossmatch | 26 (14%) | 4 (18%) |
| Single lung | 30 (16%) | 11 (50%) |
| Waitlist* time, days | 42 (13–169) | 17 (13–133) |

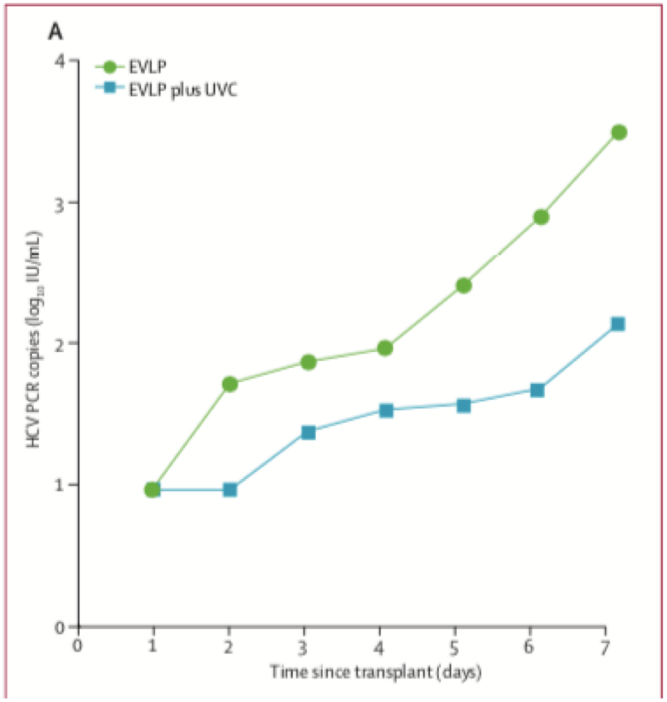
Data are median (IQR) or n (%). *Canadian waitlist status: status 1, patients stable on low concentration of oxygen; status 2, patients requiring high oxygen concentrations or pulmonary hypertension or both; status 3, patients with a rapid decline in lung function, generally hospitalised on ward or in intensive care.

***Table 2:*** **Recipient characteristics**

day-1 viral load, e is the natural logarithm, x is time variable, and a is the slope of the curve when plotted on a logarithmic axis. We assigned viral loads that were less than the limit of quantitation to a nominal value of 1·0 $\log_{10}$ IU/mL. We calculated viral load doubling time for each patient using the equation (ln2)/a. We compared doubling times between groups using the Mann–Whitney *U* test. All statistical analyses were done with GraphPad Prism 8 or R 3.5.0, with summary statistics within groups presented as median values with IQRs (for numerical data) or counts and percentages (for categorical data).

M Cypel et al., *Lancet Resp Med*, 2019

# PDT and UVC – lung graft decontamination



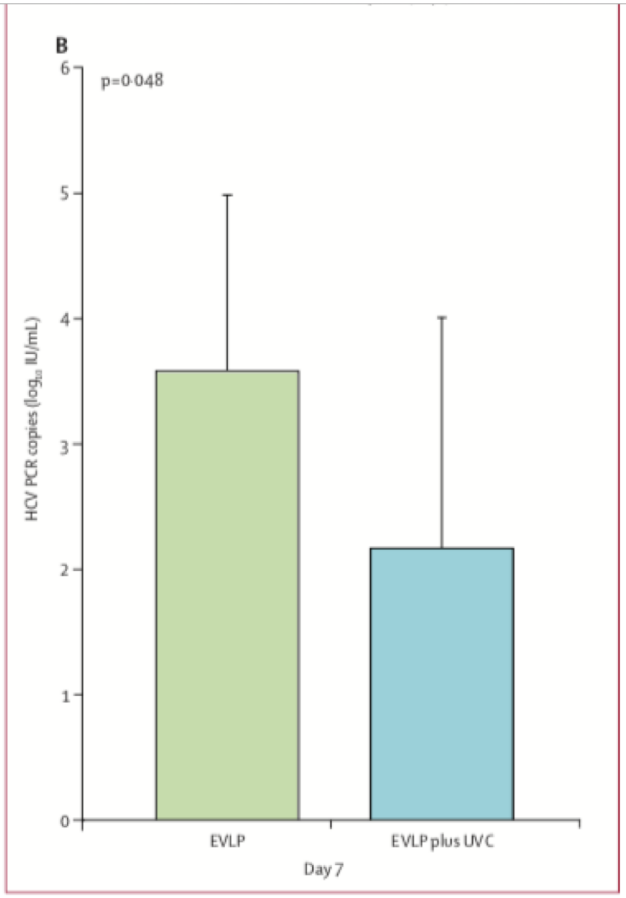
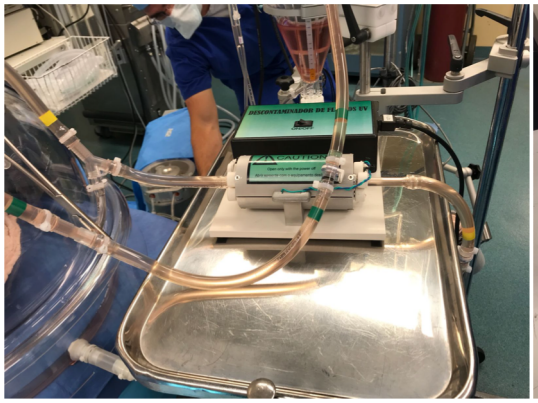
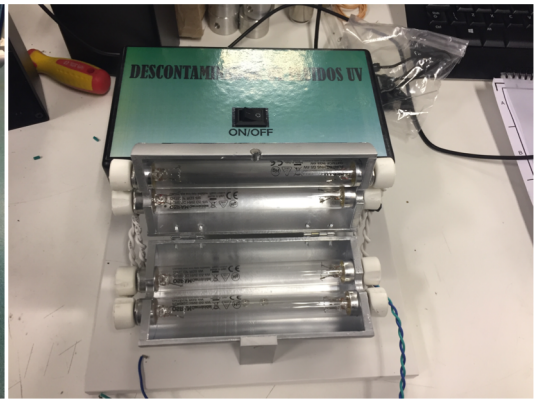
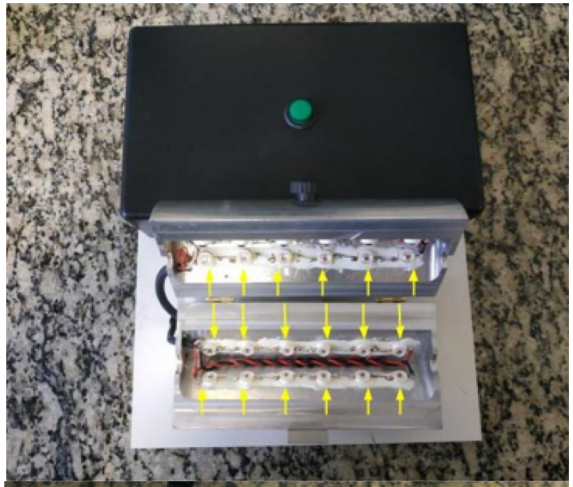



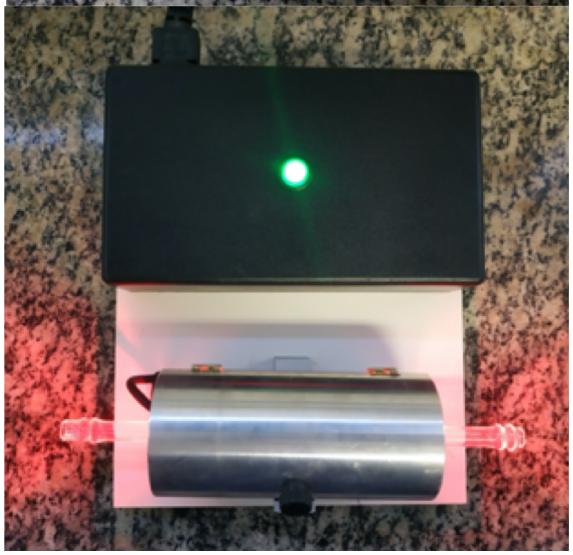

*Figure 4:* **Serum viral load in the first week and at day 7**
Medians presented with IQRs. (A) Kinetics of HCV viraemia in first week after transplantation in recipients receiving HCV-positive donor lungs. (B) Recipient median viral load at day 7 after transplantation in EVLP or EVLP plus UVC groups. EVLP=ex-vivo lung perfusion. HCV=hepatitis C virus. UVC=ultraviolet C.

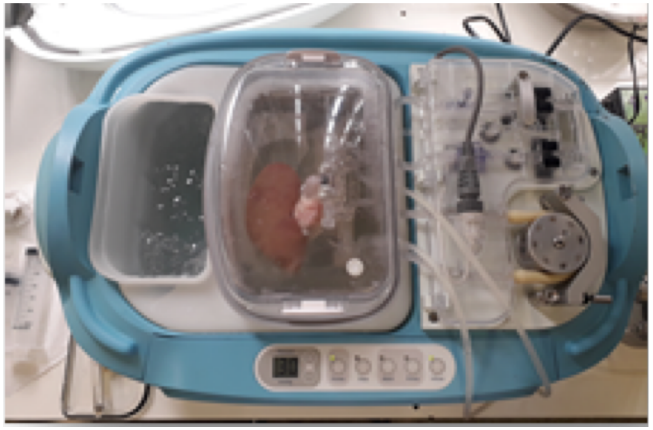




# UVC and Photodynamic Inactivation of *S aureus*

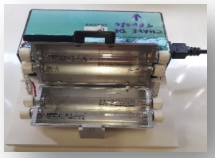




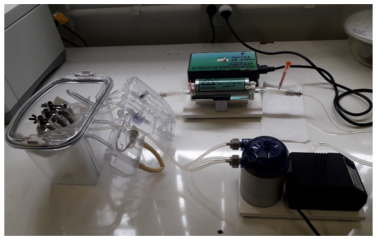


# Delineamento experimental

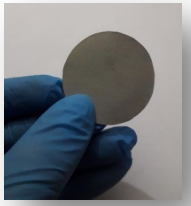
Cassete



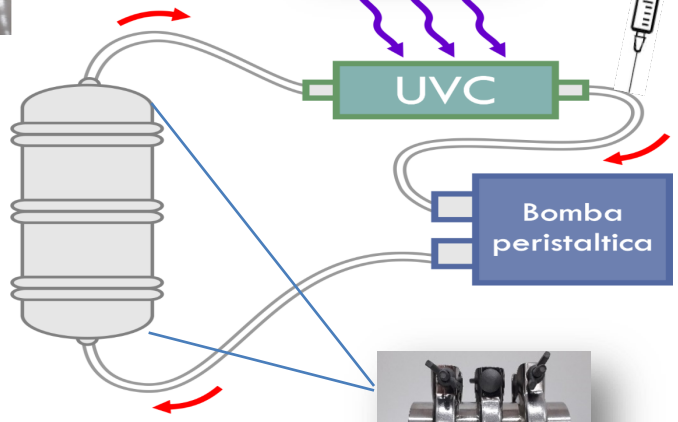
Fonte de irradiação UVC



Sistema de perfusão com módulo de irradiação



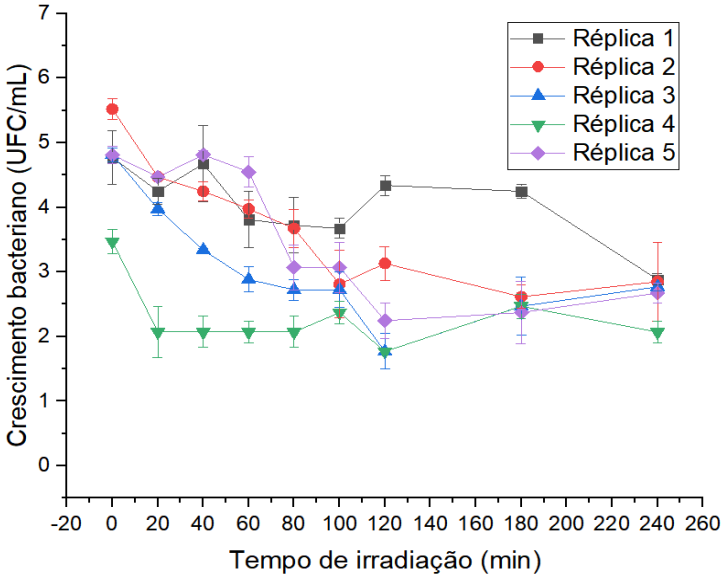
Membrana com poros de 5 µm



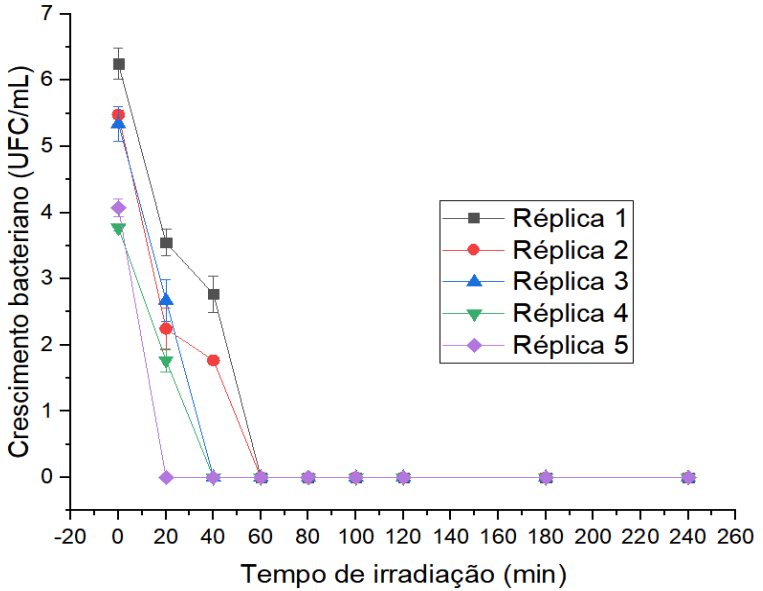

Câmara de filtragem

Bomba peristáltica

# Crescimento microbiano após dos testes (grupo controle e irradiado)



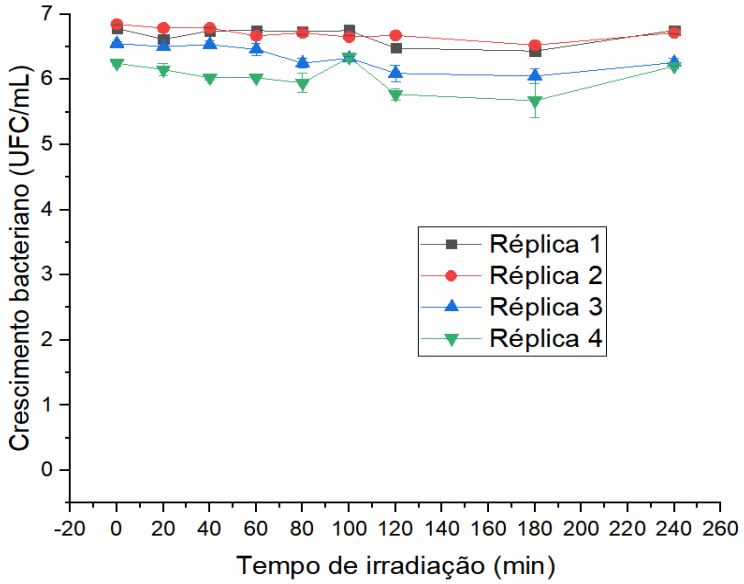

**Controle (sem irradiação)**



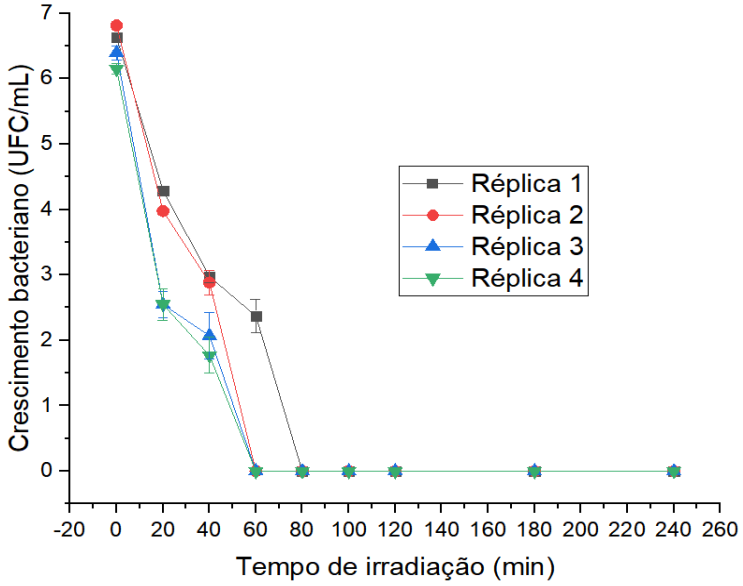

**Inativação UVC**

Yordania Matos Gámez

➢ **Testes usando a câmara para filtragem (esfregaço das colônias de bactérias)**




**Controle (sem irradiação)**




**Inativação UVC**

Quantidade média de UFC/mL do remanescente bacteriano nas membranas

| Testes | Media log 10 UFC (UFC/mL) | | |
|---|---|---|---|
| | $M_1$ | $M_2$ | $M_3$ |
| **Sem irradiação** | 3,53 ± 0,07 | 3,66 ± 0,02 | 7,10 ± 0,04 |
| **Com irradiação** | 2,9 ± 0,2 | 3,0 ± 0,1 | 6,50 ± 0,08 |