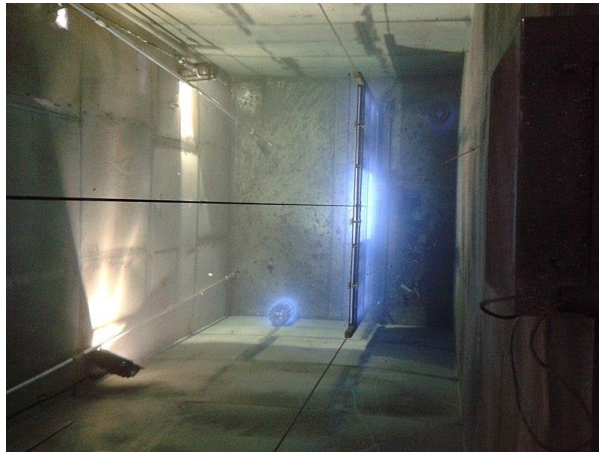


Microbiological aspects of the process of radiation sterilization with the emphasis of virus sterilization possibility

Sylwester Sommer,
Institute of Nuclear Chemistry and Technology, Warsaw, Poland



Swimmaaj Wiki

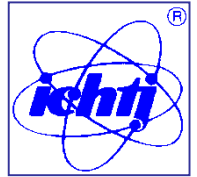


Introduction of lecturer



- Dr Sylwester Sommer, the Head of the Laboratory of Biological Dosimetry, Centre of Radiobiology, INCT is a specialist in radiobiology, biological dosimetry and radiation protection.
- Dr. Sommer is an lecturer in the field of nuclear energy and radiation protection.
- He gives lectures on radiobiology at Medical Physics Department of University of Warsaw.
- Dr Sommer is an author or co-author of about 30 scientific publications.

Main ideas



- Radiation sterilization is used to kill or deactivate microorganisms;
- Most of microorganisms (but not prions) contain genome consist of nucleic acid, as higher organisms;
- Ionizing radiation destroys DNA and this is the way for killing microorganisms.

Range of ionising radiation doses

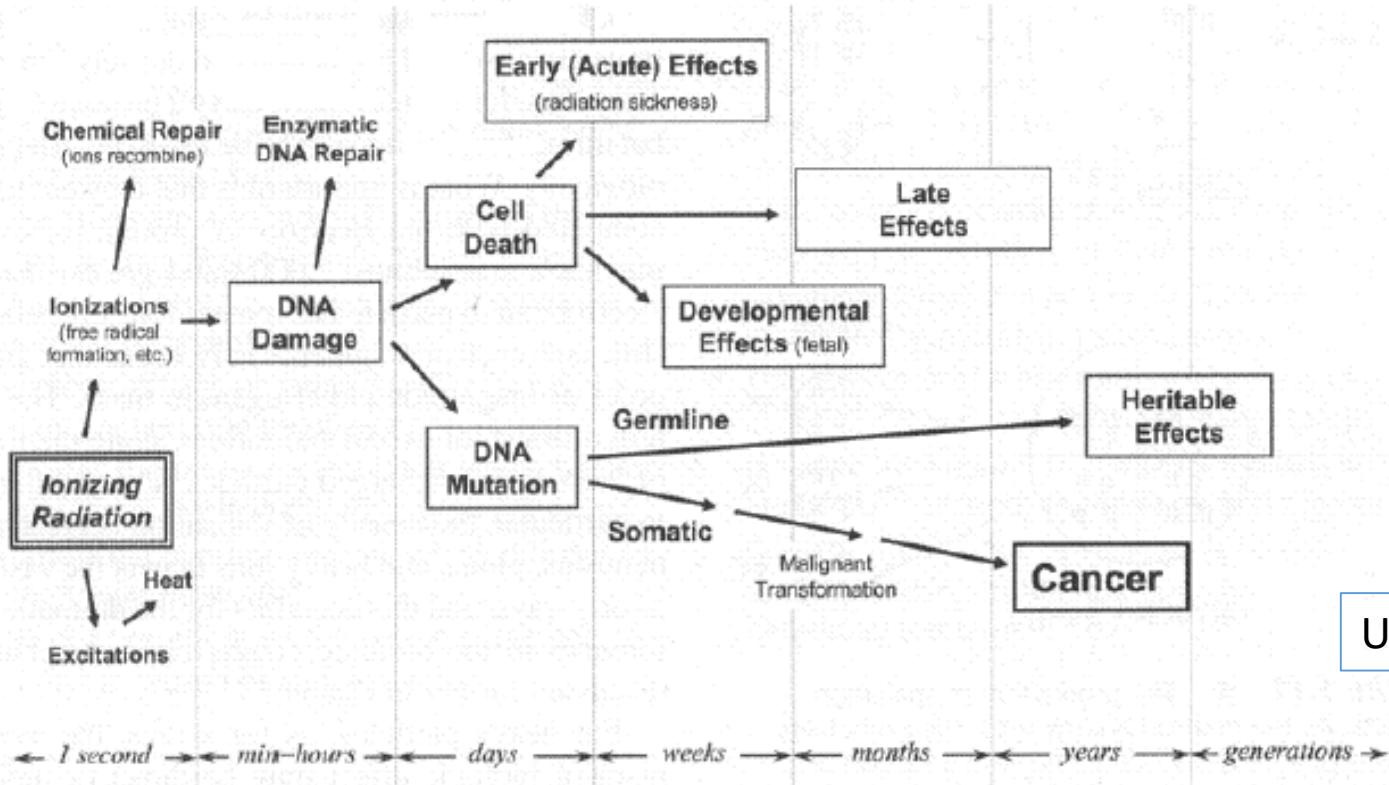


	Dose / LD _{30/50} / Gy (Sv)
Annual dose from diagnostic X-ray	0.00004 / year
Annual dose from cosmic radiation	0.0003 / year
Average Chernobyl casualties dose	0.0010
Annual dose from radon	0.0016 / year
Annual average dose in Poland	0.0035 / year
Human LD _{30/50}	4-5
Bat LD _{30/50}	150
Fly LD _{30/50}	800
Paramecium LD _{30/50}	3000
Deinococcus radiodurans LD _{30/50}	12000
Doses used in radiation sterilization	10000 - 30000

What are the effects of irradiation?

Development of radiobiological damage

Classic Paradigm of Radiation Injury



Utoledo.edu

Radiation risk - humans



- ❖ LNT hypothesis: even the lowest dose of radiation can cause cancer;
- ❖ 1 mSv – the annual dose limit for general public;
- ❖ 3.4 mSv – the annual dose for general public in Poland;
- ❖ 20 mSv – the annual dose limit for occupationally exposed workers;
- ❖ < 100 mSv – the low dose of radiation according the UNSCEAR;
- ❖ ≈ 100 mSv – first deterministic effect – temporary infertility of man;
- ❖ $\approx 1000 - 8000$ mSv – Acute Radiation Syndrome;
- ❖ $\approx 4000 - 5000$ mSv - Human $LD_{30/50}$;
- ❖ 10.000.000 – 30.000.000 mSv – radiation sterilization.

Genetic (stochastic) effects (cancer) versus Acute Radiation Syndrome (deterministic effects)



Genetic (stochastic) effects of radiation: mutations, aberrations are the beginning of cancer.



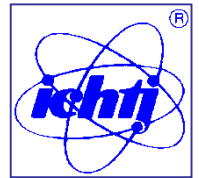
Radiation burn. LK Wagner, PhD; Vlietstra et a, Wiki



Stochastic effects: Mitosis, chromosome aberrations: dicentric and acentric fragment visible.

Cells dying in the tissue (different ways of cells death), what lead to the its malfunctioning and Acute Radiation Syndrome.

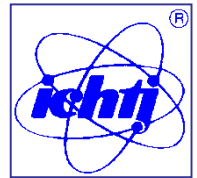
Radioresistance



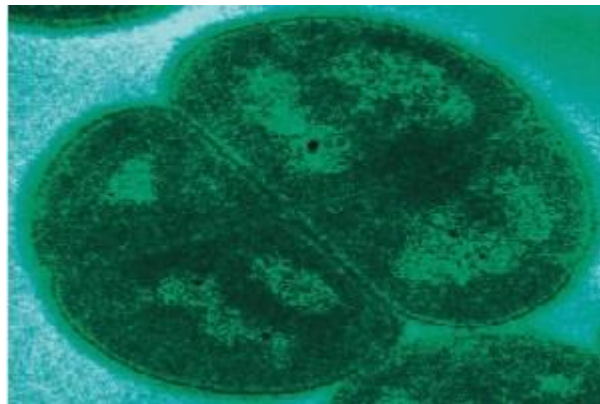
	D ₁₀ (kGy)
Humans	0.007– 0.01
Bats	0.15
Molds	0.03 – 0.5
Escherichia coli	0.25
Vegetative form of bacteria	1-2
Bacterial spores	3-7
Viruses	5-9
Deinococcus radiodurans	10-12

D₁₀ – dose killing 90 % of individuals;

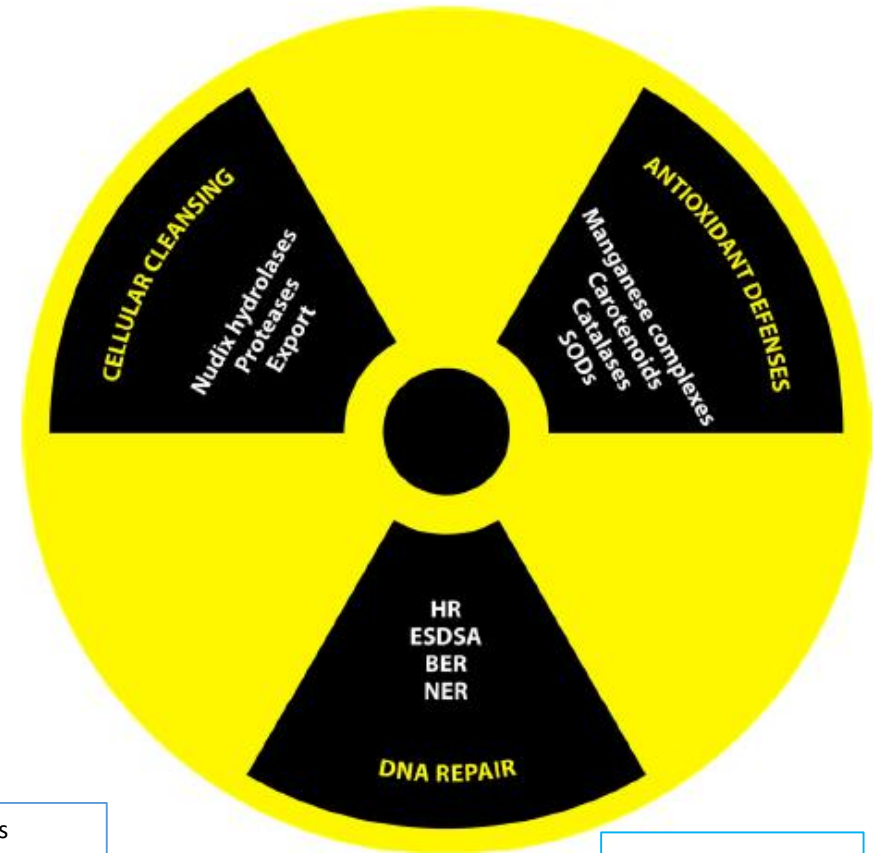
Deinococcus radiodurans



- *D. radiodurans* is the most extreme in terms of radiation resistance;
- *D. radiodurans* is the flagship organism to investigate radioresistance, there is around 870 publications about it in Pubmed.



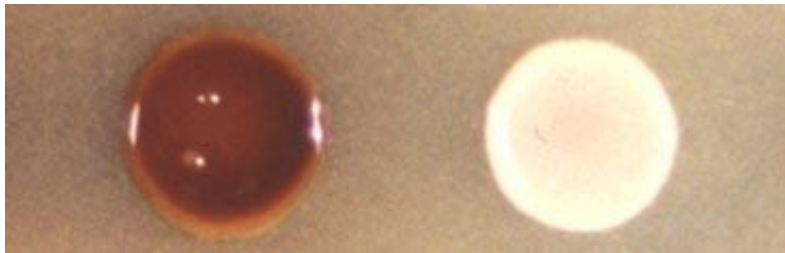
D. radiodurans acquired in the laboratory of Michael Daly, Uniformed Services University, Bethesda, MD, USA. Wiki



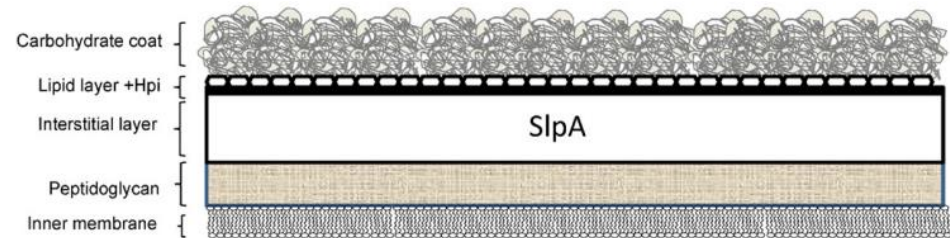
Slade 2011

Physical protection from radiation

- "Protective colors", e.g. melanin - soil fungi can have two forms with melanin, they are radio-resistant and more sensitive without it (Ruegsegger 2010);



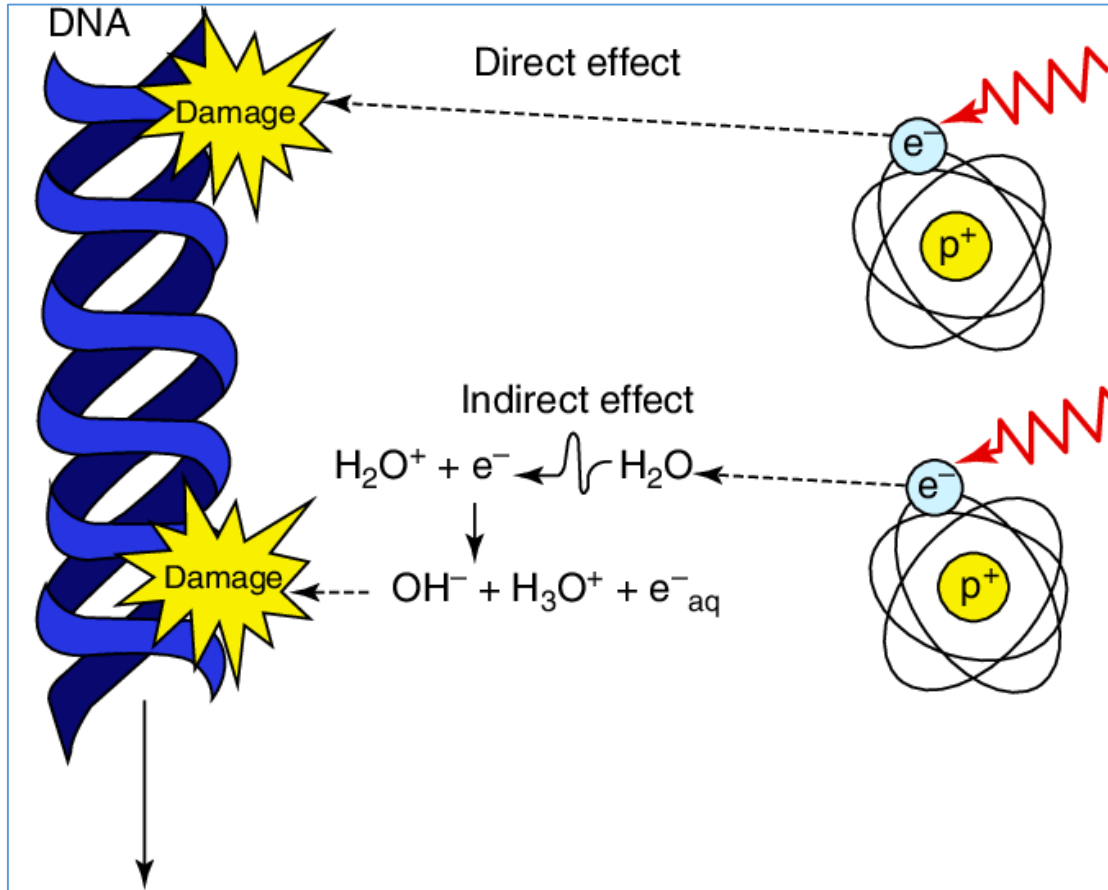
Ruegsegger 2010



Apte 2015

"Protective layers" - the most radiation-resistant bacteria *Deinococcus radiodurans* - apart from the cell wall and plasma membrane, has five other layers outside the cell membrane (Apte 2015);

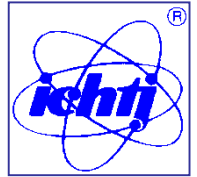
DNA damage by ionising radiation



Moreels 2020

Sparsely ionising radiation:
 Direct action = 20 %;
 Indirect action = 80 %.

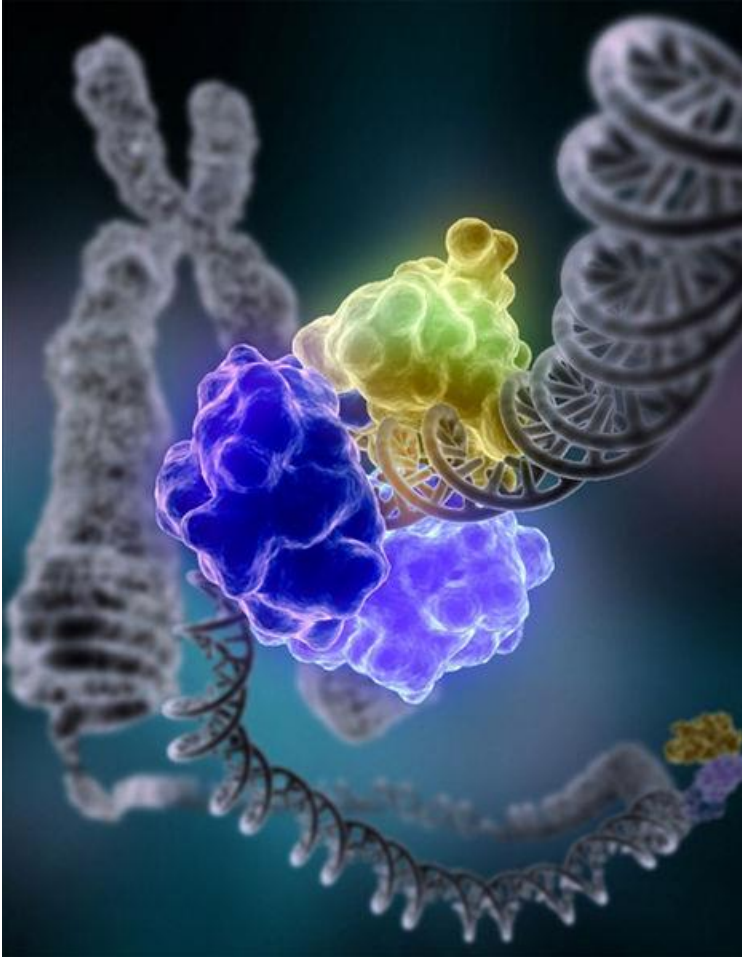
Chemical protection from radiation – antioxidant defense



Directed mainly against free radicals, reactive oxygen species:

- Enzymatic free radical scavengers: glutathione, superoxide dismutase or catalases;
- Nonenzymatic antioxidants: carotenoids, vitamins A, E, selenium, flavonoids and polyphenols, metal complexes (e.g. manganese complexes in *D. radiodurans*);
- Nonenzymatic antioxidants are available for us in food: vegetables, whole grains and legumes, nuts and spices, coffee, tea, wine;
- Free radicals level is dependent on oxygen and water condition. Therefore spores are more resistant than vegetative forms. Viruses are not the cells.

DNA damage and repair



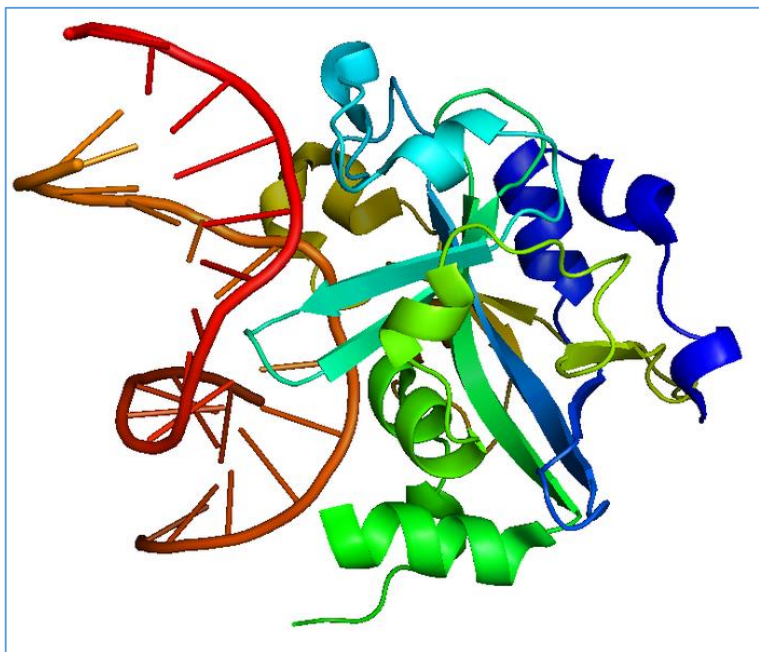
Tom Ellenberger Wiki 2016

Ionising radiation cause variety of DNA damages:

- base damage;
- nucleotide damage;
- single strand breaks;
- double strand breaks;
- complex damage.

DNA damages are effectively repaired by highly complex repair systems, which are evolutionary conserved from bacteria to mammals.

DNA repair



BQUB15-Arafi, Wiki

DNA damage is repaired by specialized repair systems.

These DNA repair systems are highly conserved during evolution, very similar at bacteria, yeast and mammalian cells.

unrepaired

genomic instability

cell death

DNA damage

misrepaired

DNA mutation

cancer

repaired

Last level of defence: tolerance for genomic instability and cellular cleansing



Microorganisms better tolerate genomic instability than higher organisms cells.

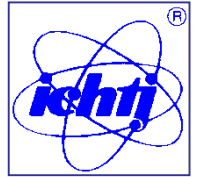
There are several explanation of this fact:

microorganisms cannot be directed for the pathway of programmed death;

microorganisms usually have many copies of each gen.

Microorganisms usually have developed the way for effective cellular cleansing: nucleases and proteases for e.g. badly folded proteins, peroxidated lipids and the way to get rid of these proteins and lipids.

Effective radiation sterilization



Usual dose between 10 and 30 kGy of electrons, X-rays or gamma radiation.

Successful sterilization depends on dose, oxygen conditions, temperature, water content of microorganisms, level of microorganism contamination, microorganism radiation resistance.

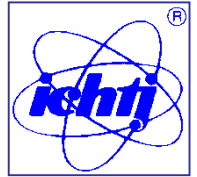
You cannot increase the dose because of the price, possibility to destroy object during the process or excite some elements in the object.

TABLE 8.1. REFERENCE MICROBIAL RESISTANCE DISTRIBUTION
USED IN METHOD 1 OF ISO 11137:1995 [8.8]

D_{10} (kGy)	1.0	1.5	2.0	2.5	2.8	3.1	3.4	3.7	4.0	4.2
Probability (%)	65.487	22.493	6.302	3.179	1.213	0.786	0.350	0.111	0.072	0.007

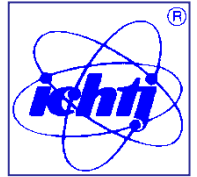
TRENDS IN RADIATION STERILIZATION OF HEALTH CARE PRODUCTS,
IAEA 2008

Effective radiation sterilization



- For each product line the microorganisms viability after irradiation should be checked in reliable microbiological laboratory;
- Validation and standardization processes;
- Following ISO standards;
- QA and QM;
- Technical requirements;

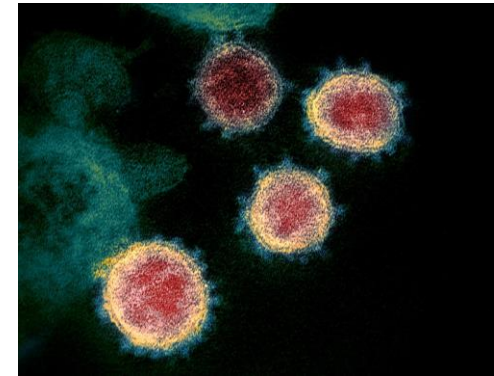
What about viruses?



- Viruses are more radioresistant than bacteria and even than bacteria spores;
- It is not clear how ionising radiation sterilize viruses, probably damages genetic material, protein coat (envelop) and capsid;
- Usually there is no need to use radiation method in purpose to inactivate the viruses, but with progress of Covid-19 pandemic change of stock which is sterilized is obvious from single use medical devices and grafts more to personal protective equipment (PPE);
- In case of viruses 20 kGy is enough for virus inactivation (1) but higher doses are taken into account as well (2).

1. European Centre for Disease Prevention and Control, 2020b. Cloth masks and mask sterilisation as options in case of shortage of surgical masks and respirators. Available on 8th April 2020 at <https://www.ecdc.europa.eu/en/publications-data/clothmasks-sterilisation-options-shortage-surgical-masks-respirators>.

2. COVID-19 Pandemic: Radiation Sterilization of PPE (personal protective equipment). IAEA presentation 2020.



NIAID, Wiki 2020

Covid-19

Change of kind of products which are sterilized from:



Single use
medical
devises and
grafts



<https://www.nursetogether.com/>. 2020, Wiki

[The U.S. Food and Drug Administration](#) 2011, Wiki



Paul Farrant 2008, Wiki



PPE – personal
protective
equipment



CDC Global ,2014, Wiki

Fot. LUVIMINA G. LANUZA PHILIPPINE NUCLEAR RESEARCH INSTITUTE DEPARTMENT OF SCIENCE AND TECHNOLOGY IAEA Webinar: "COVID-19 Pandemic: Radiation Sterilization of PPE"

The activity was sponsored in the frame of the Central European Initiative (CEI) Extraordinary Action 2020

Dozen of studies



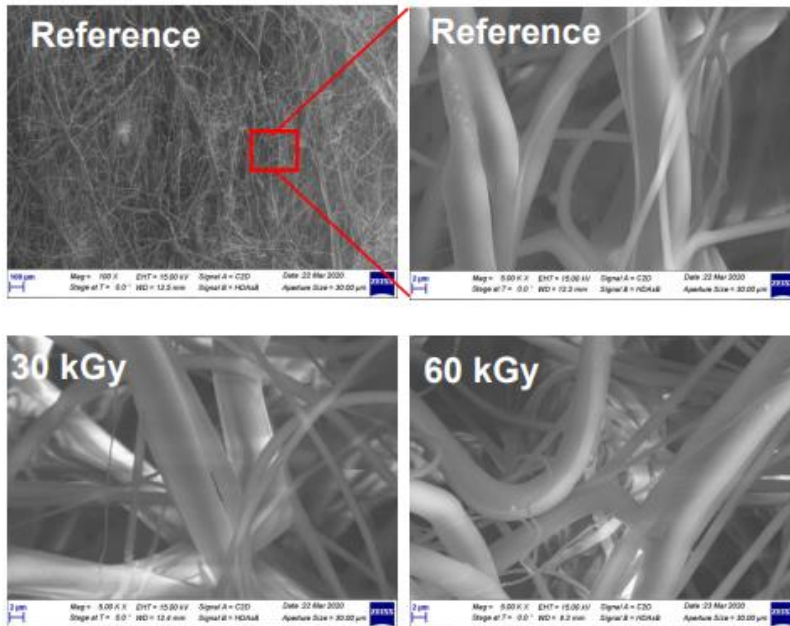
Why? Shortages of PPE.

Investigated characteristics:

- Filtration efficiency;
- Chemical toxicity after irradiation;
- Mechanical degradation;
- Chemical degradation;
- Visual and morphological changes;
- Mask and uniform fitting;
- Chemical structure;



Photo Dr. Javed Anees, Wiki



No structural changes after irradiation;

3M aura 9332+ (FFP3) mask – internal PP layer

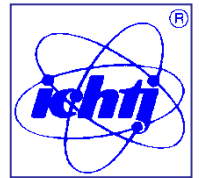
I. GOUZMAN, H. DATZ, R. VERKER, A. BOLKER, L. EPSTEIN, L. BUCHBINDER, Y. FRIED
and E. SARID

Soreq Nuclear Research Center (SNRC), Yavne, Israel

The activity was sponsored in the frame of the Central European Initiative (CEI) Extraordinary Action 2020



Organisations



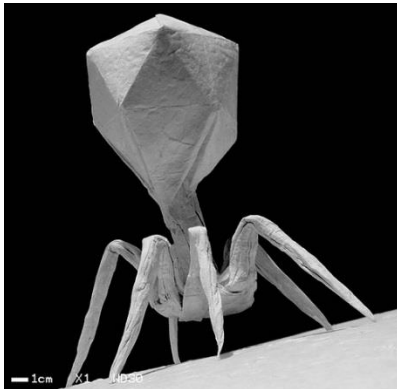
Webinar: COVID-19 Pandemic: Radiation
Sterilization of PPE
(personal protective equipment), May 2020.

Options for the decontamination and reuse of
respirators in the context of the COVID-19
pandemic – Technical report, June 2020.

General conclusions of IAEA Webinar:



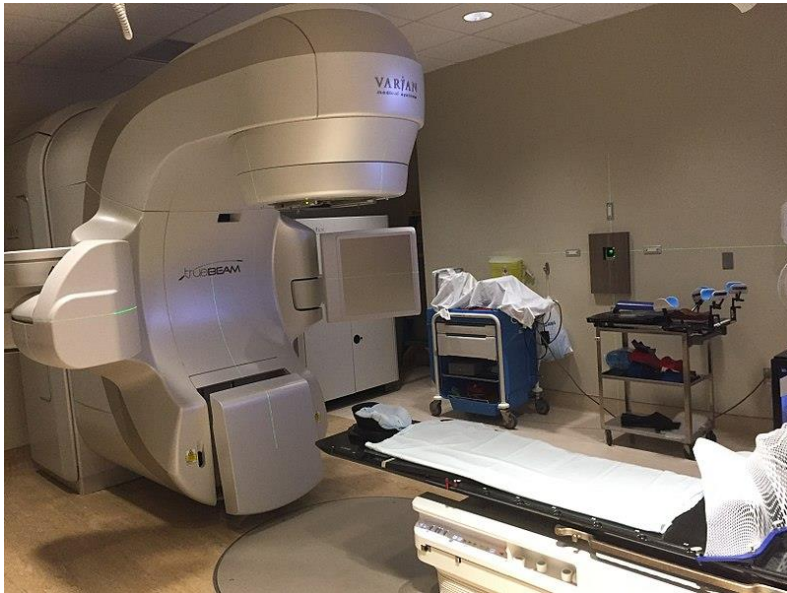
Ionizing radiation is an effective and established tool to sterilize personal protective equipment (PPE) that is in high demand during the COVID-19 pandemic, including surgical and handmade masks. Also seems to be promising for reuse of some items.



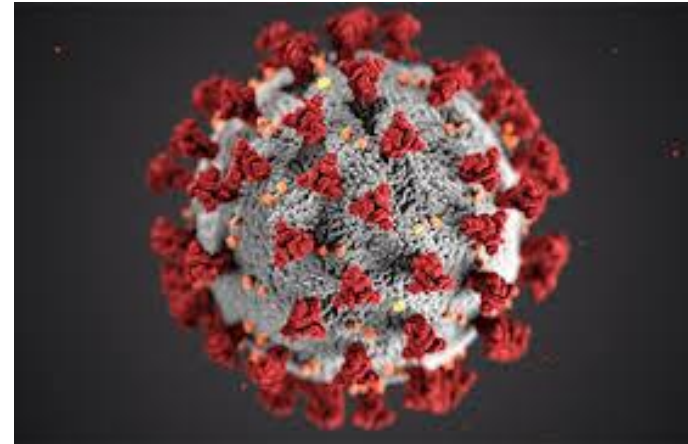
Creation Wiki

Respiratory masks, made of melt-blown polypropylene (PP) nonwoven fabric, used as the filtration layer, are not suitable to be radiation sterilized because it affects filtration performance (tested in gamma and Ebeam, from 1 to 60 kGy, in air and vacuum).

Low dose of radiation chest therapy – is it possible and effective in case of Covid-19?



Michael Goodyear 2017 Wiki



CDC/ Alissa Eckert, MS; Dan Higgins, MAM, 2019 Wiki

Main ideas

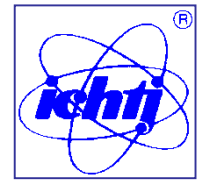


- ✓ Most of the Covid-19 patients suffered of a respiratory disease, and problems begun if it progress to an acute respiratory distress syndrome (ARDS). ARDS is in this case connected with systemic inflammatory response with a Cytokine Release Syndrome (CRS) and that is life threatening.
- ✓ Radiotherapy for pneumonia has been used since the 30' s of the last century with promising results (e.g. Oppenheimer A (1943), Quimby AJ (1916)), even if the mechanisms of such a treatment was not fully understood.
- ✓ Nowadays we understand more the paths how radiation can help for respiratory disease and the treatment seems to be attractive for Covid-19 patients. Some (around 10) preclinical trials have already been performed.

How radiation can impact on immune response for Covid-19 infection

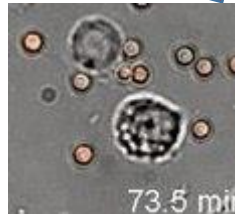


Zarateman. 2014, Wiki

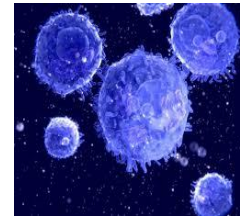


IL-1 β , IL-6 and TNF-alpha

transforms



Wiki



Wiki

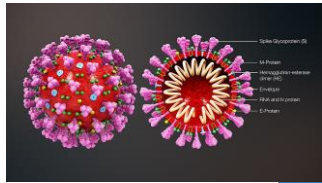


Mikael Häggström, M.D. 2018, Wiki

Covid-19 pneumonia



Toglenn, 2019, Wiki



<https://www.scientificanimations.com>
2020, Wiki

activates

virus Covid-19

macrophages pro-inflammatory phenotype M1

lymphocytes

macrophages anti-inflammatory phenotype M2



Wiki

IL-4, IL-10

Literature



Lara PC, Burgos J, Macias D. Low Dose Lung Radiotherapy for COVID-19 Pneumonia. The Rationale for a Cost-Effective Anti-Inflammatory Treatment. Clin Transl Radiat Oncol. 23: 2020 Apr 25, 27-29. doi: 10.1016/j.ctro.2020.04.006;

Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 395(10229): 2020, 1033–4;

Deloch L, Fuchs J, Rückert M, et al. Low-dose irradiation differentially impacts macrophage phenotype in dependence of fibroblast-like synoviocytes and radiation dose. J Immunol Res. 14: 2019 Aug 3161750;

Kirkby C, Mackenzie M. Is low dose radiation therapy a potential treatment for COVID-19 pneumonia?. Radiother Oncol. 147: 2020, 221. <https://doi.org/10.1016/j.radonc.2020.04.004>;

Pre-clinical trial

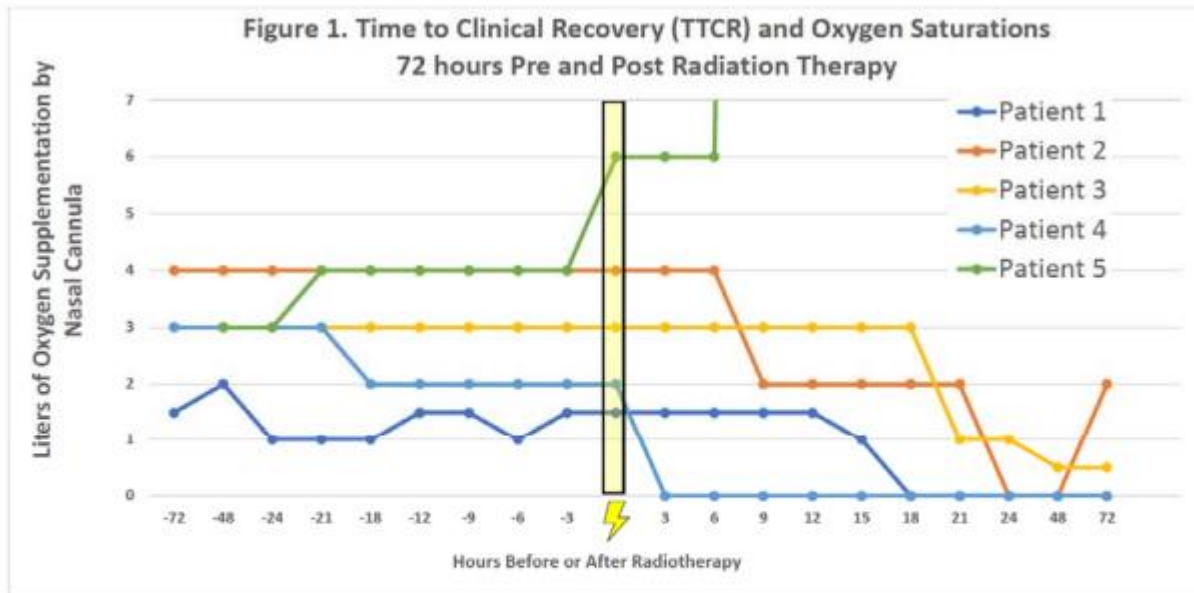


Low-Dose Whole-Lung Radiation for COVID-19 Pneumonia: Planned Day-7 Interim Analysis of an Ongoing Clinical Trial

Clayton B. Hess MD MPH^{1,7}, Zachary S. Buchwald MD PhD^{1,7}, William Stokes MD^{1,7}, Jeffrey M. Switchenko PhD MS^{2,7}, Tahseen H. Nasti PhD³, Brent D. Weinberg MD⁵, James P. Steinberg MD⁴, Karen D. Godette MD^{1,7}, David Murphy MD PhD⁶, Rafi Ahmed PhD^{3,7}, Walter J. Curran Jr MD^{1,7}, Mohammad K. Khan MD PhD^{1,7}.

1. Departments of Radiation Oncology,
2. Biostatistics and Bioinformatics,
3. Microbiology and Immunology,
4. Infectious Disease,
5. Diagnostic Radiology, Pulmonary Critical Care,
6. Winship Cancer Institute,
7. Emory University. Atlanta GA

Pre-clinical trial at Winship Cancer Institute Emory University. Atlanta

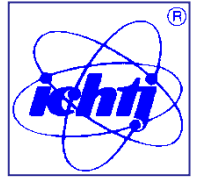


ID	Ordinal Scale for Clinical Improvement by Hospital Day																		TTCR
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Patient 1	5	5	5	5	4	4	4	4	4	3	3	3	3	3	3	3	3	3	18 hours
Patient 2	4	4	4	4	3	3	4	4	3	3	3	3	3	3	2	-	-	-	24 hours
Patient 3	5	5	5	4	4	4	4	4	4	4	3	3	3	3	-	-	-	-	96 hours
Patient 4	4	4	4	4	4	3	3	3	3	3	3	2	-	-	-	-	-	-	3 hours
Patient 5	4	5	5	5	5	5	6	6	6	-	-	-	-	-	-	-	-	-	Ongoing

1=not hospitalized. 2=hospitalized without need for oxygen or medical care. 3=hospitalized without need for oxygen. 4=hospitalized requiring low-flow supplemental oxygen. 5=hospitalized on high-flow oxygen. 6=hospitalized on mechanical ventilation 7=deceased.

Single 1.5 Gy dose for both lungs

Conclusions



- ❖ For today there is 35 mln people infected with Covid-19 and there is over 1 mln fatalities all over the world;
- ❖ There is no vaccination, no clear medication against Covid-19;
- ❖ Respiratory problems are the key point during infection and we know that the immune system plays role;
- ❖ The single dose of radiation helps in control of Covid-19 pneumonia – 10 clinical trials all on the world: USA, Spain, Italy, India and Iran.
- ❖ Low dose of radiation (0.5 – 1.5 Gy) is used for both lungs and there is no adverse symptoms of irradiation visible;
- ❖ Method is cheap, easy and can be applied all over the world.

**Unethical not to Investigate Radiotherapy
for COVID-19 - Jerry M. Cuttler**

