



advanced  
electron beams

**White Paper:**

**Surface Sterilization of Plastic Beverage Pouch using AEB Electron Beam System**

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### **In-Line Surface Sterilization**

Advanced Electron Beams (AEB) offers compact, low energy ( $\leq 150\text{keV}$ ) electron beam emitter technology for a wide range of industrial applications. The small, modular nature of the emitter makes it an ideal technology for the in-line sterilization of medical devices and packaging components, primarily for the pharmaceutical, food, and beverage industries. This technology has the potential to replace bulk sterilization methods, which use traditional high energy electron beam ( $\geq 5\text{MeV}$ ), gamma radiation, chemical treatment, or thermal-based methods. Although the use of low energy electron beams of  $200\text{keV}$  and  $225\text{keV}$  to sterilize the interior surface of open bottles has been proven to be successful<sup>1</sup>, no work had been done at the energy regime below  $200\text{keV}$ . Accordingly, this study was conducted to demonstrate the capability of using low energy electron beam emitters to sterilize the plastic films typically used in pouch, blister, and form-fill-seal (FFS) packaging. The results of this study verify that:

- (1) the achieved kill rate is comparable to those previously obtained by other researches who used ionizing radiation techniques of either electron beam or gamma radiation; and
- (2) the electron energy of  $150\text{keV}$  is sufficient for transmission through the plastic material in order to sterilize the interior surface of the material.

### **Low-Energy Electron Beam Irradiation**

In this study AEB utilized the eBeam 250, an electron beam emitter with beam dimensions of  $75\text{mm} \times 250\text{mm}$ , to irradiate the plastic pouch material used in beverage pouch packaging. The electrons were directed perpendicular to the pouch surface, allowing them to penetrate through the pouch material in order to sterilize the interior surfaces of the pouch.

The pouches are made of three sandwiched layers of polyethylene-aluminum-polyethylene (PAP) with a total thickness of approximately  $120\mu\text{m}$ . The samples were irradiated using a vacuum acceleration voltage of  $150\text{kV}$ , yielding electrons into ambient air with one of the six different doses: 3, 6, 12, 15, 20 and  $25\text{kGy}$ . The pouches, placed directly on the conveyor system tray of an AEB Applications Development Unit (**Figure 1**), moved across the  $75\text{mm}$  width of the emitter during the irradiation process. The electron beam exposure times ranged from 0.3 to 0.5 seconds.



**Figure 1.** AEB Applications Development Unit

### **Biological Test Method**

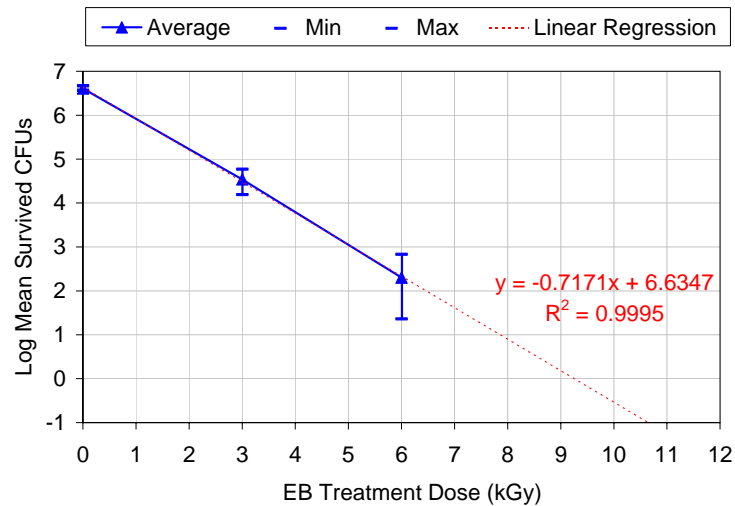
Microorganism inoculation, spore recovery, and microbiological testing of the pouches were performed at our qualified laboratory partner, Apex Laboratories, Inc. The samples were express-shipped to AEB for electron beam treatment and returned immediately to Apex Laboratories for the subsequent microbiological tests. Direct Count of survivors and Fractional Outgrowth methods were used in this test. A suspension of *Bacillus pumilus* ATCC 27142 spores was chosen as the test organism due to its high resistance to ionizing radiation.<sup>2-4</sup> A bacteriostasis study showed no inhibition of outgrowth when 2mls of Tryptic Soy Broth (TSB) containing ~21 Colony Forming Units (CFU) were incubated at 30-35°C.

The Direct Counting method was implemented for low-dose irradiation (3kGy and 6kGy) and control samples (0kGy). The pouch material was cut into a long strip, with a nominal width of 0.25", and the end of the strip was directly inoculated with *B. pumilus* at a concentration of  $\geq 1 \times 10^6$  CFU. The prepared biological strip, after drying at 35-40°C, was aseptically inserted through the spout and placed at the folded bottom area of the pouches. The spout was then closed with the sterile cap. The inoculated side of the strip faced outward through a single layer of the PAP laminate. After electron beam treatment, each pouch was aseptically cut and then each inoculated strip was removed and transferred into a sterile test tube containing Fluid D surfactant. Next, the solution was mechanically agitated on a reciprocal shaker for 1 hour and sonicated for 5 minutes to remove viable spores. The resultant preparation was serially diluted and plated on Tryptic Soy Agar (TSA) and incubated for 48 hours at 30-35°C. The surviving colonies from each exposure dose were counted and the values were used to construct a survivor curve and determine the D-value.

For the irradiation doses greater than 6kGy, the Fractional Outgrowth method was utilized. The pouches were cut open and their interior surfaces were spot inoculated with  $\geq 1 \times 10^6$  CFUs of *B. pumilus* spores. The spore suspension was then dried at ambient temperatures under a laminar flow hood and the open end of the pouch was impulse sealed to create a hermetic package. After electron beam irradiation, an approximate 2ml of TSB was injected into the exposed pouch using a sterile syringe. After the syringe was removed, the needle entry point was sealed with the silicone sealant. The pouches were visually examined for growth over a seven day period of incubation at 30-35°C. Since growth was not readily apparent inside the pouches, a small sample of the broth was then aseptically removed and plated onto TSA. The plated samples were incubated at 30-35°C for two days and examined for the presence of viable organisms. The method was designed to demonstrate that pouch contents were devoid of surviving *B. pumilus*.

### **Survival Curve And Outgrowth**

The number of surviving CFUs recovered from five samples at each dose setting and the associated mean values are graphically plotted as a survival curve in **Figure 2**.



**Figure 2.** Survival curve of EB treated of *B. pumilus*

As can be seen in **Figure 2**, *B. pumilus* inactivation by low energy electron beam of 150kV vacuum accelerating voltage demonstrates a log-linear relationship between the treatment dose and the surviving spore counts. The data is best fitted using the least squares linear regression, where a correlation coefficient is close to one ( $R^2 = 0.9995$ ) and the sum of squared errors is acceptably small ( $SSE = 0.005$ ). The equation of fitted model is expressed in the figure. Using the fitted model's equation, the theoretical dose required for complete kill of spores is calculated to be greater than 9kGy. The results of fractional outgrowth test in **Table 1** support this finding, demonstrating that 100% kill was achieved with the doses ranging from 12kGy to 25kGy.

**Table 1.** Results of fractional outgrowth

Treatment Dose (kGy)	# of Positive Pouches/Total number of Pouches
12	0/7
15	0/7
20	0/7
25	0/7

The  $D_{10}$  value, the dose required to reduce a microorganism population by one log cycle, is also calculated by taking a negative reciprocal of the slope of the linear regression equation. In our experiment the  $D_{10}$  value is 1.4kGy. It is comparable to the values previously reported by several researchers who have used either higher energy electron beams or gamma rays. Other reported values include (a) 1.1 to 1.6kGy for electron beam treatment<sup>1</sup>, (b) 1.6kGy for electron beam treatment<sup>5</sup>, (c) 1.4kGy for gamma ray treatment<sup>6</sup>, (d) 1.6kGy for electron exposure<sup>7</sup>, and (e) 0.6 to 3.0kGy for ionizing radiation<sup>8</sup>.

## Summary

This study successfully demonstrates the feasibility of utilizing the AEB low-energy electron beam technology to (a) penetrate through 120 $\mu$ m plastic packaging material and (b) achieve the 6-log reduction of *B. pumilus* inside the package.

AEB low-energy electron beam irradiation is an alternative technique for microorganism inactivation, providing a room temperature, chemical free process. With its compact size, modular feature, flexible process integration, and low-cost of operation and maintenance, the AEB emitter is ideal for an in-line process to either sterilize package surfaces or reduce microbial contamination for both extended shelf-life and aseptic packaging applications. Furthermore, the technology eliminates chemical and water usage, enabling a reduction of the energy and waste treatment expenses typically associated with chemical sterilization processes. As such, any concern for residual chemicals coming into direct contact with food and beverages is also eliminated. With its room temperature process, the thermal budget and its associated carbon footprint are reduced to realize energy and operating cost savings. In comparison to a traditional hot-fill process, in which the package must withstand high temperature, the cold sterilization method that uses low-energy electron beams allows the reduction of the quantity of resin needed for the packaging materials.

## References

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