# Accelerator Technologies for Food Safety & Food Quality: Response of Microbial Populations to Ionizing Technologies

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*eBeam Technology for Cleaning, Healing, Feeding, and Shaping this World and Beyond…*

*an International Atomic Energy Agency Collaborating Centre for Electron Beam Technology*

# Acknowledgements










# A big thank you!

ORS
Office of Radiological Security
Protect · Remove · Reduce

USDA
United States Department of Agriculture

SERDP
DOD ▪ EPA ▪ DOE

EPA
United States Environmental Protection Agency

NASA JPL

# eBeam and X-ray Technology Applications in the Food Industry


Food Safety

Food Quality

Phytosanitary Treatment


Food Packaging

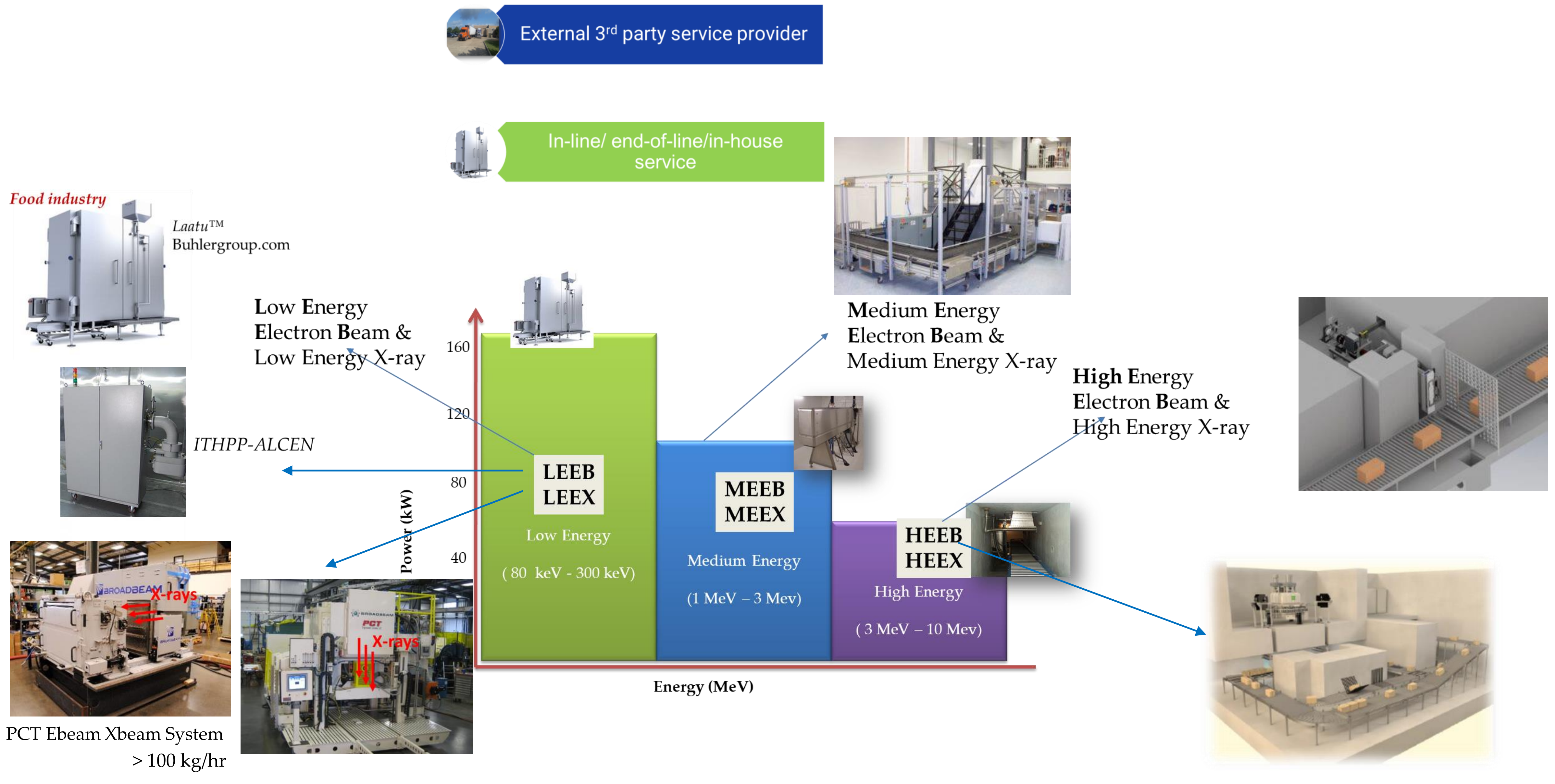


National Center for Electron Beam Research
Texas A&M AgriLife Research
A&M

# Presentation Focus

Questions that need clear answers

# Technologies to Accelerate Adoption



# eBeam for aseptic packaging
## *a Green Solution – Tetra Pak in-line eBeam application*

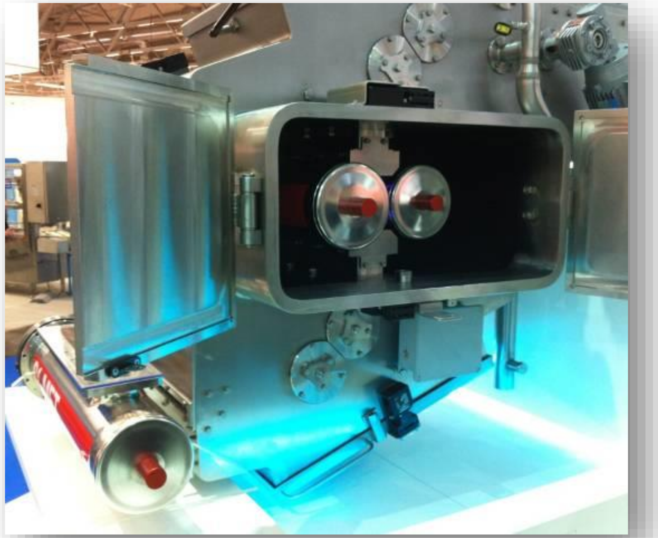
Substitutes $H_2O_2$, the gold standard for > 30 years

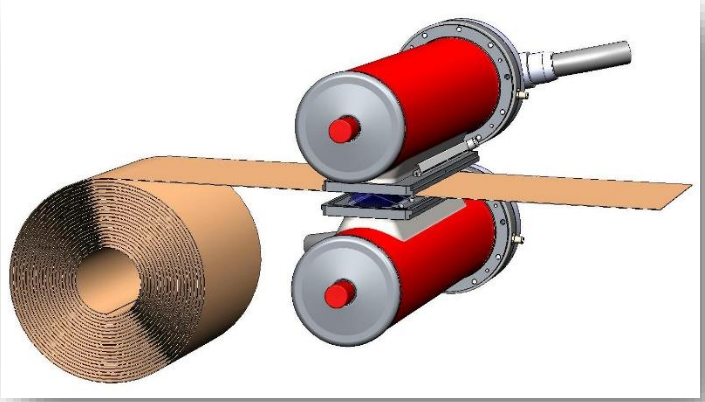
75% less energy than $H_2O_2$ for the sterilization process

40% lower $CO_2$ footprint

33% less power draw from the mains

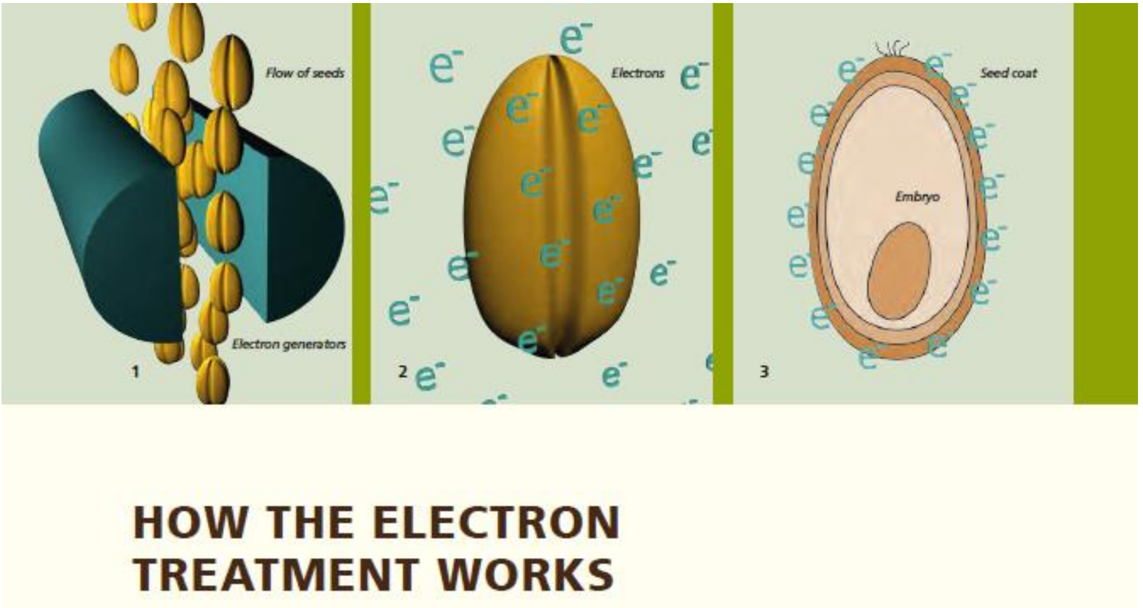
> 100 billion aseptic packages produced and sold around the world






*Courtesy : Tetra-Pak*

# Low Energy Electron Beam Technology Applications



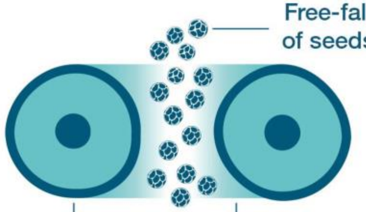

Enhancing seed germination

LEEB - eBeam technology on wheels

Fraunhofer FEP

In-house spice decontamination

**SPICE decontamination**




BÜHLER

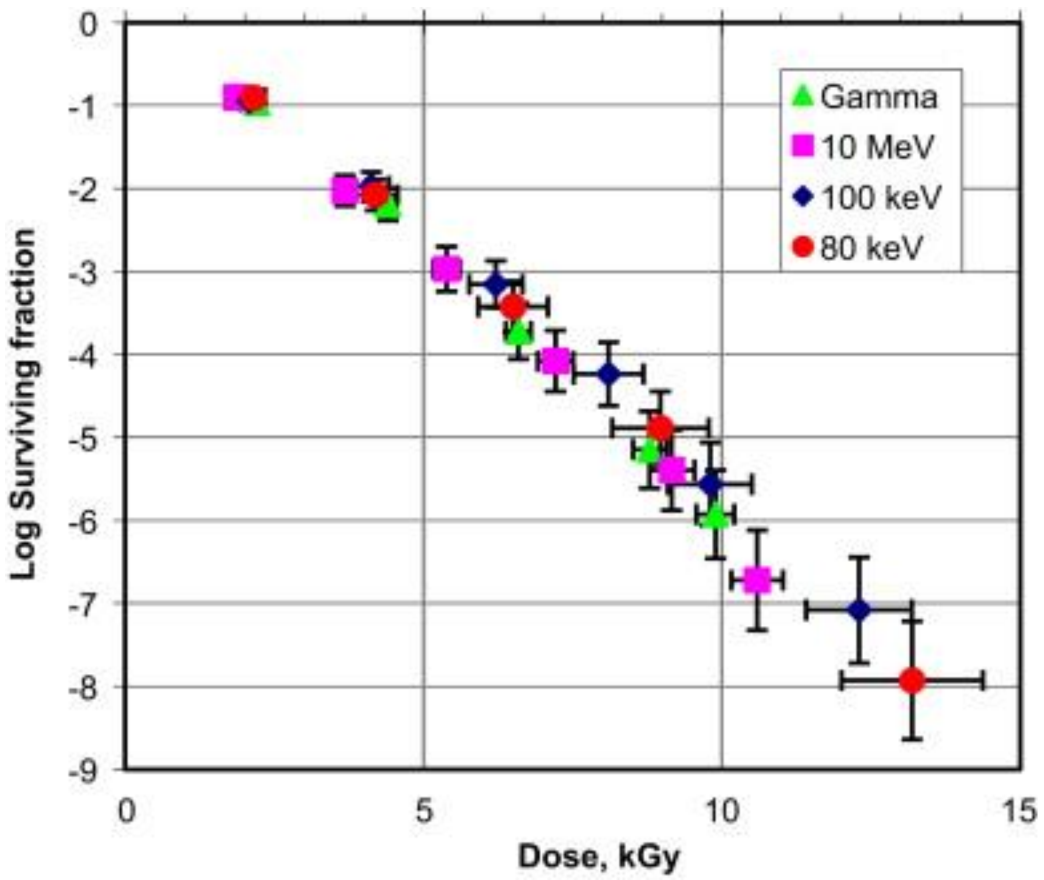
National Center for Electron Beam Research

TEXAS A&M AGRILIFE RESEARCH

# Questions that Need Clear Answers

- Do eBeam energies have an effect on D-10 values?
- Is 2 kGy from a gamma source the same as 2 kGy from an eBeam or X-ray source?
- Will 2 kGy from a gamma source at ~ 4kGy/hour have the same biological effect of 3 kGy from an eBeam linac at 3kGy/sec or an X-ray source at 0.3 kGy/sec?
- How do we confirm microbial inactivation? Viability testing or molecular analyses?

# Some studies show that there is no effect




Response of B. pumilus spores to varying ionizing energies and dose rates

*2.2. Radiation sources*

| | |
|---|---|
| Risø Gamma Cell 1: | |
| Radiation source: | Cobalt 60 |
| Source strength: | Approx. 1000 Ci ($37 \times 10^{12}$ Bq) |
| Dose rate: | 12 Gy/min |
| High energy electron accelerator: | |
| Sterigenics, Denmark, Rhodotron electron accelerator | |
| Type: | TT 200 |
| Manufacturer: | IBA |
| Energy: | 10 MeV |
| Beam current, max: | 8 mA |
| Scanned beam width, max: | 80 cm |
| Low energy electron accelerator: | |
| Risø High Dose Reference Laboratory low energy electron accelerator | |
| Manufacturer: | AEB |
| Energy: | 80–125 keV |
| Beam current, max: | 10 mA |
| Fixed beam width, approx: | 20 cm |

**Table 2**
Values of slopes of dose-log survival curves and derived $D_{10}$ values for each radiation type together with those found by Tallentire and Khan (1975).

| | Slope, $kGy^{-1}$ | $D_{10}$ kGy |
|---|---|---|
| Cobalt 60 | −0.65 | 1.54 |
| 10 MeV | −0.65 | 1.54 |
| 80 keV | −0.63 | 1.58 |
| 100 keV | −0.61 | 1.65 |
| Average | −0.65 | 1.58 |
| Tallentire and Khan (1975) | −0.62 | 1.61 |

*Tallentire et al., 2010*

# Some studies do suggest that there is an effect..

| Bacillus/$D_{10}$ | 100 keV | 10 MeV |
|---|---|---|
| *B. pumilus* | 1.34kGy | 2.12kGy |
| *B. megaterium* | 3.46kGy | 4.11 kGy |
| *B. subtilis* | 1.01kGy | 2.05 kGy |

Table 5. Preliminary results of $D_{10}$ values for different Bacillus spp spores on Al coupons using 100keV and 10MeV electron beam irradiation.

*Urgiles et al., 2007*

# Inactivation kinetics of food-borne pathogens exposed to varying ionizing energies

| | $D_{10}$ Value[a] (Gy) | | | |
|---|---|---|---|---|
| **Radiation Source** | ***E. coli* (25922)** | ***E. coli* (#5)** | ***Salmonella* Typhimurium** | ***Salmonella* 4,[5],12:i:-** |
| **10 MeV eBeam** | 68 ± 4[B] | 107 ± 2[D] | 170 ± 16[E] | 147 ± 15[F] |
| **8.5 MeV eBeam** | 103[A] | 129[C,D] | 163[E] | 163[F] |
| **La-140 (gamma)** | 95 ± 10[A] | ND | 178 ± 9[E] | ND |
| **Reactor core (gamma)** | 75 ± 3[B] | 138 ± 15[C] | 174 ± 5[E] | 164 ± 0.2[F] |
| **5 MeV x-ray** | 90 ± 7[A] | 151[C] | ND | ND |
| **100 keV x-ray** | NA | ND | ND | ND |

[a] Values are means ± standard deviation. $D_{10}$ values with different letters indicate statistically significant ($P \leq 0.05$) differences. Statistical analyses were performed for each organism against all the different radiation sources. ND, not determined. NA, not applicable.

*Hieke, Ph.D dissertation*

# eBeam energy does not appear to have a significant effect on Inactivation kinetics of *Salmonella* spp.

| | $D_{10}$ Value[a,b] (Gy) | |
|---|---|---|
| **Radiation Source** | ***Salmonella*** **4,[5],12:i:-** | ***Salmonella*** **cocktail** |
| Non-attenuated 10 MeV eBeam | 220 ± 45[A] | 270 ± 46[A] |
| Attenuated 10 MeV eBeam | 222 ± 62[A] | 289 ± 20[A] |

[a] Values are means ± standard deviation.
[b] There was no statistically significant ($P \leq 0.05$) difference in $D_{10}$ values between attenuated and non-attenuated conditions.

Attenuated 10 MeV source was 2.97± 0.22 MeV (*most probable electron beam energy (Ep)*)

*Hieke and Pillai, 2015*

**How is the U.S. food industry using this technology?**

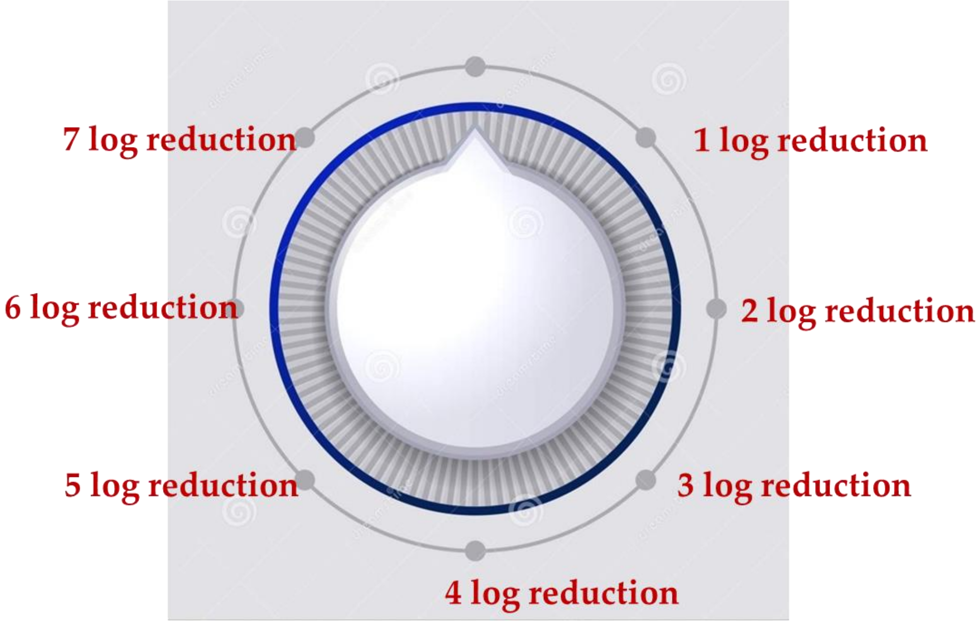
# How is this technology used commercially by the US food industry




## Table 1: Food Products Approved for Irradiation in the United States

| Food product | Agency and approval date | Purpose of irradiation | Maximum permitted dosage (kiloGray) |
|---|---|---|---|
| Dry or dehydrated enzyme preparations | Food and Drug Administration (FDA), June 10, 1985 | Control of insects and micro-organisms | 10.0 |
| Pork carcasses or fresh nonheated processed cuts | FDA, July 22, 1985<br>United States Department of Agriculture (USDA), January 15, 1986 | Control *Trichinella spiralis* | 0.30 to 1.00 |
| Fresh foods | FDA, April 18, 1986 | Delay maturation | 1.0 |
| Food | FDA, April 18, 1986 | Arthropod disinfestation | 1.0 |
| Dry or dehydrated aromatic vegetable substances | FDA, April 18, 1986 | Microbial disinfection | 30.0 |
| Fresh, frozen uncooked poultry | FDA, May 2, 1990<br>USDA, September 21, 1992 | Control foodborne pathogens | 3.0 |
| Refrigerated and frozen uncooked sheep, cattle, swine, and goat | FDA, December 3, 1997<br>USDA, December 23, 1999 | Control foodborne pathogens and extend shelf-life | 4.5 (refrigerated)<br>7.0 (frozen) |
| Fresh shell eggs | FDA, July 21, 2000 | Reduction of *Salmonella* | 3.0 |
| Seeds for sprouting | FDA, October 30, 2000 | Control microbial pathogens | 8.0 |
| Fresh or frozen molluscan shellfish | FDA, August 16, 2005 | Control *Vibrio* bacteria and other foodborne pathogens | 5.5 |
| Fresh iceberg lettuce and fresh spinach | FDA, August 22, 2008 | Control foodborne pathogens and extend shelf-life | 4.0 |

Source: GAO presentation of information from 21 C.F.R. 179.26 and *Federal Register* notices.



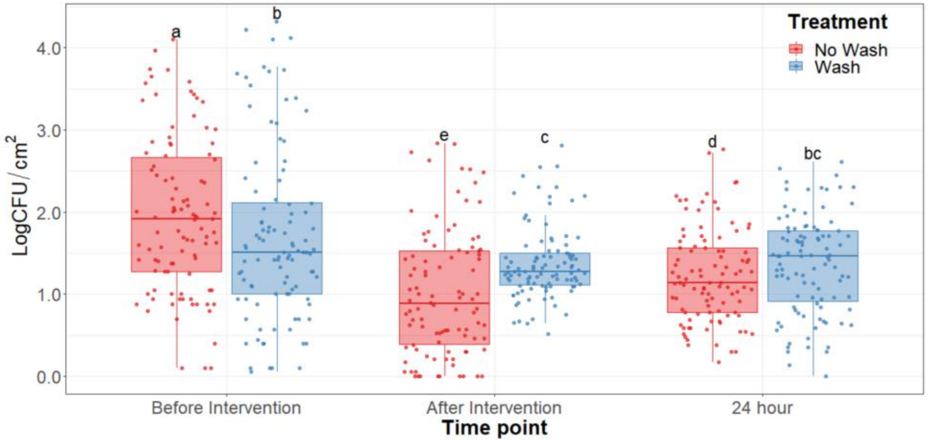
# Salmonella in peripheral lymph nodes

- *Arthur et al., 2008*
- *Haneklaus et al., 2012*
- *Gragg et al., 2013*
- *Li et al., 2015*

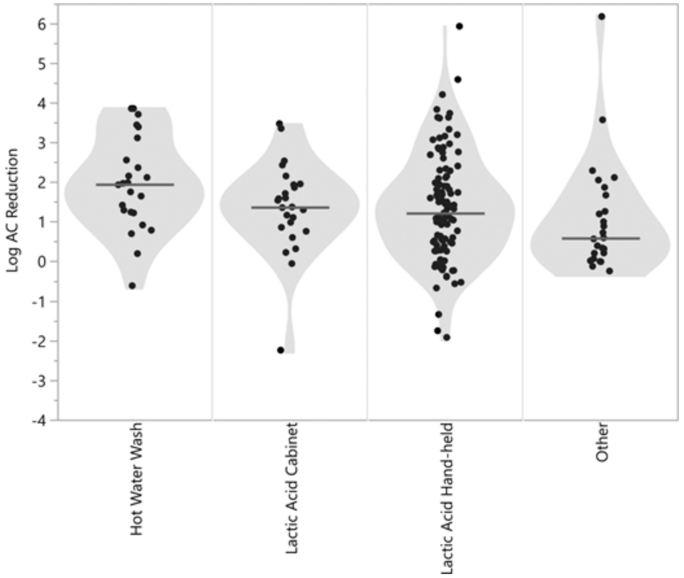
- Presence in peripheral lymph nodes protects Salmonella against carcass decontamination sprays and washes
  - Explains greater presence in ground beef relative to beef trim

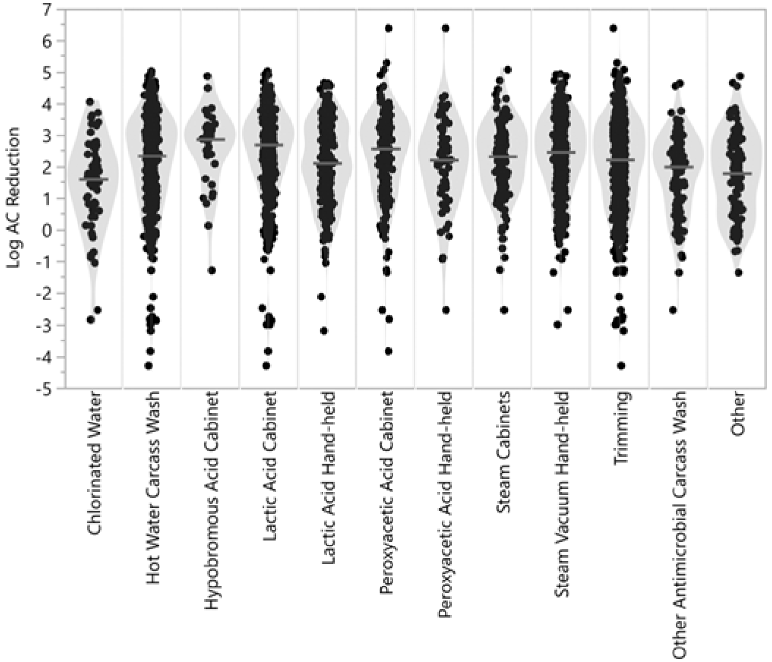
- Jan – Nov 2015 (n= 1200 pork heat and cheek samples)
  - Cheek meat – 63% positive for *Salmonella* enterica
  - Head trim – 66%
    - *Harvey, 2017*

# Carcass sprays have limited effectiveness




Aerobic plate counts before and after hot water wash intervention
And 24 hr chilling period - *Casas et al., 2021*




*Effects of antimicrobial interventions on indicator organisms during beef carcass dressing – Carter et al., 2021*

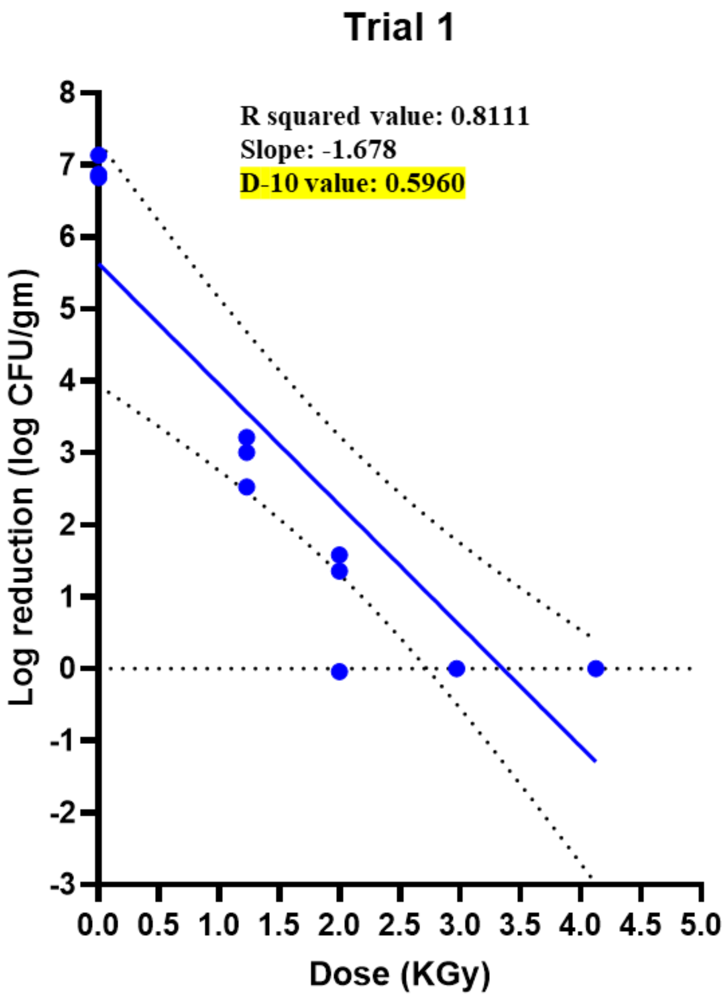
*Efficacy of single intervention ~ 0.4 – 1.9 log aerobic count reduction*
*Efficacy of multihurdle intervention ~1.6 -2.9 log aerobic count reduction*

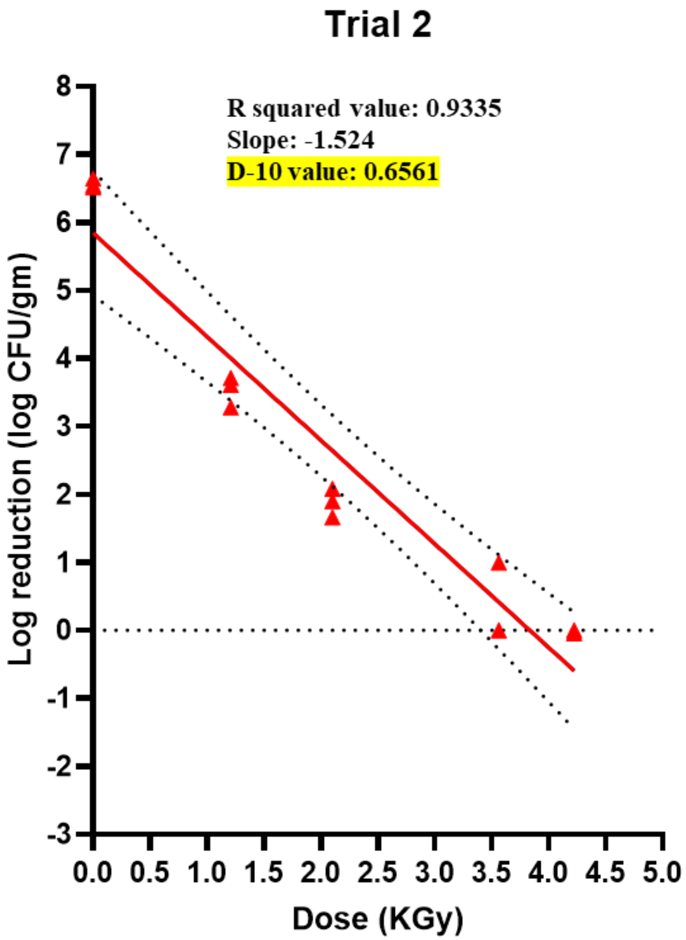
**Prevalence of Salmonella in ground beef has ranged between 1.6% and 4.2% between 2009 and 2011**

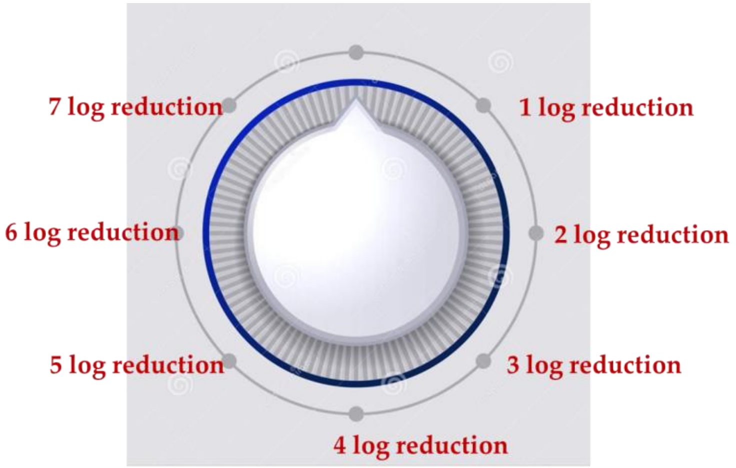
*Bosilevac et al., 2009*
*FSIS 2011*

# Case Study # 2 – What eBeam dose do I need to achieve a 5-log reduction of Salmonella in grindable cheek meat?

# eBeam Technology is effective at eliminating *Salmonella* in cheek meat samples




D-10 value: 0.6 kGy

**For 5 log reduction: 3 kGy min dose**

# Can eBeam Reduce Infection Risks from Rotavirus and Poliovirus on Lettuce?

Assuming Serving size of lettuce (14 g) contaminated ~ 10 viruses

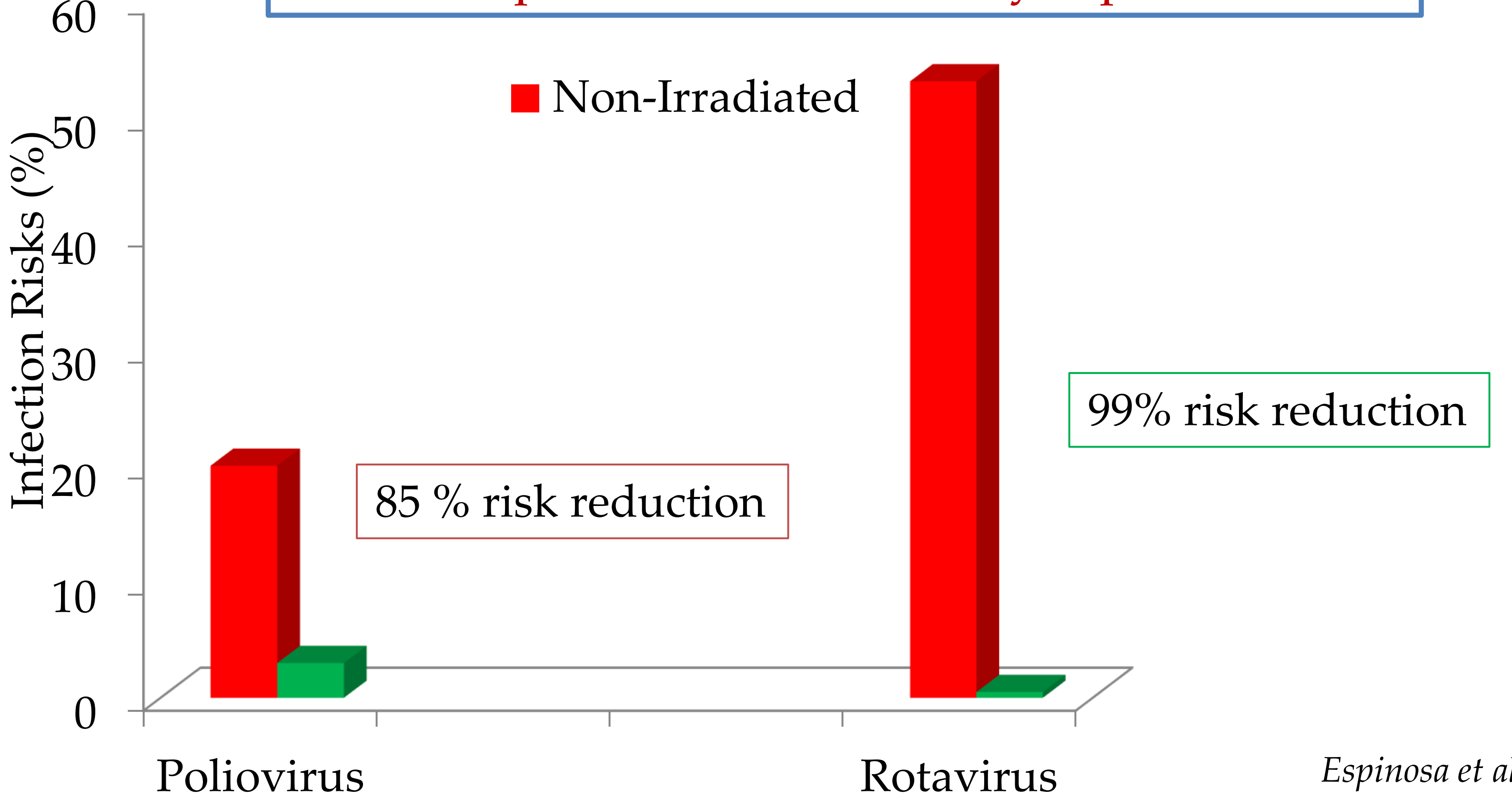
What would be the reduction in Infection Risks if eBeam pasteurization at 3 kGy is performed?

*Espinosa et al., AEM, Feb 2012*

# eBeam Reduces Infection Risks from Rotavirus and Poliovirus on Lettuce

Assuming Serving size of lettuce (14 g) contaminated ~ 10 viruses

What would be the reduction in Infection Risks if E-Beam pasteurization at 3 kGy is performed?




*Espinosa et al., AEM, Feb 2012*

# Can eBeam Reduce Infection Risks from Norovirus and Hepatitis A virus in raw oysters?

**Assuming serving size:** *12 oysters containing ~ 13.68 g meat per oyster and were contaminated with either 100 or 10 viruses per gram*

What would be the reduction in Infection Risks if eBeam pasteurization at 5 kGy is performed?

# eBeam Reduces Infection Risks from Norovirus and Hepatitis A Viruses in Raw Oysters

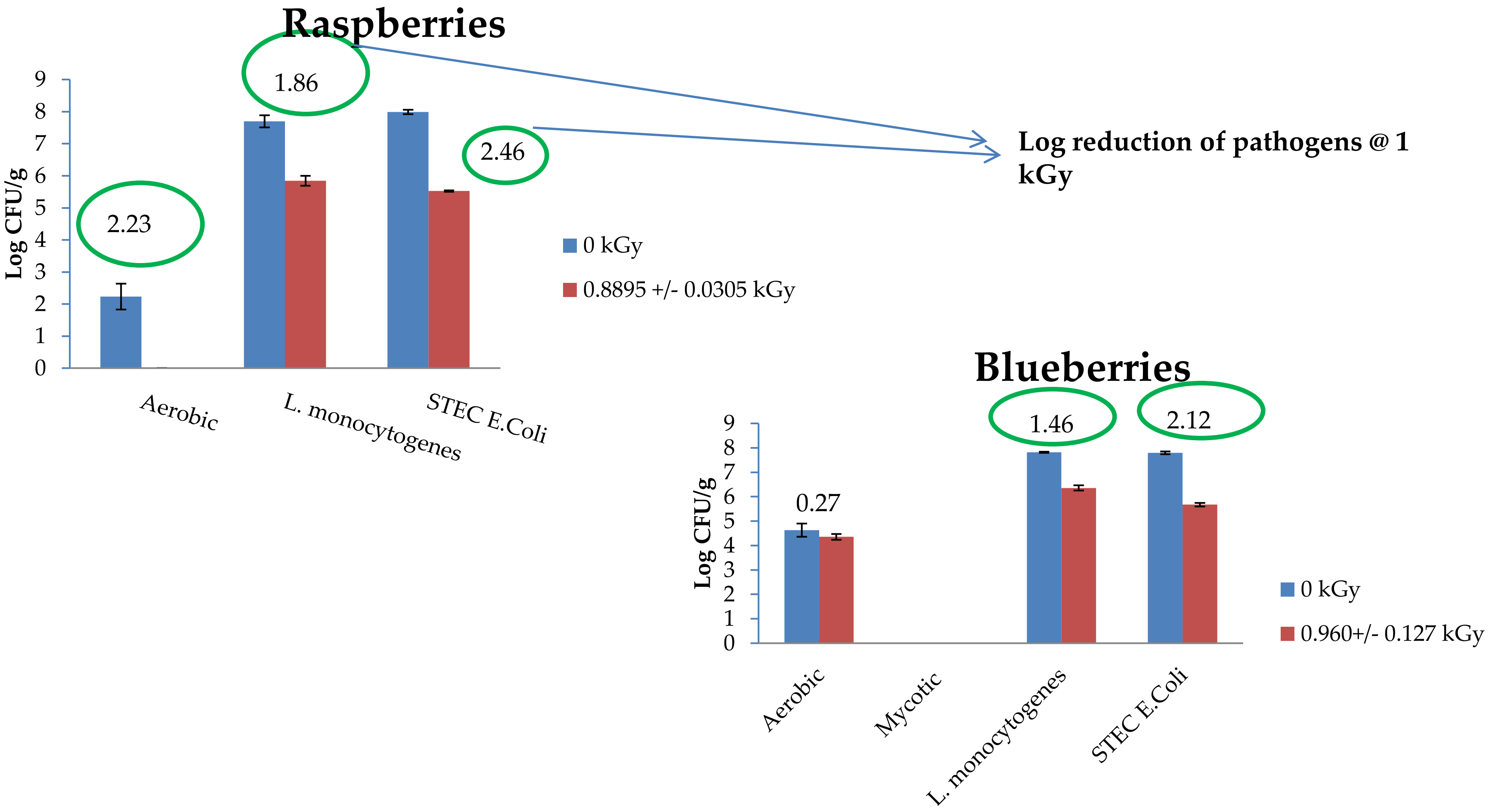
**Assuming serving size:** *12 oysters containing ~ 13.68 g meat per oyster and were contaminated with either 100 or 10 viruses per gram*

The reduction in Infection Risks when eBeam pasteurization at 5 kGy is performed

| Enteric Virus | Reduction in Infection Risks | |
|---|---|---|
| | 100 PFU per gram | 10 PFU per gram |
| Human Norovirus | 15% reduction | 19% reduction |
| Hepatitis A virus | 39% reduction | 74% reduction |

*Praveen et al., AEM, June 2013*

# Pathogen reduction on Berries



# eBeam Reduces Infection Risks from Non O157 STEC E.coli on Strawberries

| Contamination Level (per serving size) | Illness Risks | |
|---|---|---|
| | Without eBeam | with eBeam |
| 100,000 organisms | 2 out of 10 persons | 4 out of 100,000 |
| 10,000 organisms | 5 out of 100 | 4 out of 1,000,000 |

# Significant Reduction in Infection Risks from Raw Milk is eBeam pasteurized at 2.0kGy

| Pathogen | Pathogen Concentration in raw milk (CFU/serving[1]) | Infection risks without eBeam pasteurization | Pathogen Concentration in eBeam pasteurized milk[2,3] (CFU/serving) | Infection risks after eBeam pasteurization | Mean Risk reduction |
|---|---|---|---|---|---|
| *C. jejuni* | Mean: $3.16 \times 10^8$<br>Median: $2.98 \times 10^5$ | Mean: 7.80 / 10 persons<br>Median: 7.83 / 10 persons | < 1 | Mean: 4.34E-21<br>Median: 4.09E-21 | >99.99% |
| *E. coli* O157:H7 | Mean: $1.13 \times 10^8$<br>Median: $2.98 \times 10^5$ | Mean: 9.90 / 10 persons<br>Median: 9.90 / 10 persons | < 1 | Mean: 2.46E-28<br>Median: 6.49E-31 | >99.99% |
| *L. monocytogenes* | Mean: $1.15 \times 10^7$<br>Median: $1.13 \times 10^4$ | Mean: 7.94 / 10 persons<br>Median: 8.01 / 10 persons | < 1 | Mean: 1.52E-07<br>Median: 1.50E-10 | >99.99% |

**Assumptions:**

[1]Serving size: triangular distribution between 0mL – 711mL, with 237mL the most likely

[2]Pasteurization dose: 2.0kGy

[3]*C. jejuni* 28-log reduction; *E.coli* O157:H7 32-log reduction; *L. monocytogenes* 12-log reduction

*Ward et al., 2020*

**How do we confirm microbial inactivation?**

**Plate counts?  Or Molecular Analyses?**

*Earth Age : 4.5 Billion Years ago*

*Microbes : 3.8 Billion Years ago*

*Modern humans : ~ 200,000 years ago*

# Do you know what these are capable of?

- Survives -273°C to 100°C
- Survives heating to 125ºC for several minutes
- Survives extreme pressure (7.5 Gpa) for 12 hours
  - pressure at the depth of about 180 km below the surface of the Earth
- Survives for 31 days after exposure to 4 kGy
- Lives for more than 100 years without food or water by assuming a dehydrated hibernation state (cryptobiosis)

*nasa.gov*



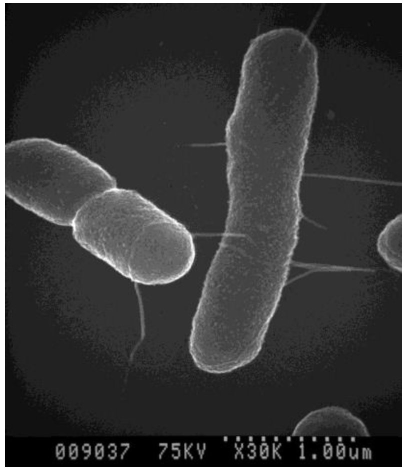
World Population

approx. 7 billion total

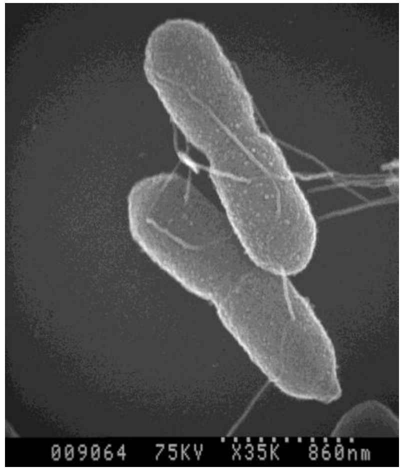

- ***In soil : approx. 6 billion bacteria <u>per gram</u>!!!***
- ***Intestines: approx. 100 trillion bacteria!!***
- ***In human feces : approx. 100 billion <u>per gram</u>!!***

# Irradiated *Salmonella* with intact cell membrane

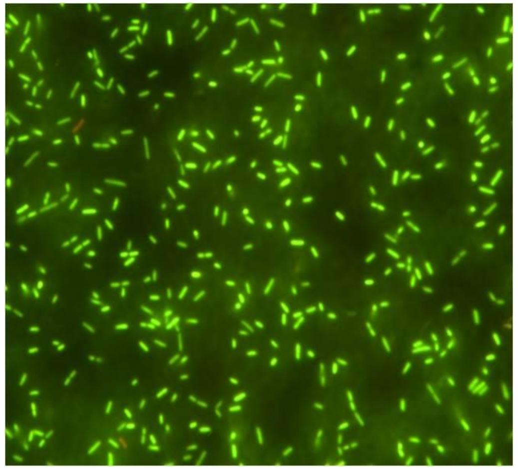
- Electron microscopy
- Membrane integrity



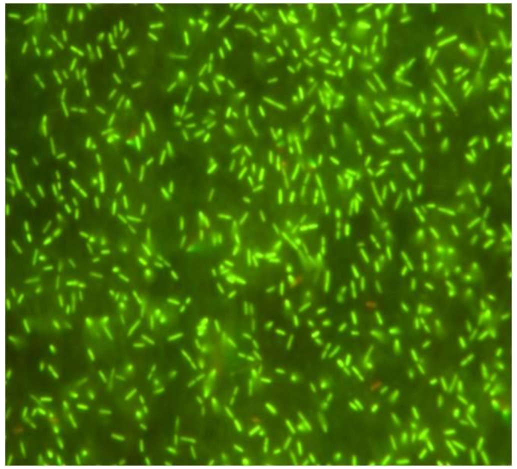

Irradiated
*Salmonella*



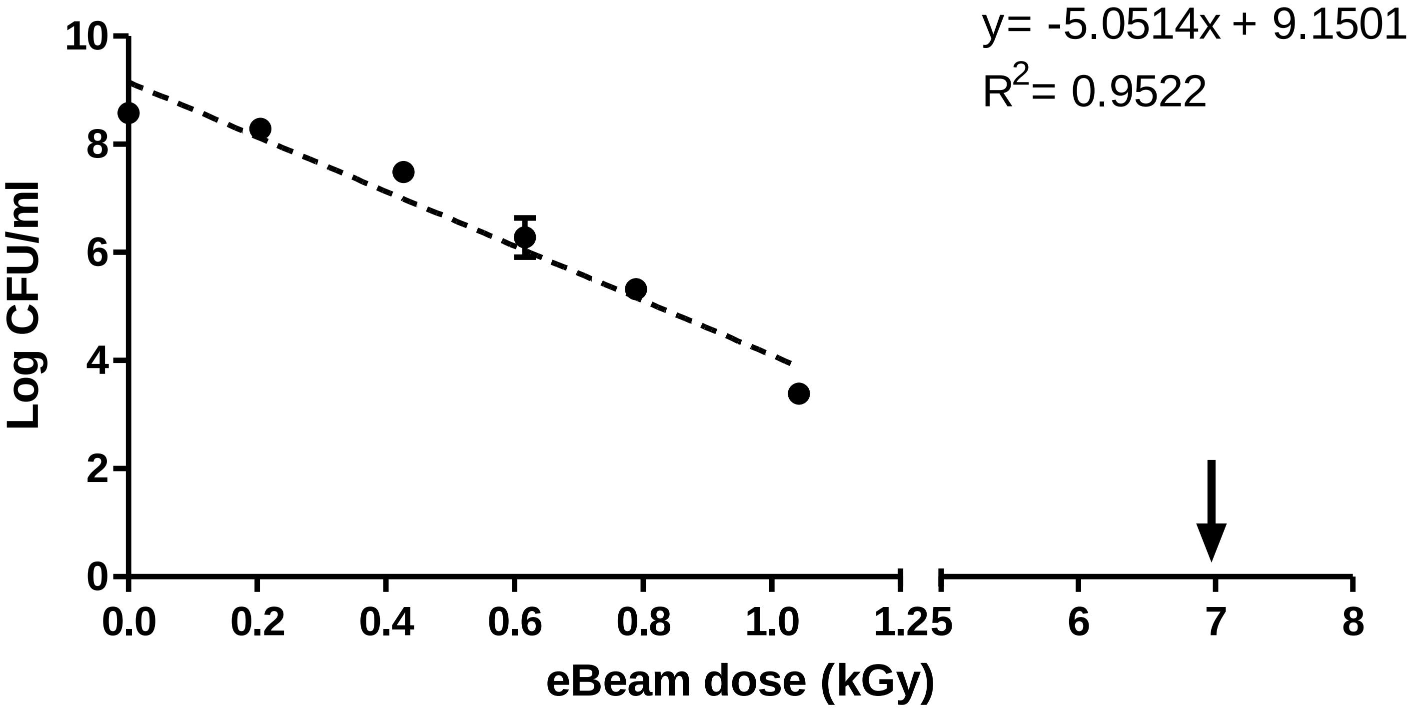

Non-Irradiated
*Salmonella*

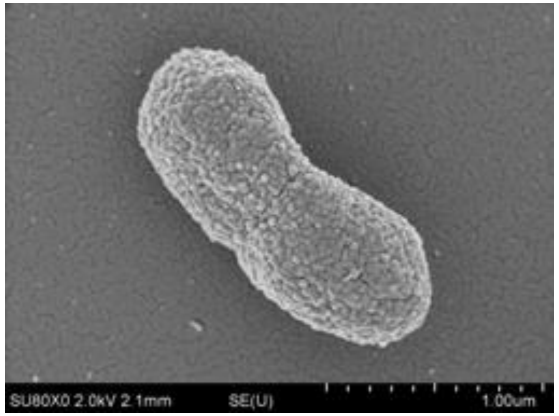


Irradiated
*Salmonella*

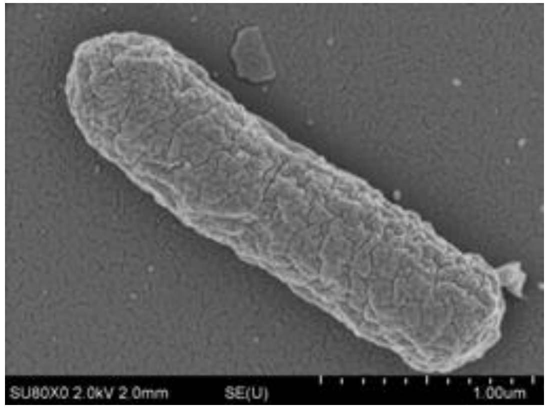


Non-Irradiated
*Salmonella*

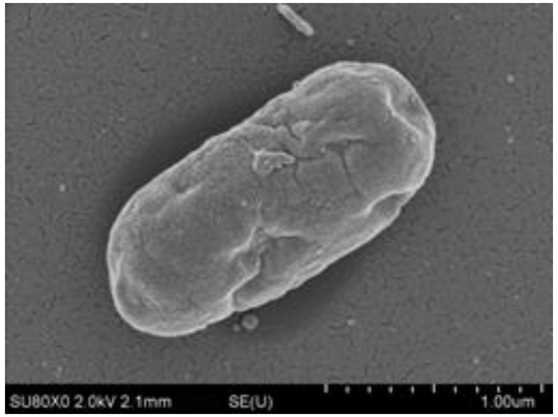



Live



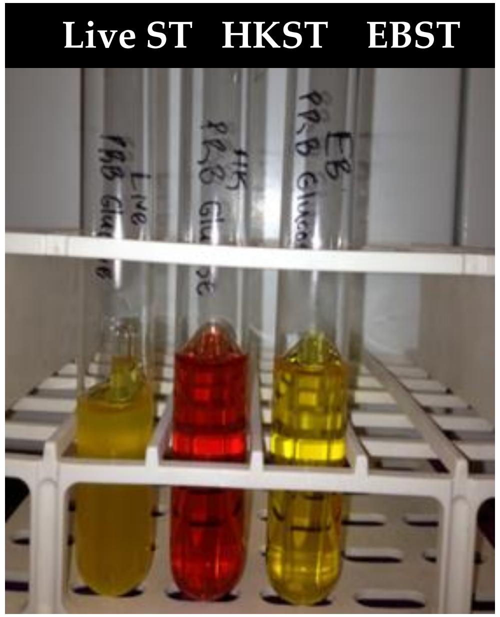

eBeam treated




Heat treated

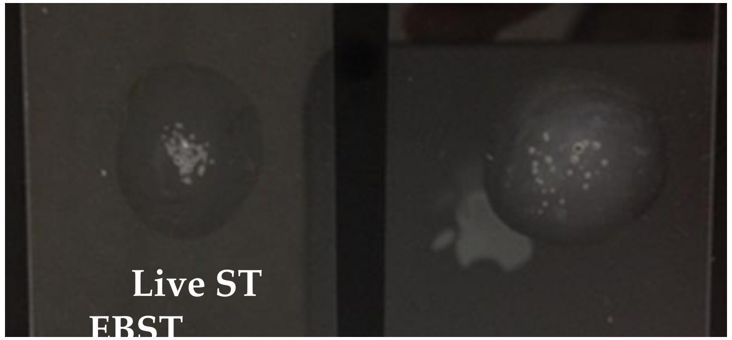
# eBeam "killed" Salmonella sp. cells are metabolically active

Carbohydrate utilization



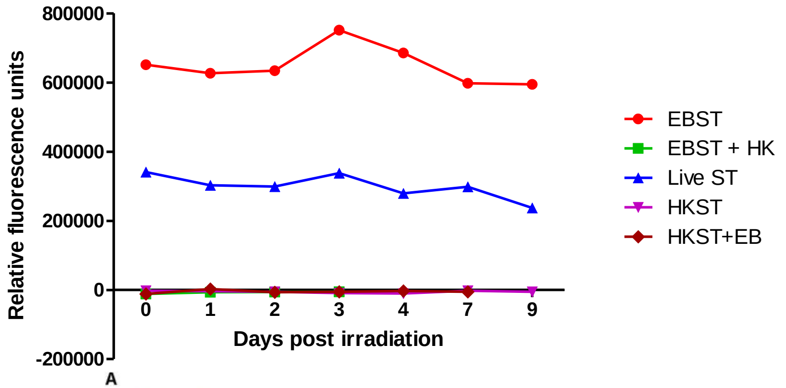

| | Live ST | HK ST | EBST |
|---|---|---|---|
| Color change | + | - | + |
| Gas production | + | - | - |
| Turbidity | + | - | - |

Catalase activity

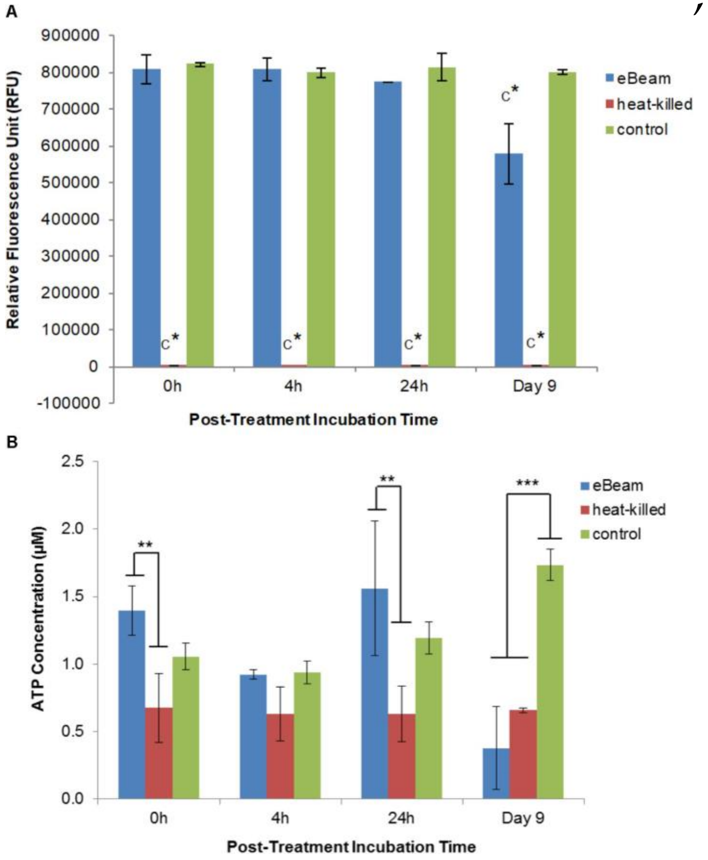



**Metabolically Active yet Non culturable cells(MAyNC)**

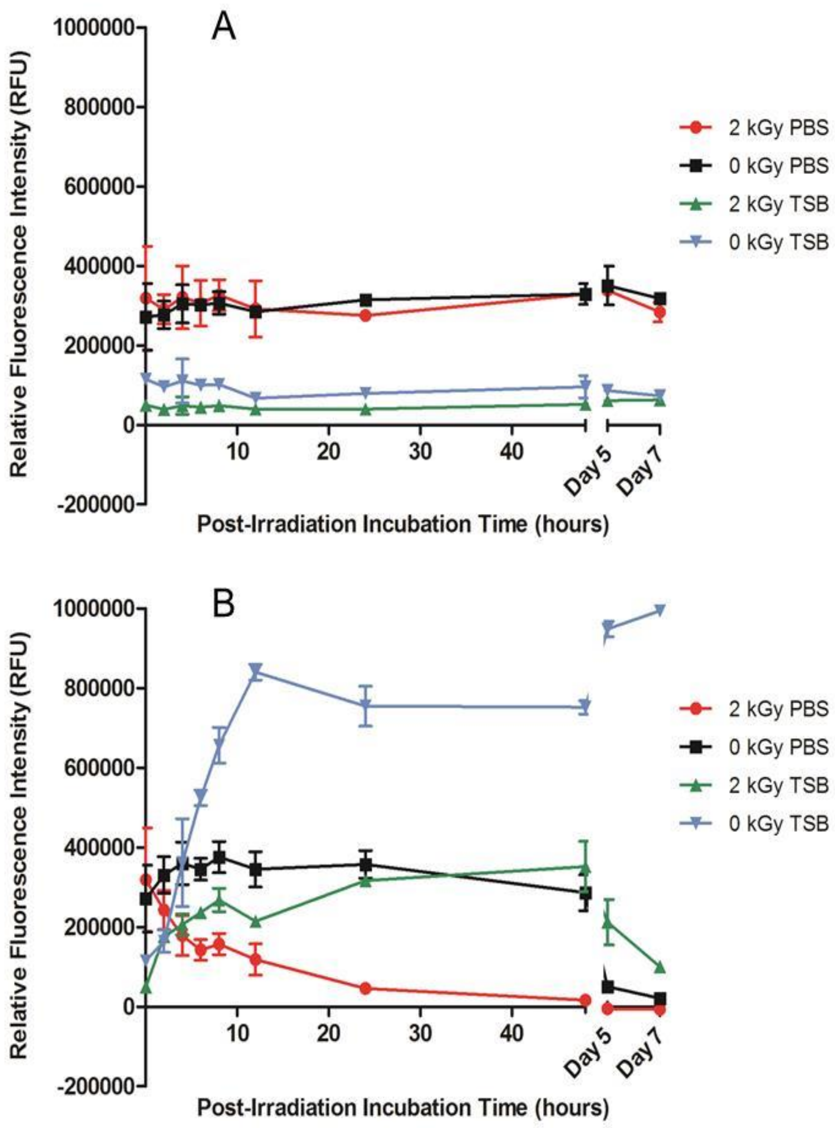
# Electron Beam irradiated cells are metabolically active




*Praveen Ph.D. dissertation , 2015*




*Hieke and Pillai, 2018 Front. Microbiol.*



# DNA Fragment Analysis
## *Experimental Design*

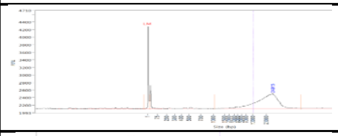
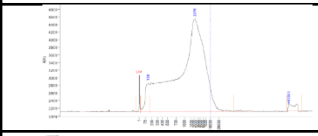
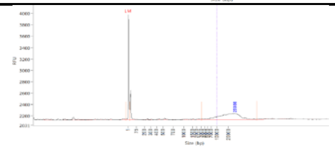
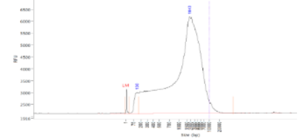
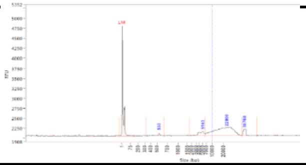
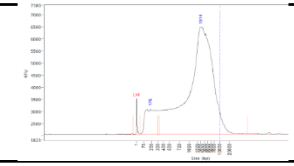
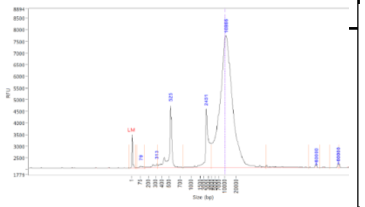
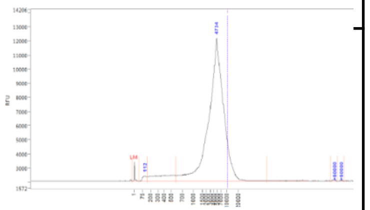
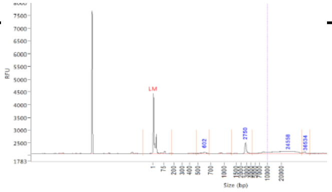
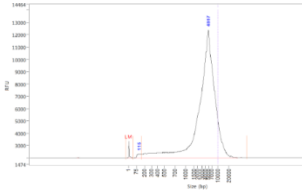
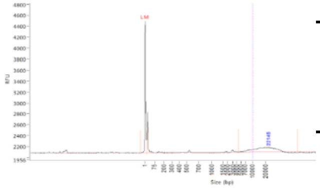
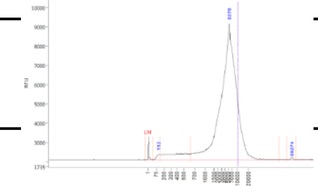
6 E. coli Strains:
- AM076
- AM1087
- 25922
- DY330N
- 11775
- k-12

**Control (0 kGy)** → DNA Purification → Qubit (QC check) → DNA fragment Analysis

**eBeam Irradiated (7 kGy)** → DNA Purification → Qubit (QC check) → DNA fragment Analysis



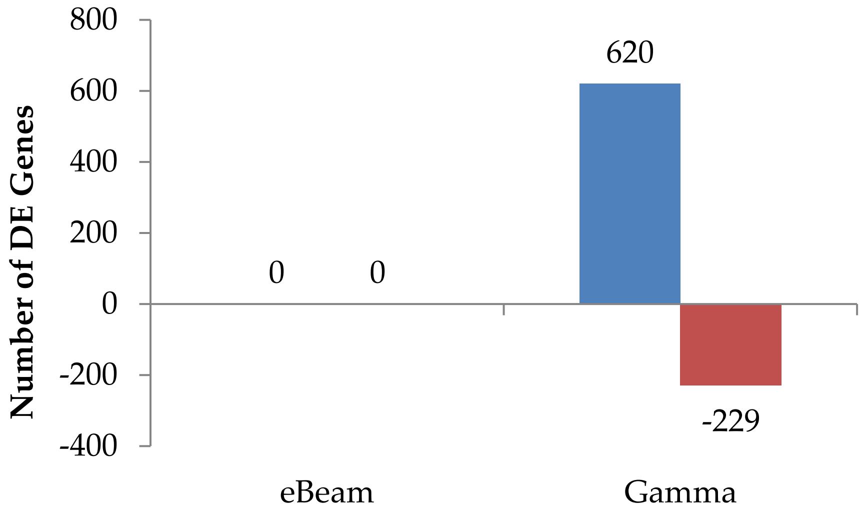
# Fragment Analysis

| Strain AM076 | 0 kGy (Control) | 7 kGy (eBeam) | 0 kGy (Control) | 7 kGy (eBeam) |
|---|---|---|---|---|
| **A** | 19071bp (87.7%) | 103bp (7.05%), 2304bp (90.9%), >60000bp (1.95%) |  |  |
| **B** | 19963bp (71.7%) | 112bp (4.81%), 2146bp (94.7%) |  |  |
| **C** | 496bp (2.38%), 4229bp (6.58%), 19591bp (65.2%), 39372bp (8.96%) | 179bp (13.2%), 2732bp (84.8%) |  |  |
| **Strain AM1087** | **0 kGy (Control)** | **7 kGy (eBeam)** | **0 kGy (Control)** | **7 kGy (eBeam)** |
| **A** | 88bp (0.39%), 258bp (1.04%), 496bp (8.31%), 2681bp (8.89%), 11998bp (79.7%), >6000bp(0.41%) | 102bp (13.6%), 5081bp (90.6%), >60000bp (0.21%) |  |  |
| **B** | 581bp (5.11%), 2768bp (12.4%), 19172bp (61.0%), 35960bp (4.92%) | 106bp (1.34%), 4636bp (98.2%) |  |  |
| **C** | 22145bp (66.7%) | 98bp (1.25%), 5709bp (89.3%), 58984bp (0.31%) |  |  |

# Not all ionizing Irradiations sources the same!

| Comparison | | Total DE genes | % of total genes | upregulated DE genes (log FC[a] ≥ 2) | % of total genes | Downregulated DE genes (log FC ≥ -2) | % of total genes | total # DE genes with log FC ≥ 2, -2 | % of total genes |
|---|---|---|---|---|---|---|---|---|---|
| EB[b]-PBS-4°C | 0h vs 4h | 465 | 8.3 | 313 | 5.6 | 2 | 0.04 | 315 | 5.6 |
| EB-PBS-4°C | 0h vs 24h | 260 | 4.6 | 12 | 0.21 | 22 | 0.39 | 34 | 0.6 |
| EB-PBS-4°C | 4h vs 24h | 98 | 1.7 | 0 | 0 | 87 | 1.5 | 87 | 1.5 |
| | | | | | | | | | |
| G[c]-PBS-4°C | 0h vs 4h | 1 | 0.02 | 1 | 0.02 | 0 | 0 | 1 | 0.02 |
| G-PBS-4°C | 0h vs 24h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G-PBS-4°C | 4h vs 24h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | |
| 0h | EB vs G | 1854 | 33.0 | 288 | 5.1 | 255 | 4.5 | 543 | 9.7 |
| 4h-PBS-4°C | EB vs G | 288 | 5.1 | 44 | 0.78 | 32 | 0.57 | 76 | 1.4 |
| 24h-PBS-4°C | EB vs G | 1601 | 28.5 | 634 | 11.3 | 71 | 1.3 | 705 | 12.5 |
| 4h-TSB-37°C | EB vs G | 2091 | 37.2 | 419 | 7.5 | 20 | 0.36 | 439 | 7.8 |
| 24h-TSB-37°C | EB vs G | 356 | 6.3 | 28 | 0.50 | 39 | 0.69 | 67 | 1.2 |
| | | | | | | | | | |
| 0h | C[d] vs EB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0h | C vs G | 1673 | 29.7 | 620 | 11.0 | 229 | 4.1 | 849 | 15.1 |
| | | | | | | | | | |
| 4h-TSB-37°C | C vs EB | 3525 | 62.7 | 717 | 12.7 | 548 | 9.7 | 1265 | 22.5 |
| 24h-TSB-37°C | C vs EB | 1502 | 26.7 | 603 | 10.7 | 98 | 1.7 | 701 | 12.5 |
| 4h-TSB-37°C | C vs G | 3253 | 57.8 | 1232 | 21.9 | 319 | 5.7 | 1551 | 27.6 |
| 24h-TSB-37°C | C vs G | 2055 | 36.5 | 705 | 12.5 | 179 | 3.2 | 884 | 15.7 |

# What happens to cells immediately after lethal irradiation?




Overall trends:

- **UP**: Bacterial secretion / virulence / cell membrane
  - Proper protein folding

- **DOWN**: ABC transporters / pyruvate metabolism/ amino acid metabolism / carbohydrate metabolism

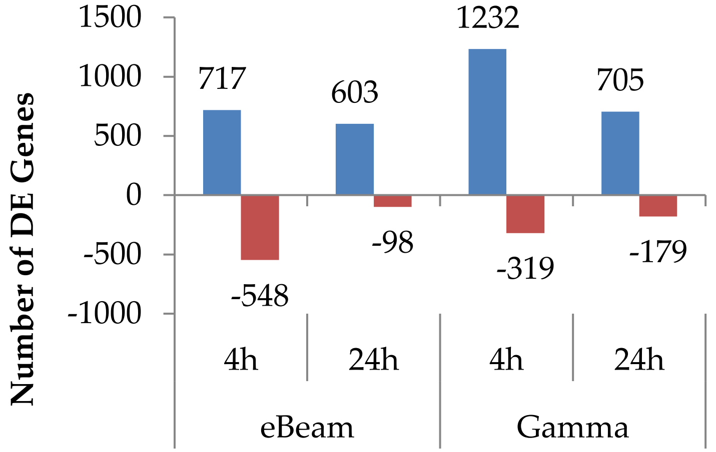
# Are the gene expression patterns in irradiated cells different from non-irradiated (control) cells when incubated under growth conditions?

**eBeam**

- **UP**:
  - **4h**: RNA binding, purine and pyrimidine metabolism, (ribo)nucleotide binding, DNA repair/SOS response, and RNA processing, membrane functions, etc.
  - **24h**: ABC transporters, SOS response/DNA repair, membrane functions, cellular metabolism, etc.
- **DOWN**:
  - **4h**: TCA cycle, oxidative phosphorylation, membrane functions, etc.
  - **24h**: TCA cycle, arginine biosynthesis, pyruvate metabolism

**Gamma**

- **UP**:
  - **4h**: RNA binding, membrane transport, nucleotide, etc. binding, DNA repair/SOS response, and RNA processing, etc.
  - **24h**: ABC transporters, SOS response/DNA repair, membrane functions, etc.
- **DOWN**:
  - **4h**: TCA cycle, oxidative phosphorylation, metal binding, etc.
  - **24h**: oxidative phosphorylation, pyruvate metabolism, glycolysis, etc.



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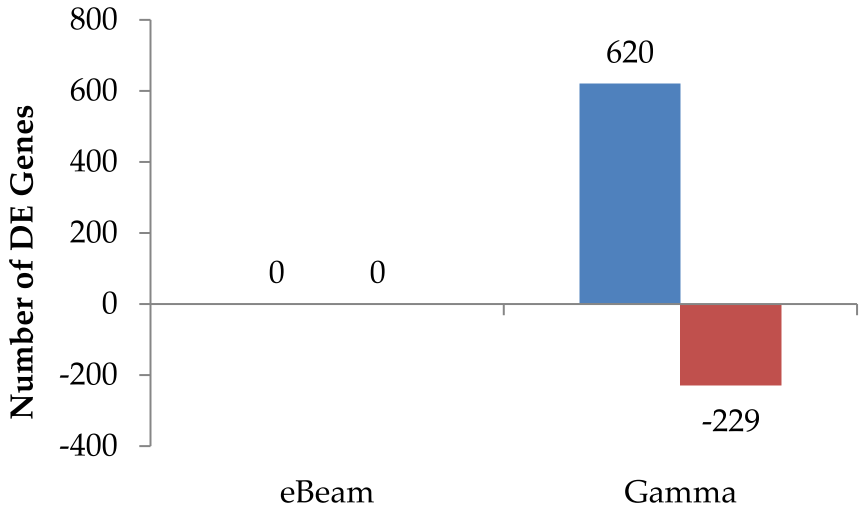


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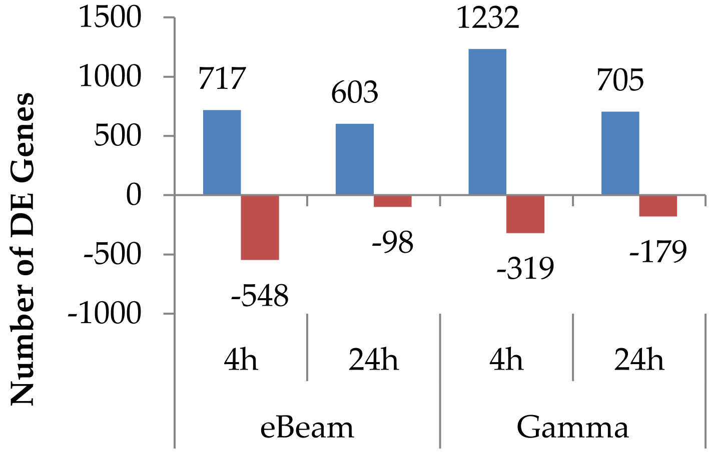
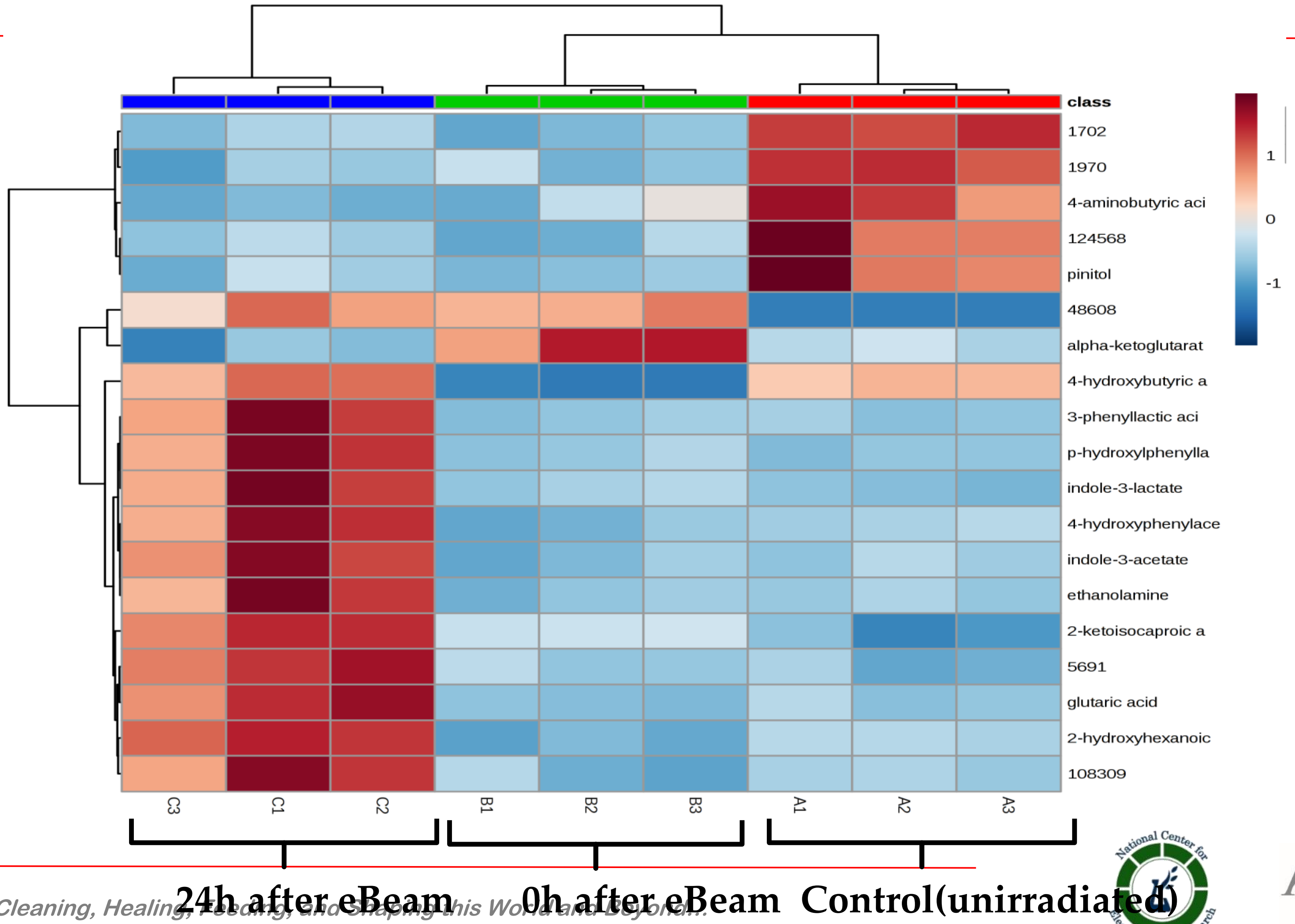


# Different temporal intervals after eBeam treatment induce different metabolomic responses in *E.coli* O26:H11 in PBS



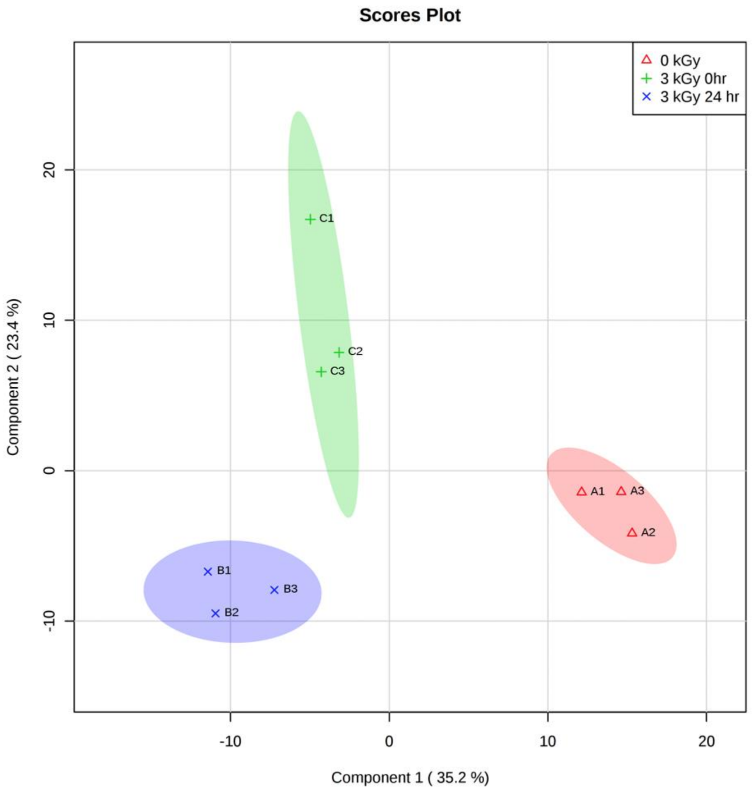



Figure 5. Partial Least Squares-Discriminant Analysis (PLS-DA) scores plot showing differences between the unirradiated cells (0 kGy), irradiated (3 kGy 0 hr), and 24 hour post irradiation (3 kGy 24hr) *E. coli* O26:H11

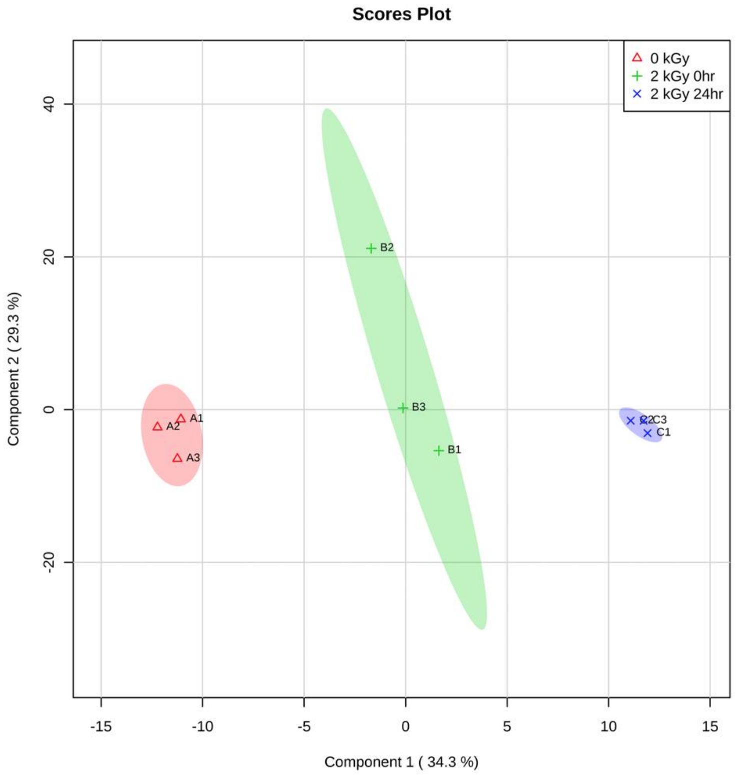



Figure 6. Partial Least Squares-Discriminant Analysis (PLS-DA) scores plot showing differences between the unirradiated cells (0 kGy), irradiated (2 kGy 0 hr), and 24 hour post irradiation (2 kGy 24hr) *S.* Typhimurium

*Bhatia & Pillai, 2019*

# Research Needs

- Deeper understanding of microbial responses to
  - Varying eBeam energies
  - Varying X-ray energies
  - Varying eBeam dose rates
  - Varying X-ray dose rates
- Need to utilize a variety of conventional and molecular analyses to better understand microbial responses

- Applications of Ionizing technology will grow once we have a better understanding of how microbes respond

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