Low-Energy-Electron Irradiation

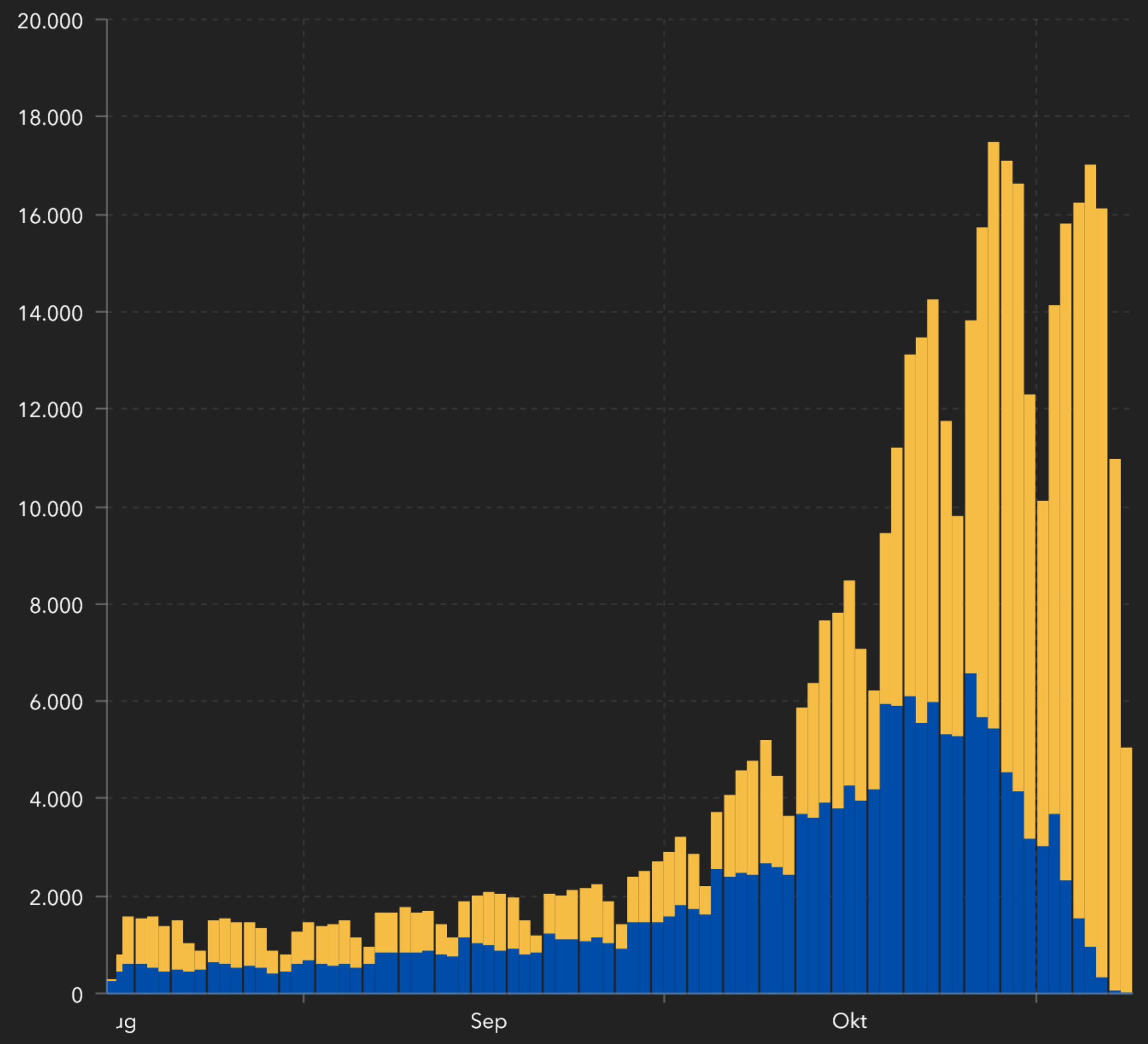
A game changer for inactivation of pathogens

KyooBe Tech GmbH, Leinfelden, Germany

The Importance of Vaccines

- Vaccines have been used for over one century to eradicate some of the most severe diseases in the world

- Currently, COVID-19 ist the best evidence why vaccines have a significant position in worldwide healthcare architecture



Robert-Koch-Institute:
Dashboard on Covid-19 cases / day for Germany

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Bringing Inactivated Vaccine Technology to the Next Level

- KyooBe seeks to revolutionize vaccine manufacturing through advanced technology approaches
- Rapid and safe without toxic components
- Protecting important antigen structures more effectively

Who we are

Interdisziplinary

Close to the Customer

Extraordinary

Innovative

StartUp company formed Dec. 2019 as part of Bausch+Ströbel Group:

BAUSCH+STRÖBEL®

Our Network

Together for the best solutions

Fraunhofer IPA

Fraunhofer IZI

BAUSCH+STRÖBEL®

LEEI Consortium

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Part 1 - Core Technology

The Use of accelerated electrons

Applications and technologies – industrial scale

The technology meets the following criteria:

- LEEI is based on *cathode ray technology* (CRT) which has been used for decades
- Safe application in various business fields, including printing and packaging industry
- Manufacturing set up can be designed as a continuous or batch-driven process

Television

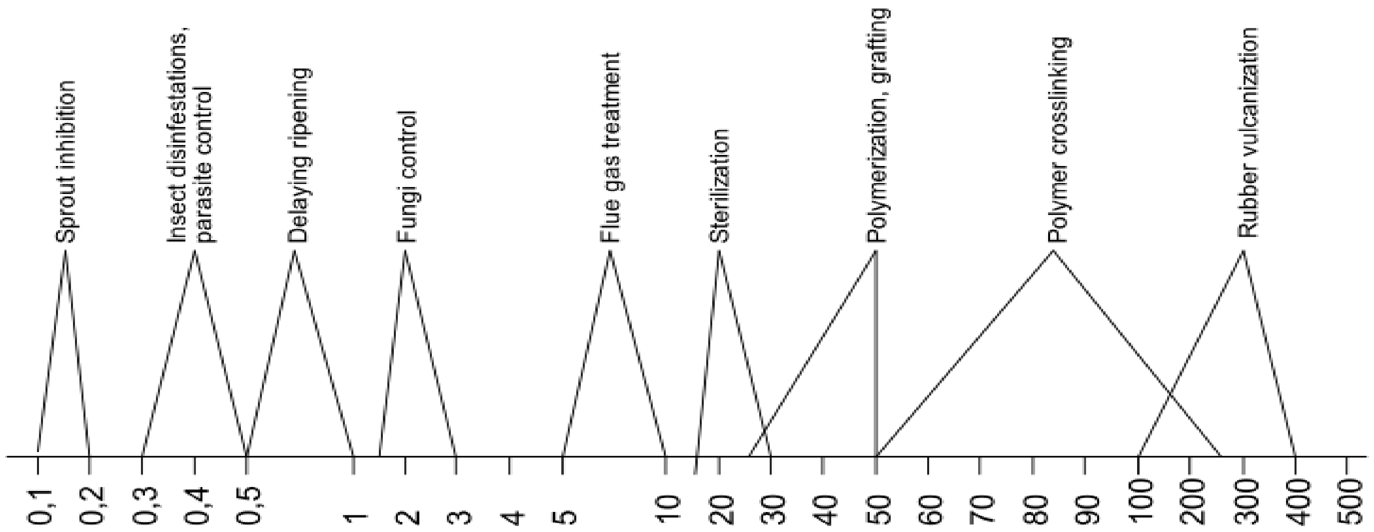
Ink Crosslinking

Packaging / Sterilization

Vaccines
(starting 2023)

The Use of accelerated electrons

Applications and technologies – industrial scale



Low doses
(up to 1 kGy)

Medium doses
(up to 10-30 kGy)

High doses
(over 25 kGy)

How it works

Low-Energy Electron Irradiation (LEEI)

Benchmarking against inactivation techniques and procedures

Inactivation	Chemical *
Process architecture	Complex
Time reuqired	weeks
Safety concerns	Used Chemicals
Process cost	High due to long process times
Impact on pathogen	Severe impact on pathogen's surface structures

*Currently the only way to produce inactivated vaccines

Reference: Fertey, Jasmin; Thoma, Martin; Beckmann, Jana; Bayer, Lea; Finkensieper, Julia; Reißhauer, Susann et al. (2020): Automated application of low energy electron irradiation enables inactivation of pathogen- and cell-containing liquids in biomedical research and production facilities. In Scientific reports 10 (1), p. 12786. DOI: 10.1038/s41598-020-69347-7.

Low-Energy Electron Irradiation (LEEI)

Benchmarking against inactivation techniques and procedures

Inactivation	Chemical *	Gamma
Process architecture	Complex	Complex
Time reuqired	weeks	Hours to days
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Process cost	High due to long process times	High due to complex handling of radioactive material
Impact on pathogen	Severe impact on pathogen's surface structures	Mainly adressing nucleic acids

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Low-Energy Electron Irradiation (LEEI)

Benchmarking against inactivation techniques and procedures

Inactivation	Chemical *	Gamma	UV light
Process architecture	Complex	Complex	Comparatively simple
Time reuqired	weeks	Hours to days	Hours to days
Safety concerns	Used Chemicals	Complex shielding	Insuffifcient shielding
Process cost	High due to long process times	High due to complex handling of radioactive material	Comparatively low
Impact on pathogen	Severe impact on pathogen's surface structures	Mainly adressing nucleic acids	Severe impact on pathogen's surface by photoadducts

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eBeam-based effects: molecular level

(1) Direct action

- deposition of energy by accelerated electron in the target molecule
- eliminating H atom and leading to a radical

(2) Indirect action

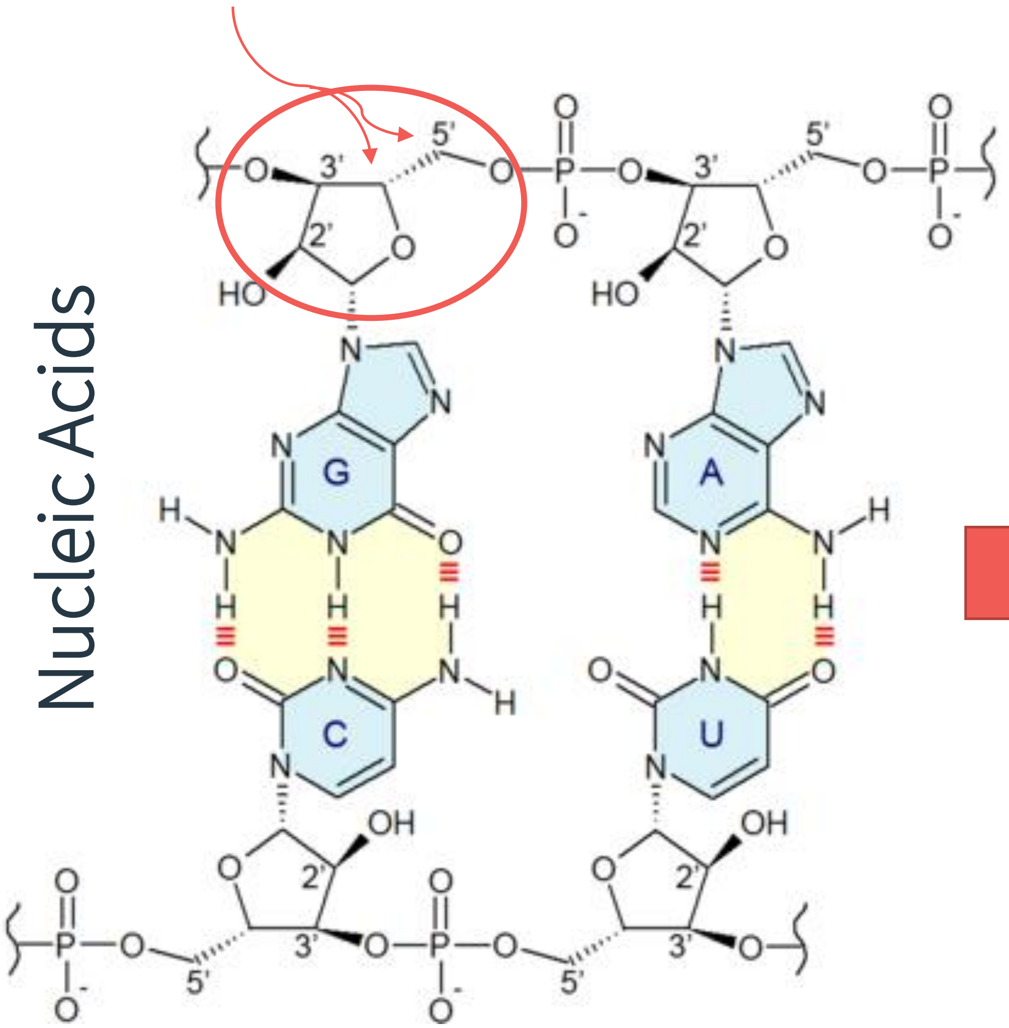
- Reactive species are formed in the surrounding of the target molecule
- OH radicals interact with the target resulting in strand breaks

Reference:

Hutchinson, Franklin (1985): Chemical Changes Induced in DNA by Ionizing Radiation. In Waldo E. Cohn, Kivie Moldave (Eds.): Progress in Nucleic Acid Research and Molecular Biology, vol. 32: Academic Press, pp. 115–154. Available online at http://www.sciencedirect.com/science/article/pii/S0079660308603475.

(1) e^- / (2) HO•

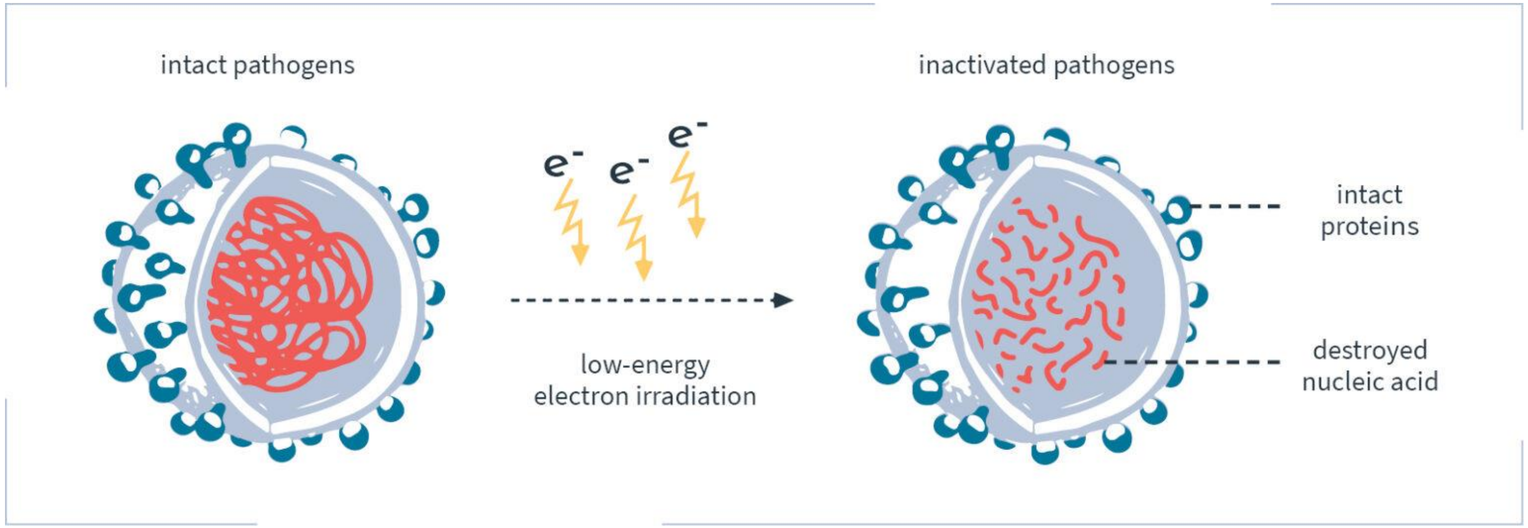
Nucleic Acids



Strand Break

eBeam-based effects: pathogen inactivation

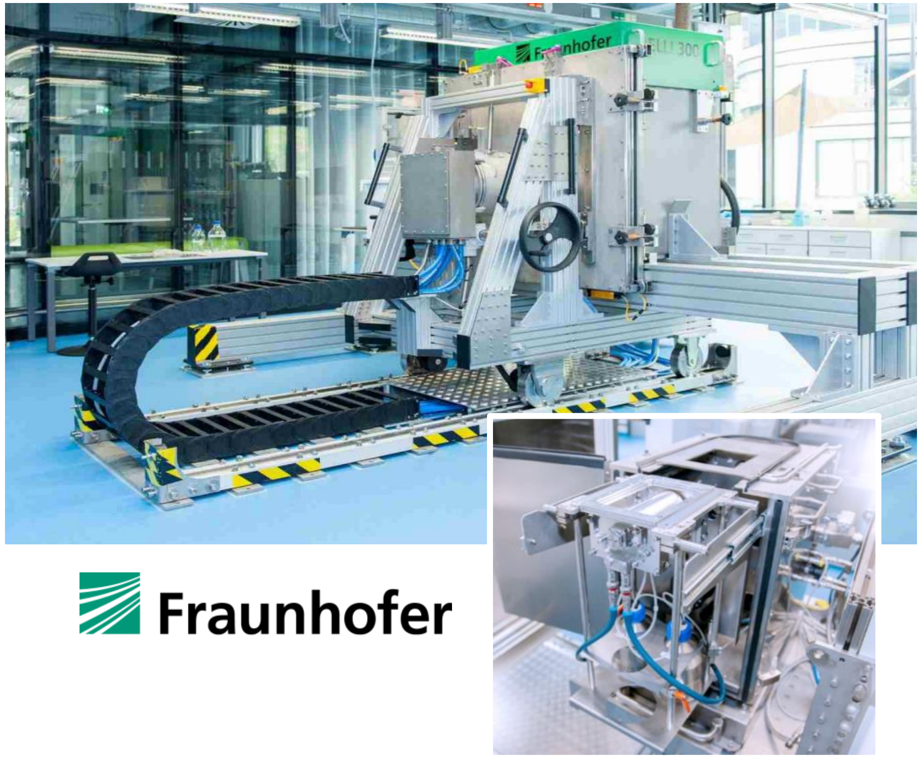
- eBeam mainly addresses large molecules like nucleic acids
- maintaining the antigen structure of the pathogen



KyooBe‘s vision on technology

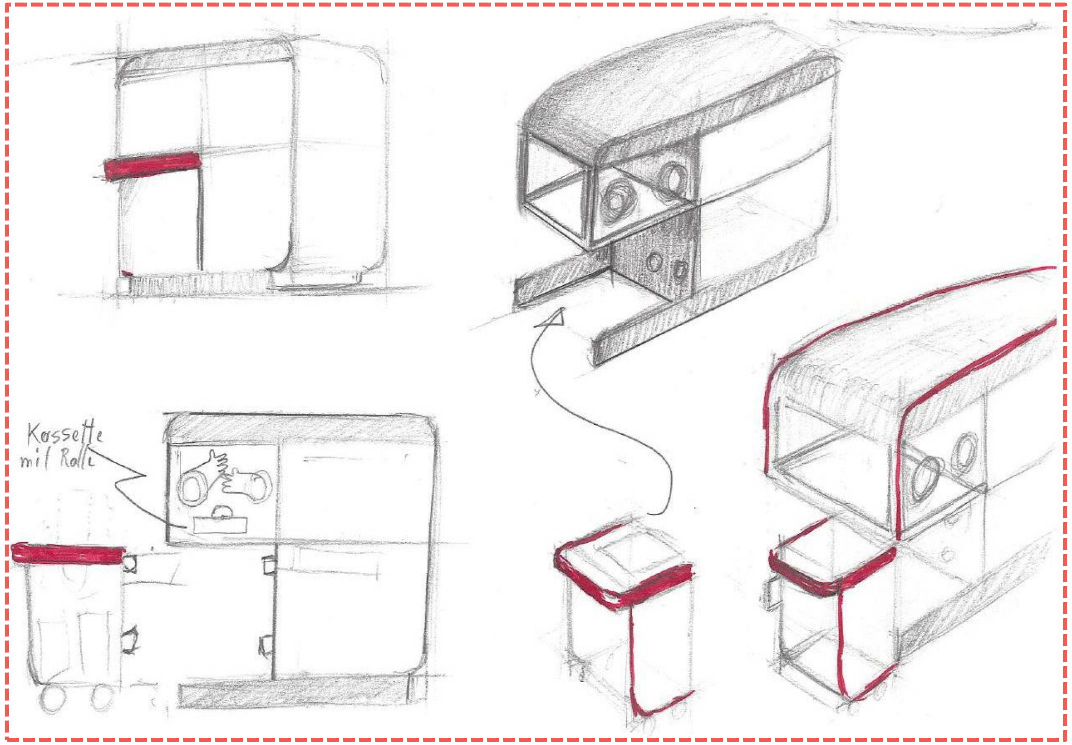
Adaptive inactivation platform for commercial manufacturing

ELLI300 (Fraunhofer IZI)



Fraunhofer

Pathogen Inactivation Platform (PIP) / 2023



KyooBe's vision on technology

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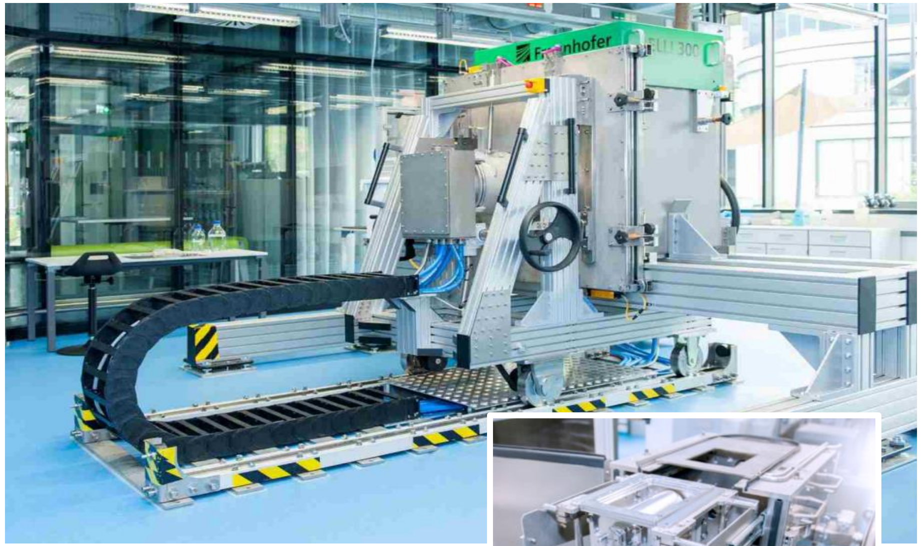
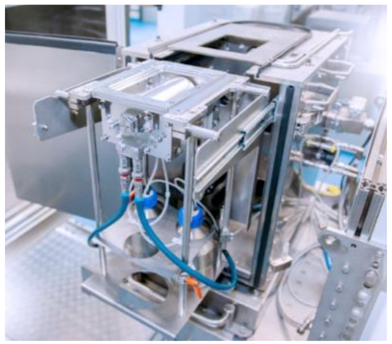




Fraunhofer

Pathogen Inactivation Platform (PIP) / 2023

Ebeam accelerator

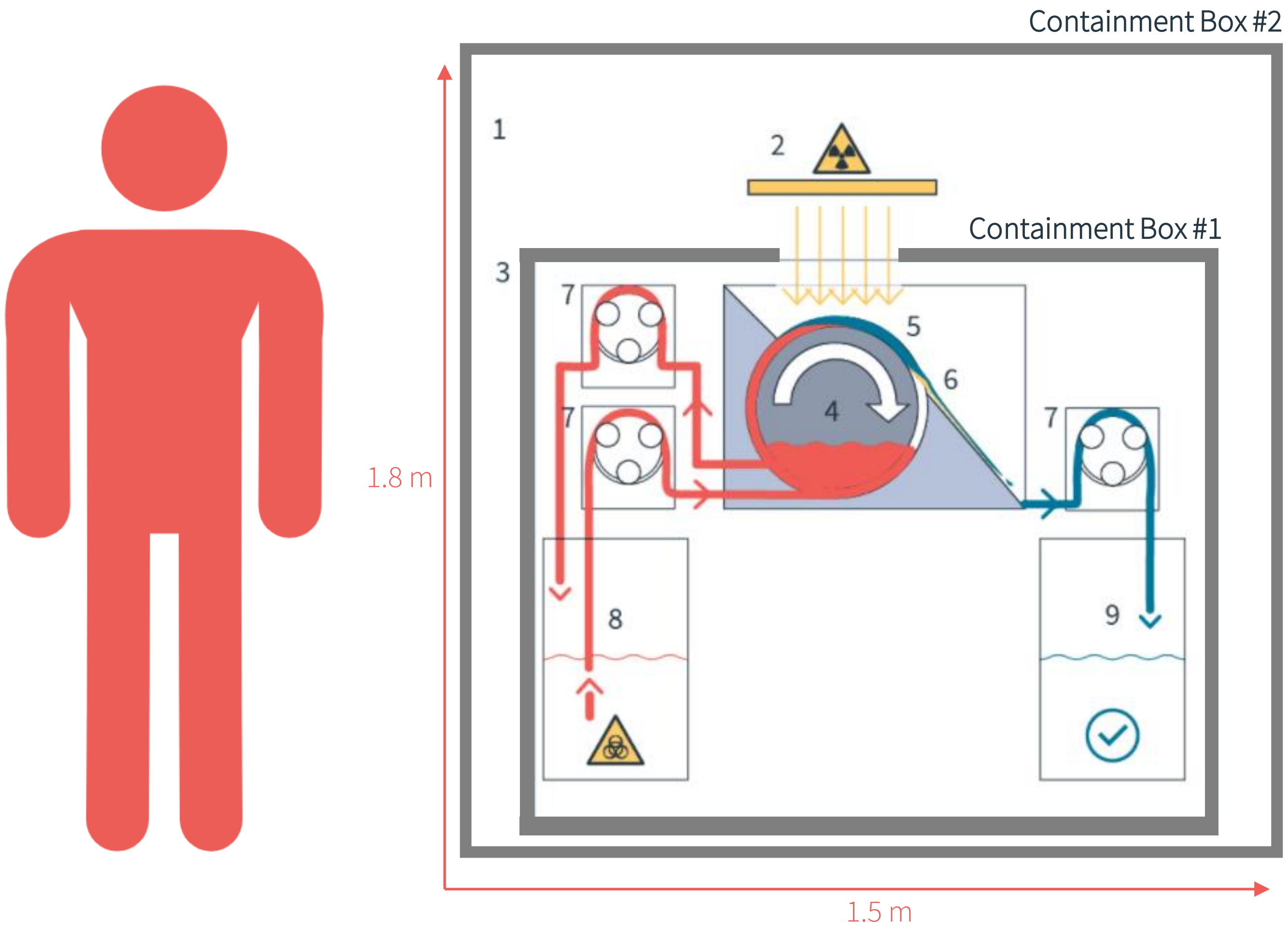
Containment box

Fluid transport container

PIP will be designed as a
full radiation protection system
with biological containment!

Pathogen Inactivation Platform (PIP)

Core functions

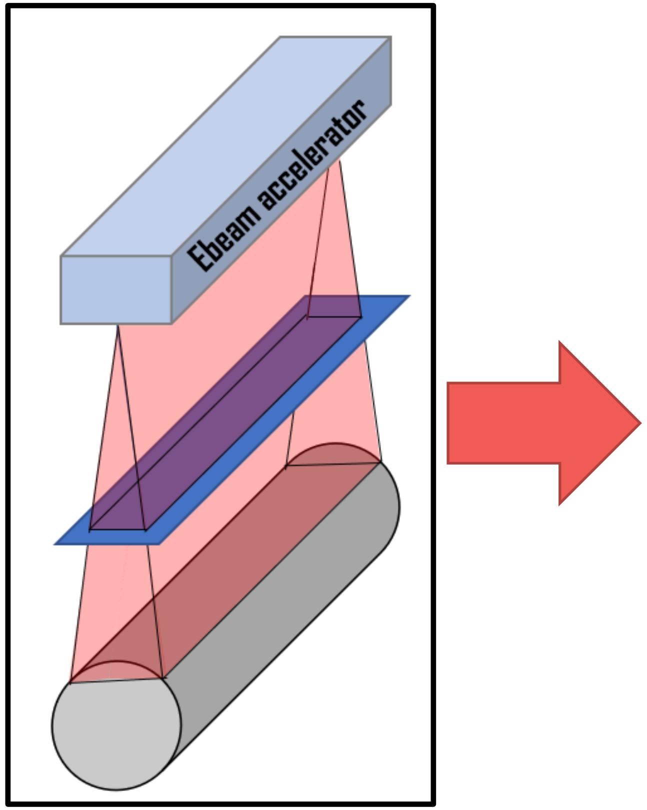


1. Irradiation chamber
2. ebeam accelerator
3. Liquid roll-system
4. Rotating roll
5. Thin liquid film
6. Squeegee / wiper lip
7. Peristaltic pump
8. Pathogen solution
9. Inactivated solution

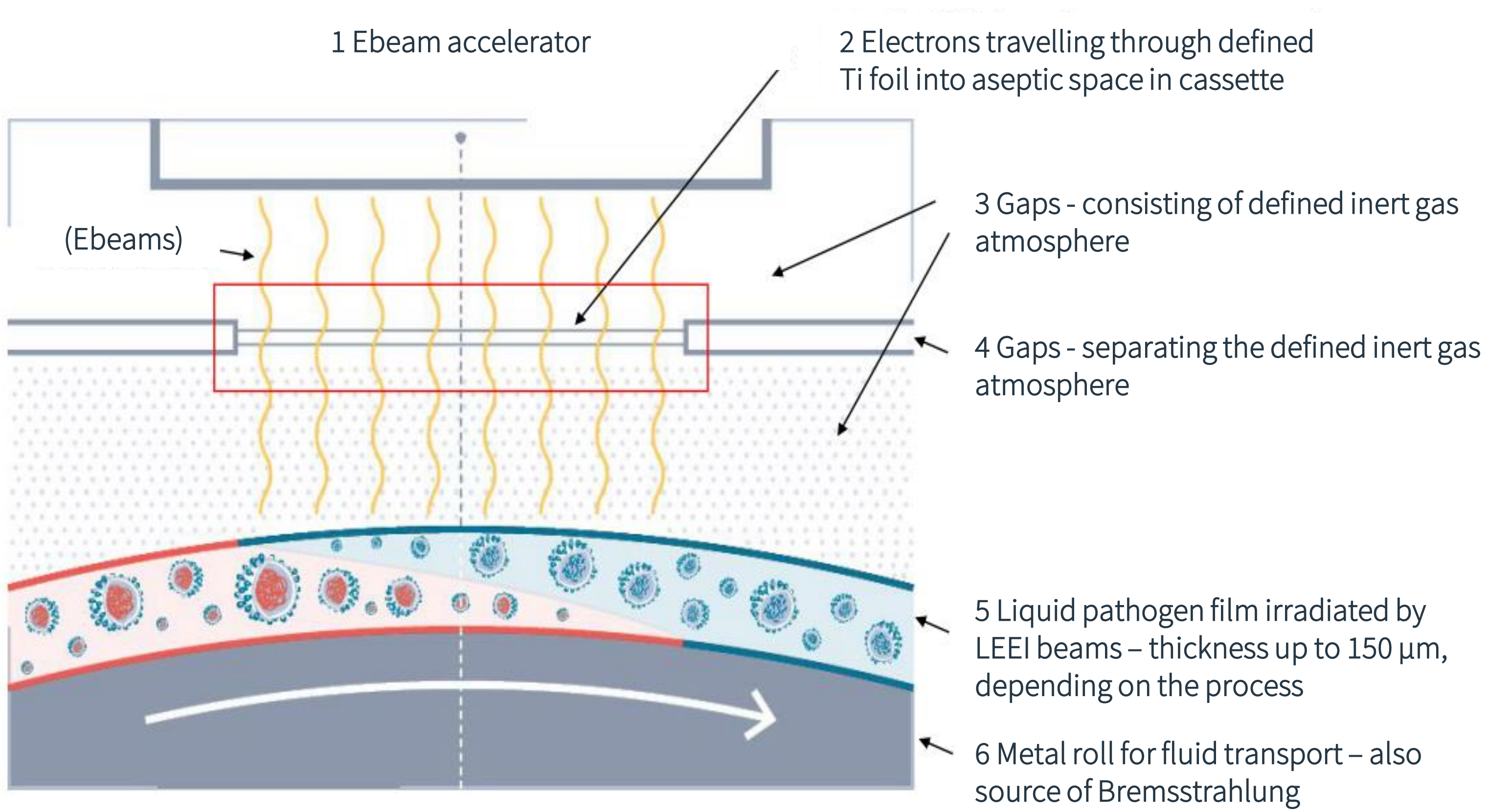
Key characteristics of the process

Controlling critical process parameters

Schematic layer set-up



Detailed view on liquid film environment



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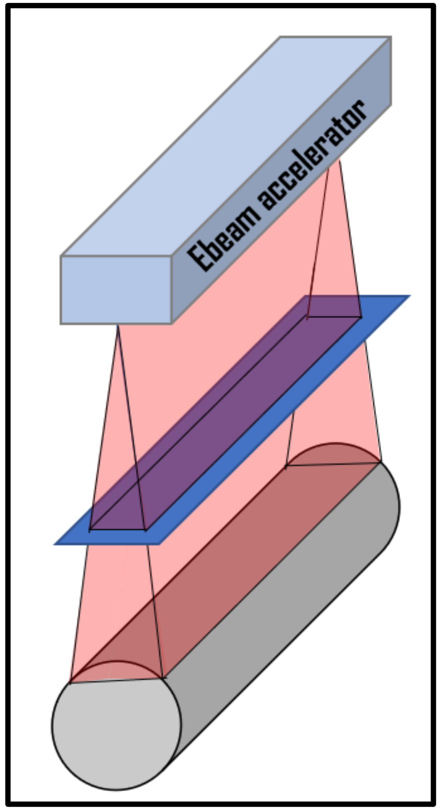


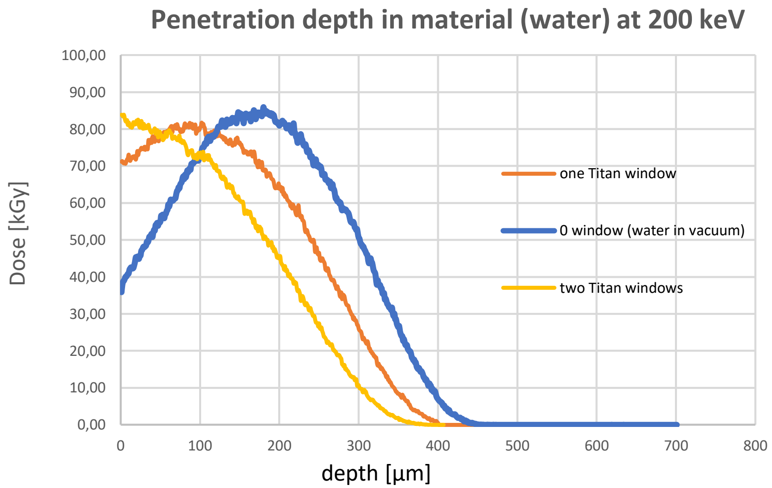
$$D = Y * \frac{I_B}{v_L * b_B}$$

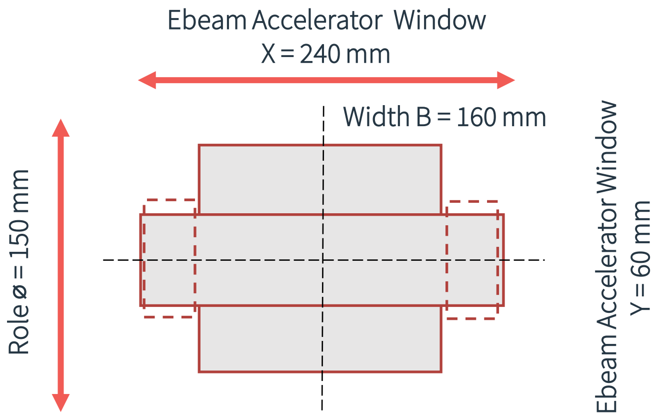
D = Irradiation dose [kGy]
Y = Dose constant [$kGy*m^2*mA^{-1}*min^{-1}$]
v_L = Rotational speed [$m* min^{-1}$]
I_B = Current [mA]
b_B = Beam width [m]

Parameter	Value	Impact on
Liquid film thickness	~ 120 µm	throughput
Acceleration voltage	200 keV	penetration depth
Ebeam current	10 mA	radiation dose
Thickness Titaniumium window	15 µm	penetration depth
Air gap distance	12 mm	penetration depth
Ebeam accelerator window	240 x 60 mm	irradiated area
Irradiated area	160 x 60 mm	throughput
Performance	max. 2 kW	current and voltage
Soll dose in target	~ 60 kGy	pathogen inactivation
Rotational speed	~ 9 m/min	throughput; radiation dose
Revolutions per minute	~ 19,1 RPM	throughput; radiation dose
Throughput	~ 10 L/h	Rotational speed; ebeam current

Key characteristics of ebeam

Uniformity, profile and robustness





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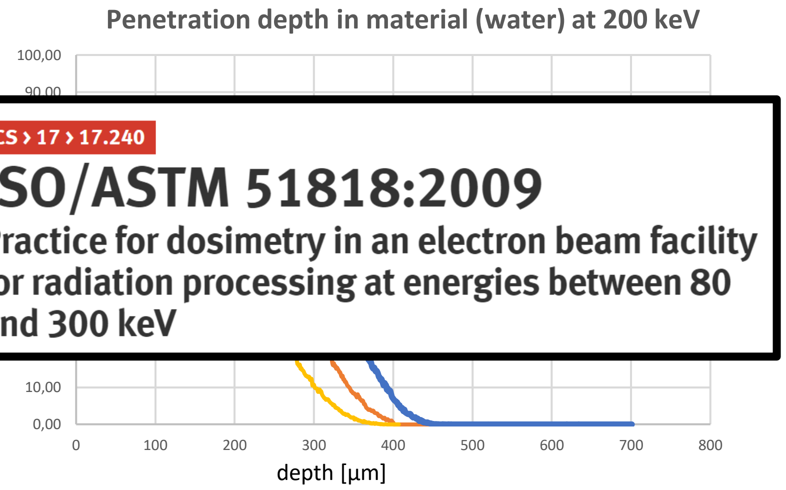
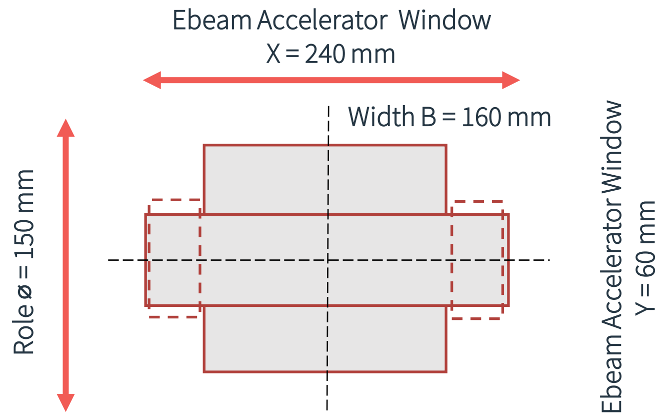


ICS › 17 › 17.240

ISO/ASTM 51818:2009

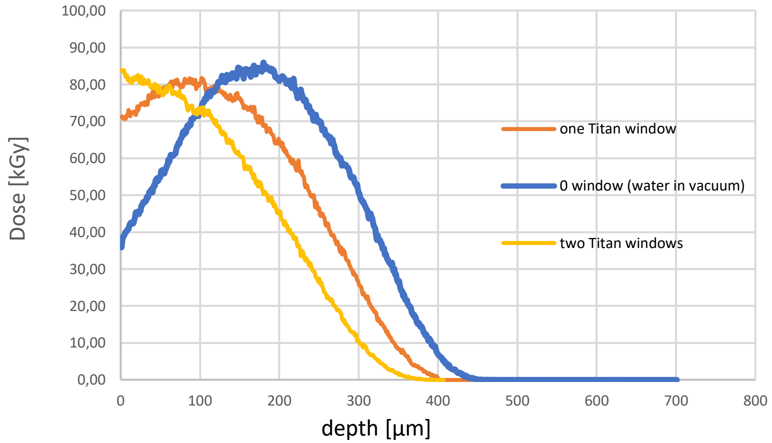
Practice for dosimetry in an electron beam facility for radiation processing at energies between 80 and 300 keV

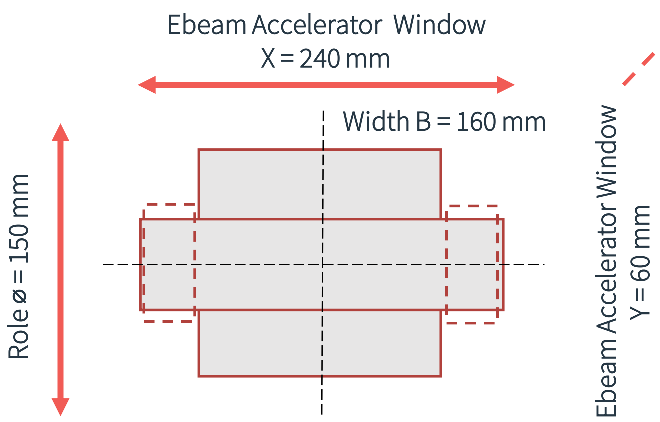


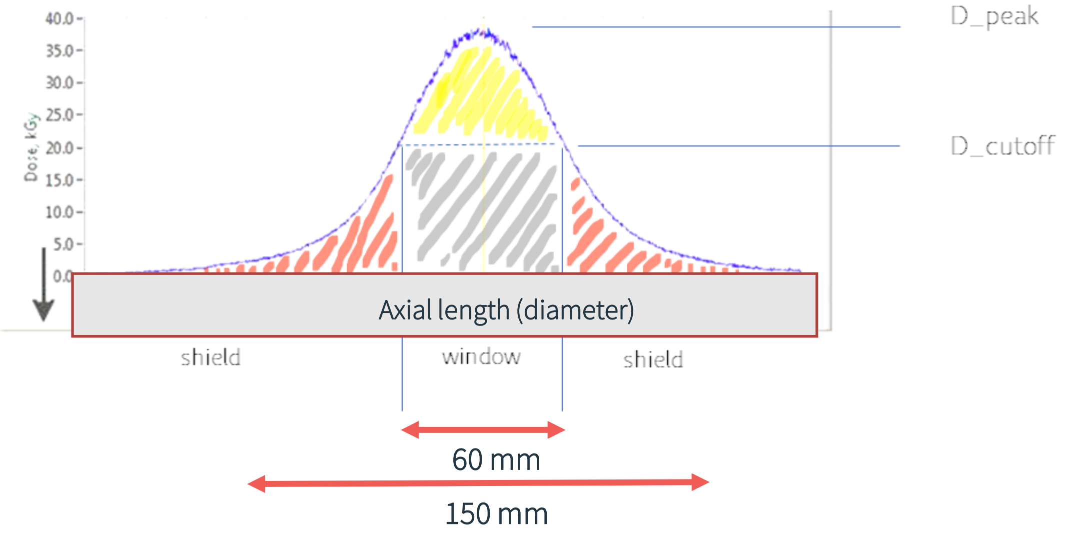
Key characteristics of ebeam

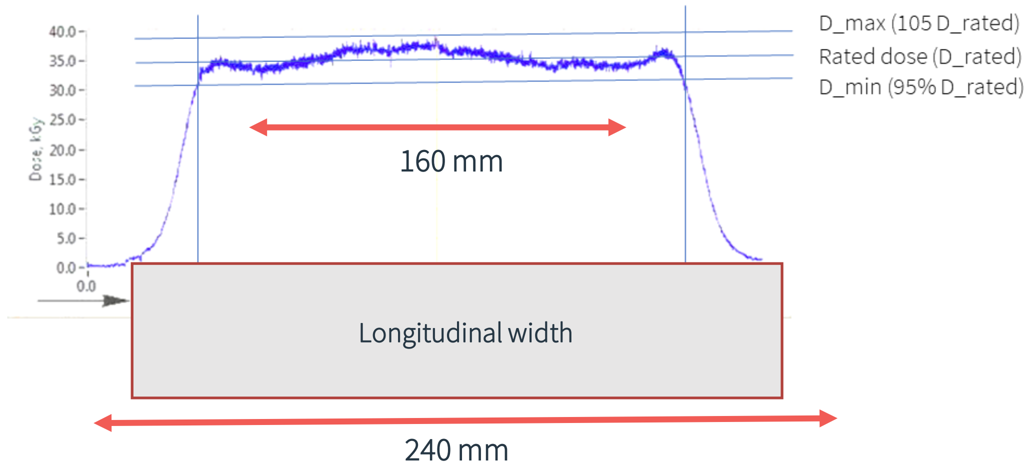
Uniformity, profile and robustness

Penetration depth in material (water) at 200 keV



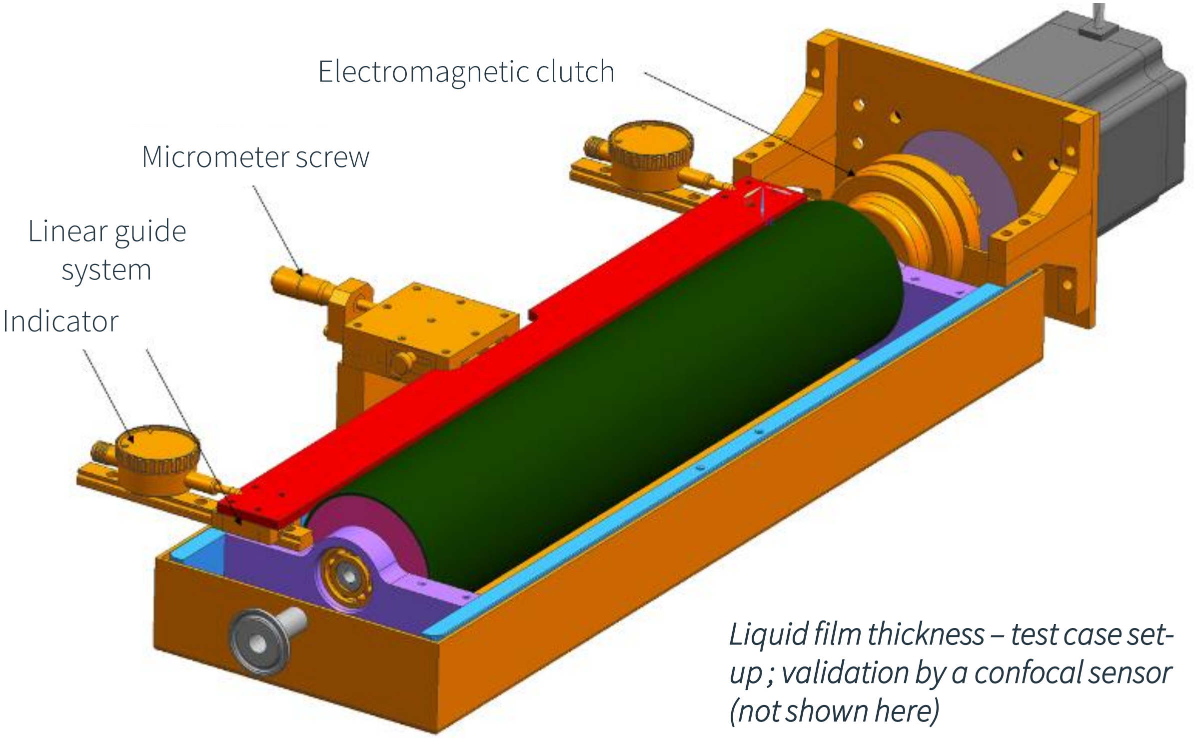






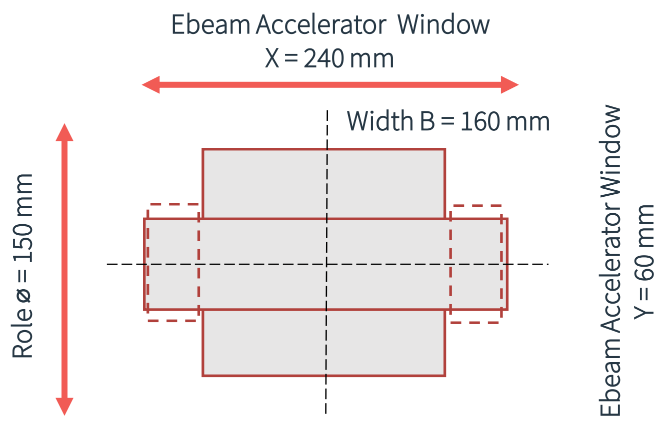
Substantiating the technology

- Process Control
  - Liquid film thickness (already started)
  - Inline & real-time dosimetry
- Experiments and Test Cases
  - Generating liquid films
  - Controlling liquid film thickness
  - Transporting fluids within the system
  - Ebeam irradiation with Ebeam supplier (ECAB)
  - Investigating continuous dosimetry

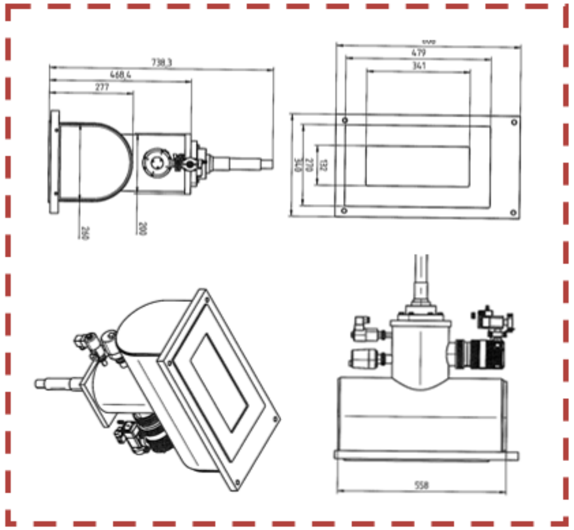


Liquid film thickness – test case set-up ; validation by a confocal sensor (not shown here)

Early draft of role dimensions



The ebeam accelerator



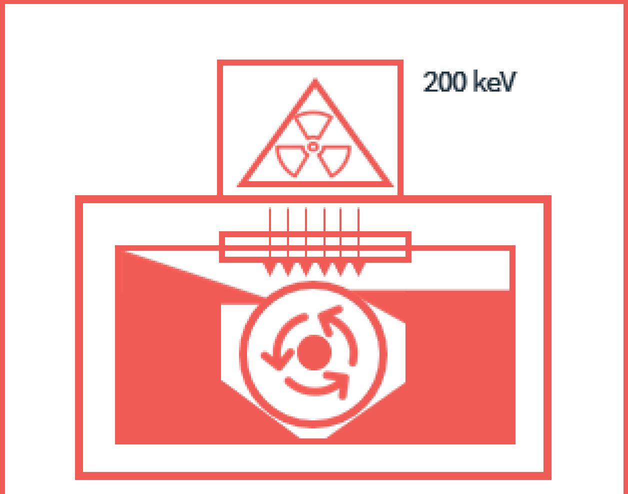
In a nutshell – Status quo

- PIP = fridge-sized platform
- PIP will include an enclosed box for aseptic handling of pathogens (containment container)
- **10 L/h*** Throughput
- **Within sub-seconds** can pathogens be inactivated
- **60 kGy** effective inactivation dose
- Ebeam accelerator for next-gen prototype platform
  - Dimensions: 700 mm x 560 mm x 340 mm
  - 200 kV; 10 mA; 2 kW

*Not yet tested

Ebeam in vaccine manufacturing

Technology Summary



The technology meets the following criteria:

Approved Technology

Scalable Process

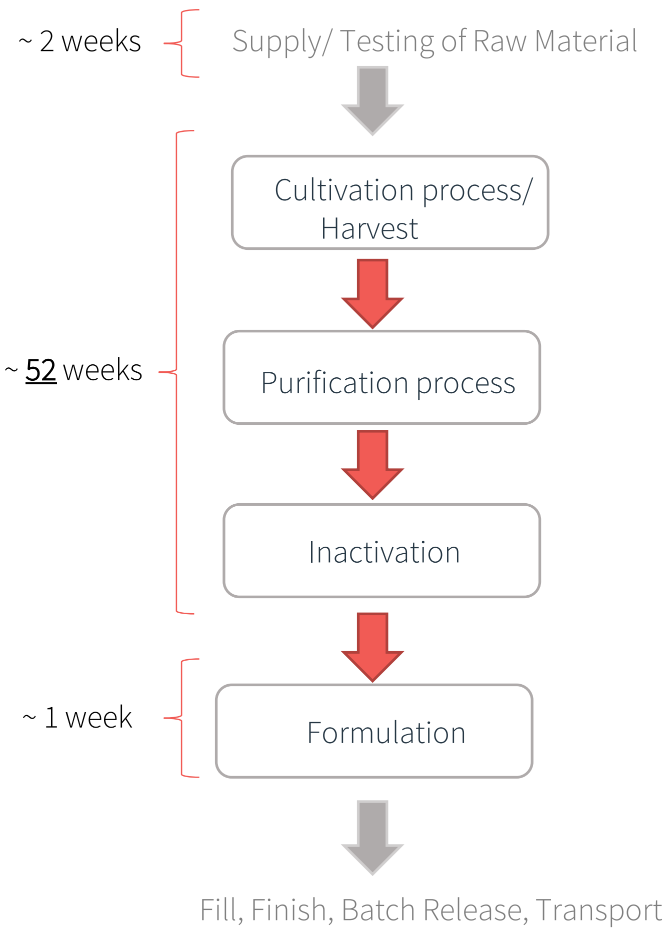
Safe Applifaction

Pathogen Inactivation

Part 2
The Application

Traditional and novel process chains

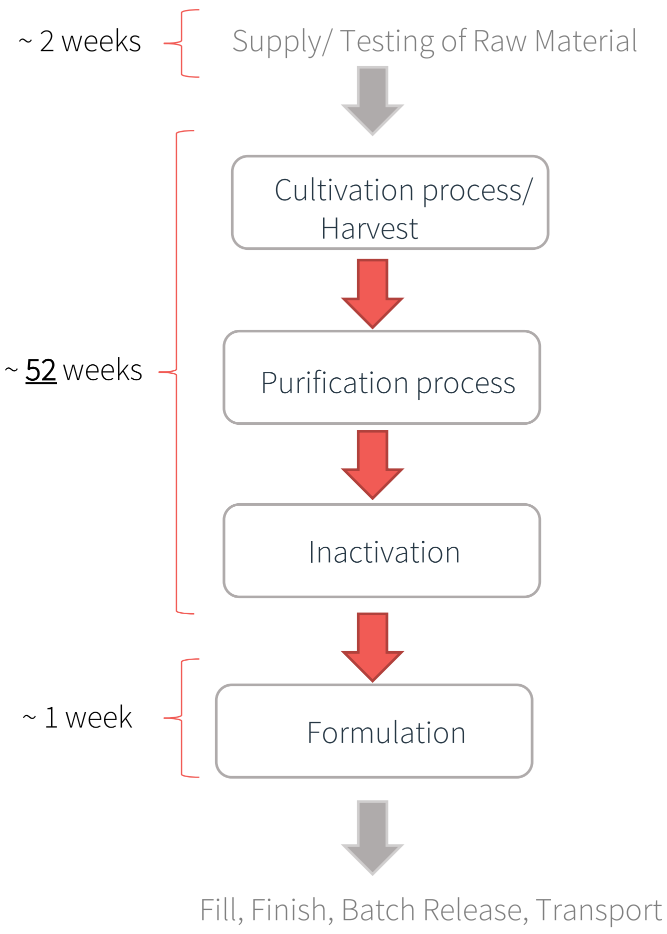
A disruptive change is about to happen

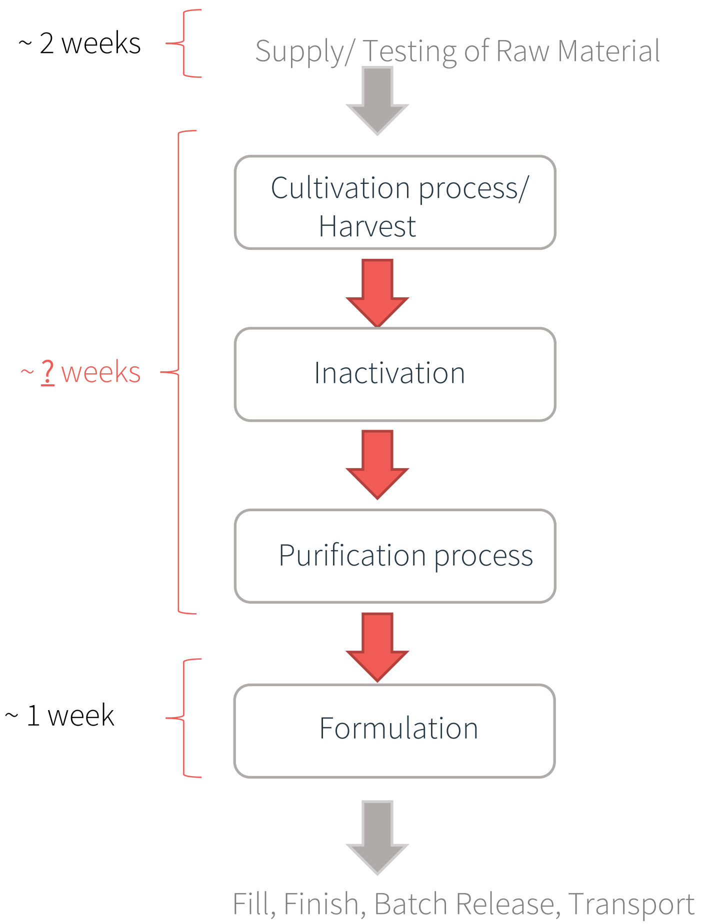


Reference: Lücke, Baedeker, Hildinger, BCG.Biotechnology report 2016

Traditional and novel process chains

A disruptive change is about to happen





Traditional and novel process chains

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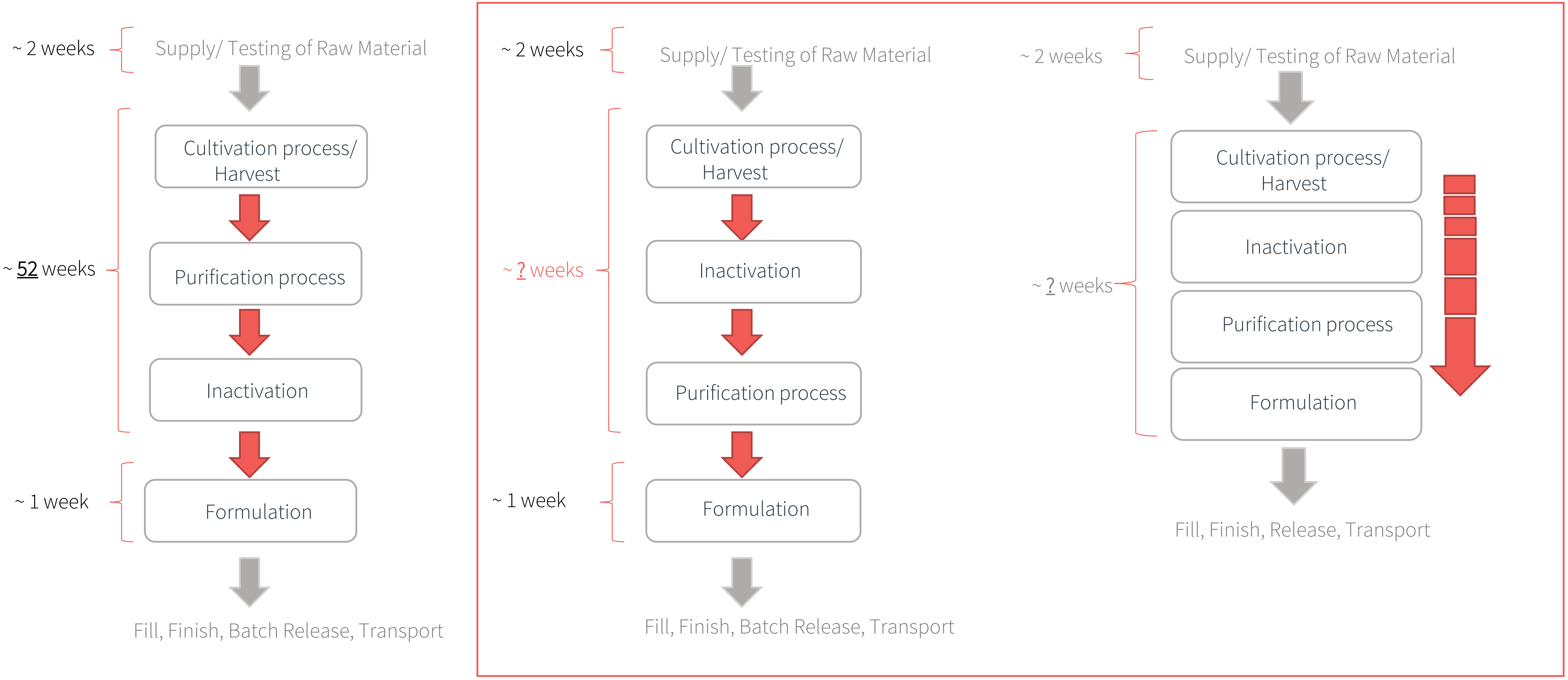
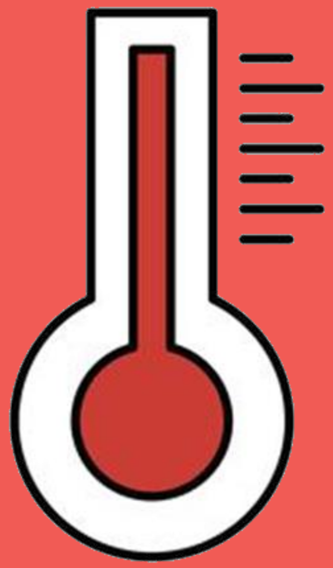


Application of LEEI Technology



9 System Test, Launch & Operations

8 System/ Subsystem development

6-7 Technology Demonstration

5 Technology Development

3-4 Feasibility Proof-Research

1-2 Basic Technology Research

Key market

Product type	Human Vaccines	Veterinary Vaccines
Application/ Proof of concept	Pathogen inactivation	Pathogen inactivation
TLR Score	8	8
Market availability	2023	2023

R&D / sidekicks

Product type	Cell- Products	Blood Products
Application/ Proof of concept	Irradiation of (immune) cell therapeutics	Irradiation of transfusion-medicine products/ Serum for cell culture applications
TLR Score	5	3
Market availability	To be determined	To be determined

Review on Feasibility

Inactivation by LEEI works for

- Bacteria
- Viruses
- Parasites
- Human & veterinary pathogens.

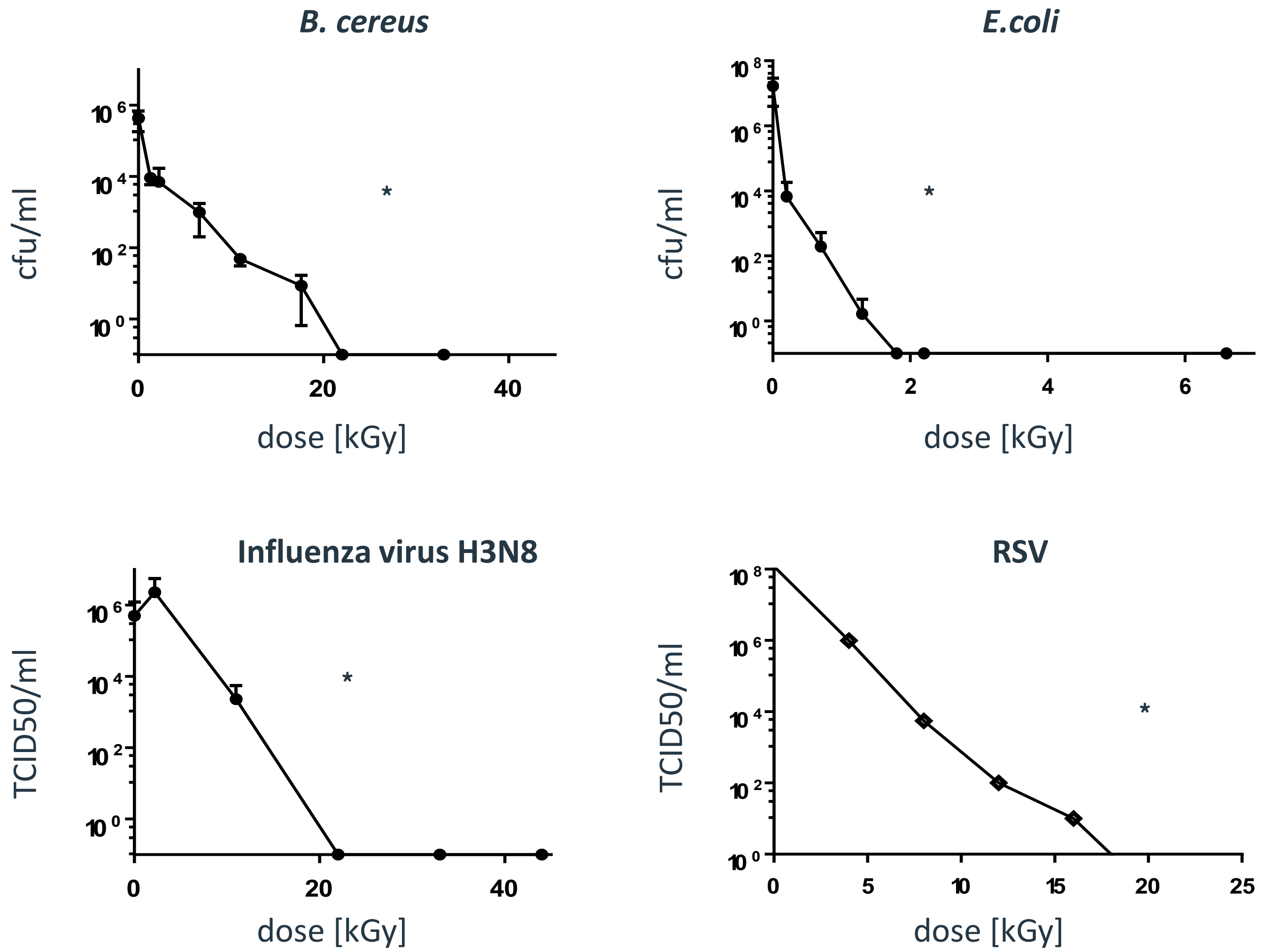
Dose range: **1 kGy and 33 kGy.**

Inactivation is *inter alia* pathogen specific

Overview of Pathogens successfully inactivated by LEEI:

Pathogen	Type	Veterinary / Human	Concentration	Dose for inactivation
RSV	ss RNA virus	Human	2 x 10^7 TCID50/ml	20 kGy
Influenza A (H3N8)	(-) ds RNA virus	Veterinary	5 x 10^5 TCID50/ml	22 kGy
ZIKV	ss RNA virus	Human	5 x 10^6 TCID50/ml	20 kGy
PRRSV	ss RNA	Veterinary	5.42 log $TCID_{50}$/ml	10,4 ±1 kGy
EHV-1	ds DNA Virus	Veterinary	3.89 log $TCID_{50}$/ml	10,4 ±1 kGy
R. pneumotropicus	Bacterium	Veterinary	1 x 10^5 CFU/ml	20 kGy
E. coli	Bacterium	Human	1.67 x 10^7 CFU/ml	2.2 kGy
B. cereus	Bacterium	Human	4.33 x 106 CFU/ ml	33 kGy
Eimeria tenella	Parasite	Veterinary	1.0- 2.0 x 10^5 Oozysten/ml	1 kGy

Proof of Concept
Dose- Inactivation curves

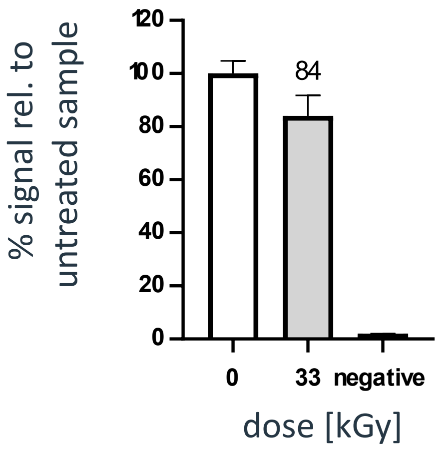


- → Successful dose- dependent inactivation of bacteria and viruses
- → Doses required for inactivation comparable to those reported with other ionizing radiation technologies.

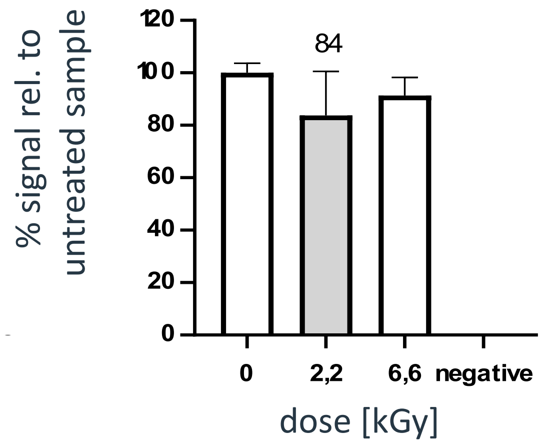
Correlation between genome size and irradiation dose required for complete inactivation: smaller genome size -> Higher irradiation dose.

Proof of Concept
Dose Antigenicity

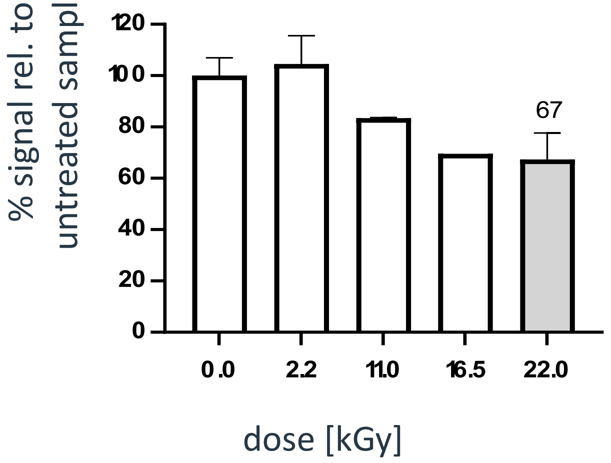
B. cereus



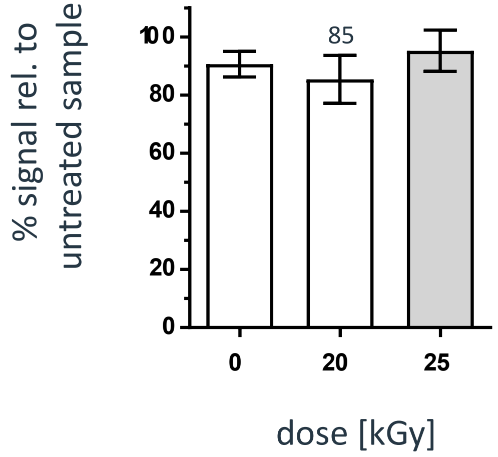
E. coli



Influenza virus H3N8



RSV

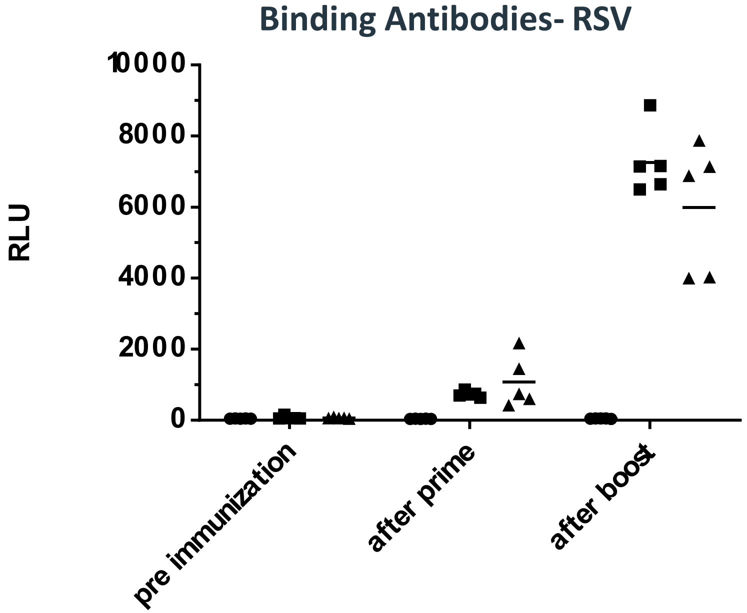


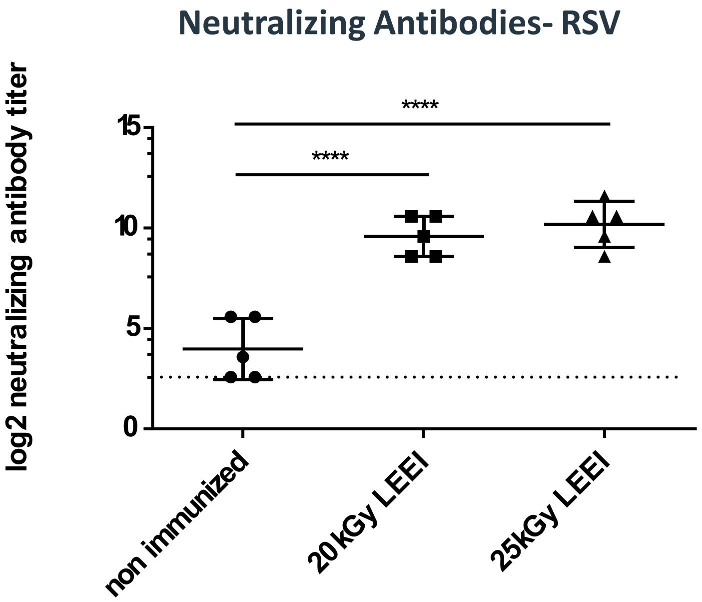
- → High degree of conservation of the native antigen structure.
- → High reproducibility of antigen conservation.
- → Monoclonal antibody recognition of RSV F-Protein is not altered after LEEI-treatment
- → Direct comparison to FI:

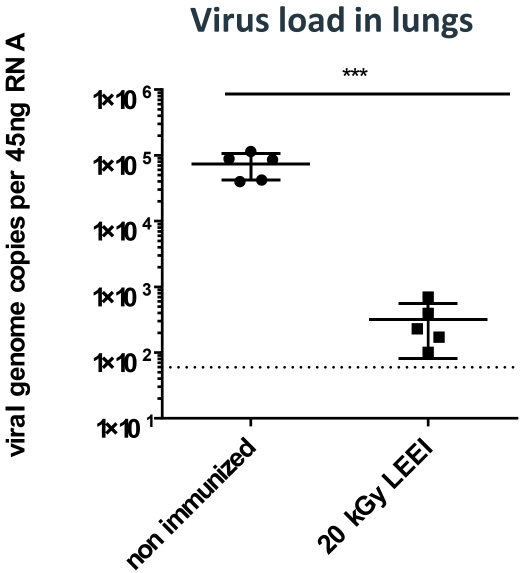
Fertey, Bayer et al (2020). 10.3390/vaccines8010113.

Bayer, Fertey (2018). 10.1016/j.vaccine.2018.02.014.

Proof of Concept
Dose- Immunization Data RSV

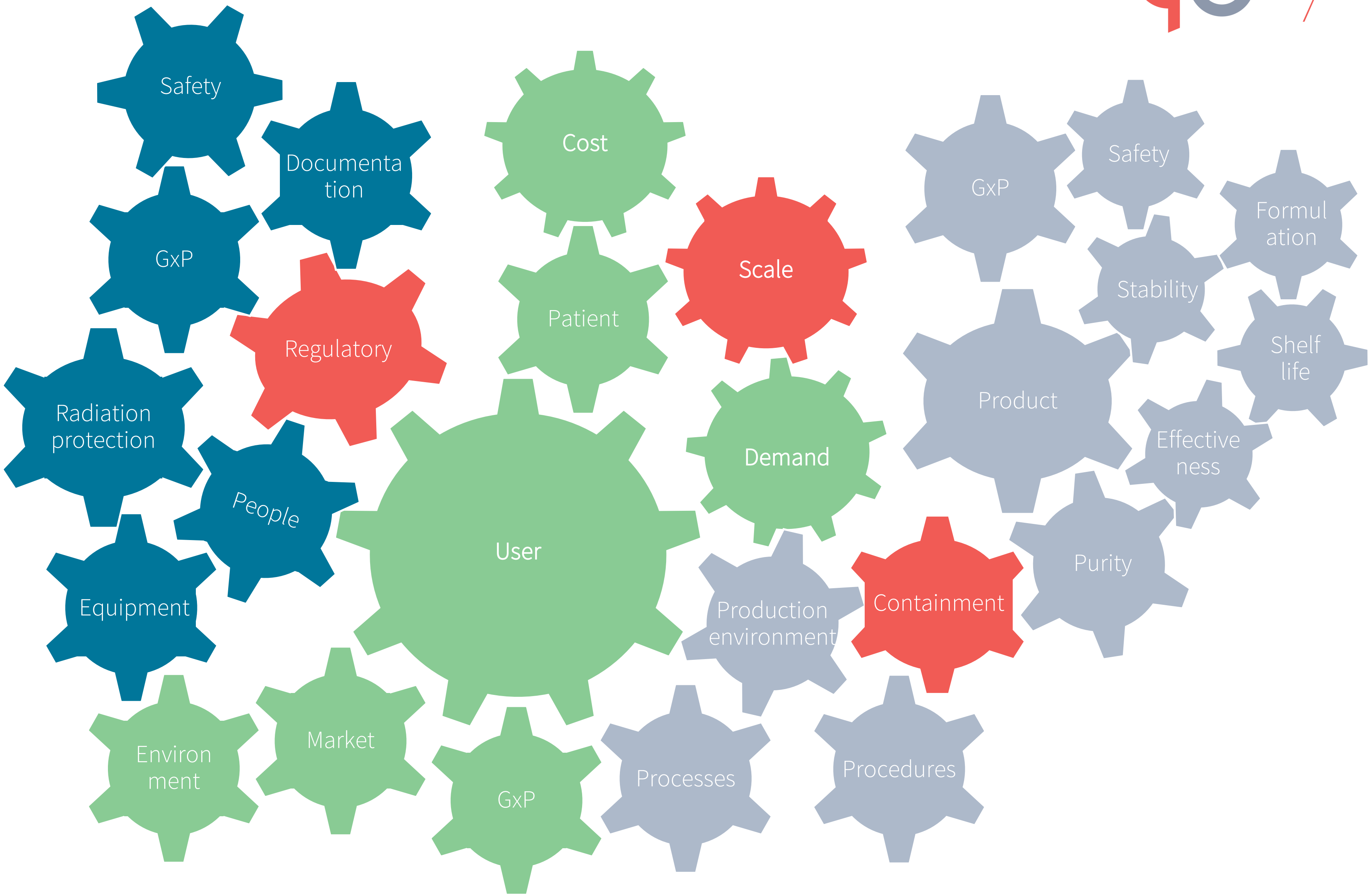




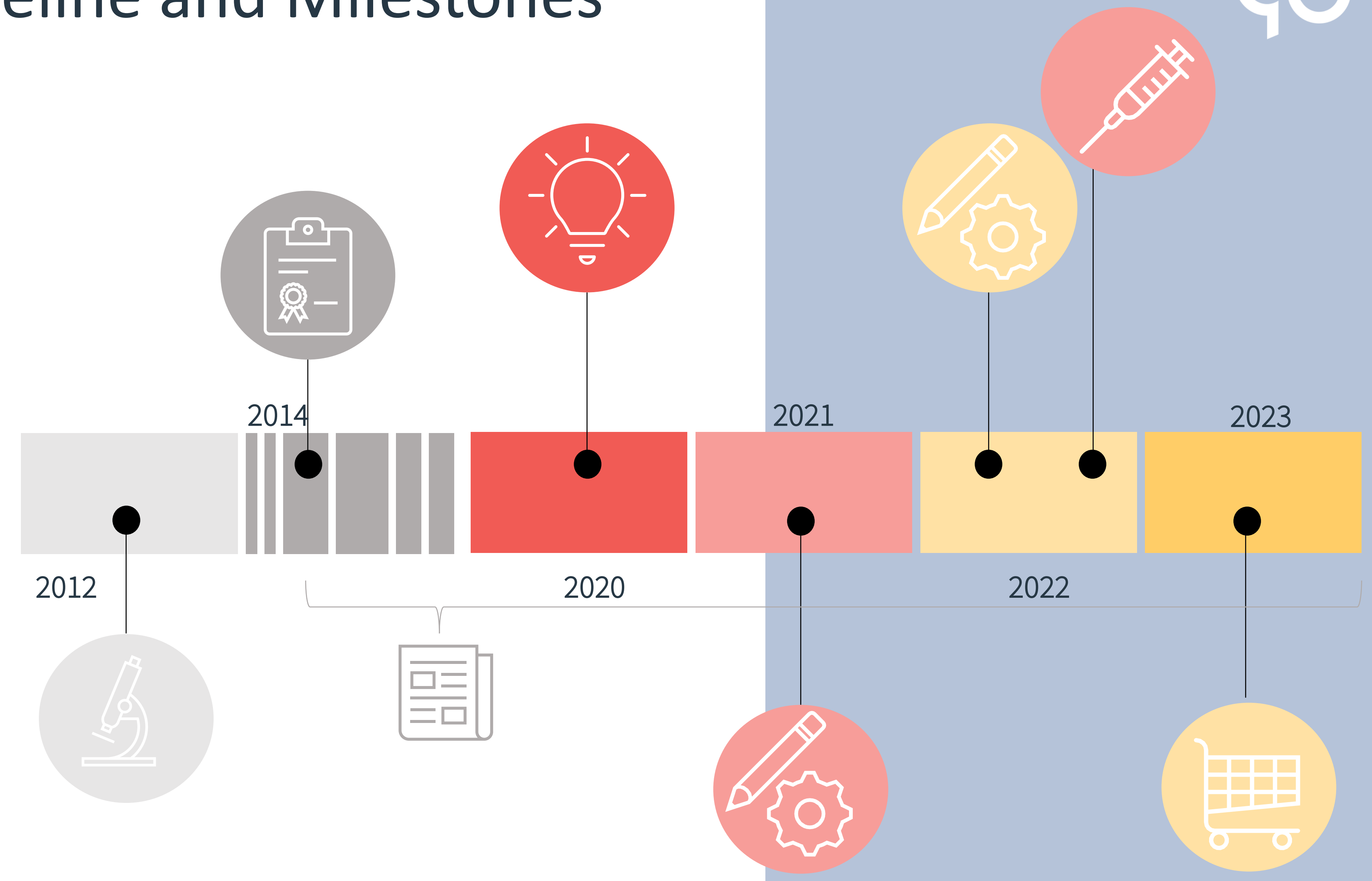


- → All animals developed significant levels of RSV- specific antibodies
- → All animals developed significant amounts of virus neutralizing antibodies
- → Animals immunized with 20 kGy-irradiated RSV have RSV-RNA levels close to detection limit

Paving the way to market



Timeline and Milestones



Partnering with KyooBe Tech

- Development (sparring) partners
- Lead customers
- Regulatory experts with vaccine manufacturing background

Enthusiastic and motivated people who want to bring WIV vaccine manufacturing to the next level

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