Shiga toxin producing Escherichia coli on beef

Shiga toxin-producing Escherichia coli O157 remains a pathogen of great concern for CDC, USDA and the beef industry because it continues to be responsible for many outbreaks and human illnesses. Even with a range of chemical antimicrobial interventions being applied in a multi-hurdle approach, Shiga toxin-producing Escherichia coli (STEC) remains a public health concern, with close to 5,000 cases recorded every year in the US alone.

Up to 99% Effectiveness without compromises

Beef processors are looking for more effective post harvest interventions. PhageGuard E reduces E. coli O157 up to 99% on beef carcasses, primals, subs and trimmings.

PhageGuard E consists of a two phage cocktail that specifically kills E. coli O157. The phages were isolated in nature and have been selected for its efficacy against a large database of E. coli O157 strains.

Phages are the natural enemy of bacteria. Unlike harsh chemical interventions, phages are completely harmless for plant workers, equipment, concrete floors and water treatment installations.

Unlike traditional chemical treatments, PhageGuard E kills E. coli O157 without affecting organoleptic properties such as taste, odor or texture in beef products.

All in all, this makes PhageGuard E a smart, green and easy to apply additional hurdle to further enhance food safety.
How to use PhageGuard E

PhageGuard E is used as a post-harvest spray or dip application. A PhageGuard E solution can be applied on carcasses after the hot box or on cold carcasses in the grading room by using a conventional spray cabinet. PhageGuard E can also be applied further down in the process on primals, subprimals and trimmings. For successful phage applications the 3 D’s of success are important:

- **Distribution** – get full coverage of the surface area and keep the surface wet as the phage must encounter the bacteria for an instant kill
- **Dosage** – using the right amount of phage to assure adequate kill
- **Dwell or contact time** - allow a minimum of 30 minutes before the next surface intervention (inactivating the phage) or grinding (exposing significantly more surface area). Most of the killing effect happens in the first 30 minutes. On wet surface areas, phage remain effective for up to 24 hours.

Effectiveness of PhageGuard E

To assess the effectiveness of PhageGuard E, refrigerated beef samples were contaminated with different *E. coli* O157 strains at a level of approximately 1x10^5 cfu/cm². Following contamination, beef samples were treated with PhageGuard E dilutions to reach 3x10^7 PFU/cm² or 3x10^8 PFU/cm², or alternatively with tap water (Control) to serve as a negative control. Samples were incubated for 24 hours at 4°C (39°F) before the *E. coli* O157 cells were retrieved and enumerated. Data is an average of three independent experiments with two samples per treatment.

**Figure 1. PhageGuard E reduces *E.coli* on beef**

![Graph showing the effectiveness of PhageGuard E over time for different strains.](image)

**Performance PhageGuard E over time**

![Graph showing the performance of PhageGuard E over time.](image)
This research demonstrated that PhageGuard E reduced E. coli O157 with 1.5 log (97%) within 2 hours after treatment and 1.7 log (98%) after 6 hours on refrigerated beef. Six hours after the PhageGuard treatment no further reductions have been observed.

**Performance PhageGuard E versus chemical interventions**

Figure 2. PhageGuard E reaches maximum reduction after 6 hours on refrigerated beef

To assess the kinetics of E. coli O157 kill by PhageGuard E, refrigerated beef samples were contaminated with a single E. coli O157 strain at a level of approximately 5x10⁴ cfu/cm². Subsequently, half of the beef samples were treated with 3x10⁸ PFU/cm² PhageGuard E, while the other half was treated with an equivalent volume of tap water (Control). All samples were incubated at 4°C (39°F) before the E. coli O157 cells were retrieved and enumerated at the indicated time points. Data is an average of three independent experiments with two samples per treatment.

Figure 3. PhageGuard E outperforms Lactic Acid on refrigerated beef

The ability of PhageGuard E to kill E. coli O157 on refrigerated beef was compared to lactic acid, a widely used antimicrobial on beef. To this end, refrigerated beef samples were inoculated with a single E. coli O157 strain at a level of 1x10⁴ CFU/cm² and either vacuum packed (Vac.) or not vacuum packed (No Vac.). All beef samples were subsequently incubated at 4°C (39°F) for 24 hours. Next, the vacuum-packed samples (Vac.) were removed from their package, after which the beef samples were treated with either buffered peptone water (BPW, Control), lactic acid (4.5%), or PhageGuard E (3x10⁸ PFU/cm²). The non-vacuum packed samples were treated similarly. After all treatments, half of the samples per treatment were vacuum packed, while the other half was not vacuum packed. This results in four vacuum regimes, namely samples being vacuum packed both after contamination and treatment (Vac. + Vac.), samples only vacuum packed after contamination but not after treatment (Vac. + No Vac.), samples only vacuum packed after treatment but not after contamination (No Vac. + Vac.), or samples not being vacuum packed at all (No Vac. + No Vac.). After the treatment, all samples were incubated for 24 hours at 4°C (39°F) before the E. coli O157 cells were retrieved and enumerated. Data presented is an average of two independent experiments with two samples per treatment.
This research shows that PhageGuard outperformed the use of 4.5% lactic acid on all four treatments. PhageGuard reduced E. coli O157 consistently with around 1 log (90%) whereas lactic acid intervention resulted in reductions varying from 0 to 0.2 log (4 to 47%).

**USDA & FDA approved**

PhageGuard E is USDA and FDA approved. FSIS has completed its evaluation and has no objection to the use of the E. coli O157 bacteriophage preparation as an antimicrobial spray, mist, or wash application (or a mix of these application methods) on beef carcasses, primals, subprimal cuts, and trimmings at levels up to $10^6$ PFU/g of food to control E. coli O157. No labeling statement is required when used under the accepted conditions of use.

**Conclusions**

- PhageGuard E is a natural and effective antimicrobial against E. coli O157 on beef, reducing the E. coli O157 up to 2 log.
- PhageGuard E has a maximum efficacy on refrigerated beef 6 hours after application.
- On refrigerated beef, PhageGuard E is a more effective antimicrobial against E. coli O157 than chemical interventions.

For more information regarding this application data sheet please use the following contact information.

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