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Technical Bulletin

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Testing of *Salmonella* bacteriophage preparation for presence of free *Salmonella* DNA

Introduction

Of the various organisms causing foodborne illness in the United States, the Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* alone causes approximately 1.3 million infections, 26,000 hospitalizations and 400 deaths in the United States every year [1]. It is estimated that *Listeria monocytogenes* causes significantly fewer infections, approximately 1,600, but a higher percentage of these infections end in death. On average, the case-fatality rate is 15 – 30%, and it can be more than 80% in the case of perinatal and neonatal infections [2].

The food industry is constantly looking for technologies to reduce the risk of these and other foodborne pathogens to keep the food supply safe. One of these technologies is the use of bacteriophage to reduce the population of pathogens in the processing environment. Bacteriophage, or phages, are viruses that infect bacteria and replicate within them. There has been evidence of phages being used for human benefit since the early 20th century [3]. Phages are a novel approach to the reduction of pathogens in the food production process. The use of phages to eliminate Listeria in ready to eat (RTE) poultry and meat products was first approved by the USDA in 2006 [4]. Since then, *Salmonella* and *E. coli* phages have also been approved for use in meat & poultry products. Unlike disinfectants, phages are pathogen specific, leaving beneficial microflora in place.

For commercial purposes, phages are grown via fermentation. Once the fermentation is completed, a series of processes occur in order to separate the phage from the growth medium and the bacterial host cells used for viral propagation. During this process, a series of filters is used, however due to the size of phage and residual DNA from the growth medium, there is some transfer of free (“dead”) bacterial host DNA into the phage solution. DNA left behind from the dead bacterial cells can interfere with DNA-based detection methods used for pathogen testing. This can lead to potential false positive results.

Objective

The objective of the study was to determine the presence of free DNA in commercial preparations of Micreos PhageGuard S (anti-*Salmonella*). Multiple commercial lots of the phage were tested at various concentrations. DNA was detected using the 3M™ Molecular Detection System with the 3M™ Molecular Detection Assay 2 - *Salmonella*. The assay is based on Loop-mediated Isothermal Amplification (LAMP) of DNA and a reporter based on bioluminescence. The detection of DNA at

different concentrations of the preparation was used to create a probability curve to model the chance of obtaining false positive results when sampling areas that have been treated with these phage preparations.

Materials

Micreos PhageGuard S

Four lots of commercial Micreos PhageGuard S

- Lot A: 19M27-S
- Lot B: 19N05-S
- Lot C: 20A16-S
- Lot D: 19L07a-S

3M Molecular Detection System

- 3M™ Molecular Detection System Instrument (MDS100)
- 3M™ Molecular Detection Assay 2 - Salmonella (MDA2SAL96)
- 3M™ Molecular Detection Matrix Control (MDMC96)

Diluent

- Bottled drinking water

Methods

Four lots of Micreos PhageGuard S, with different production dates, were diluted in water to achieve the concentrations described in Table 1. From each dilution, 20-µL aliquots were analyzed using the 3M™ Molecular Detection Assay 2 - *Salmonella*, following the instructions for use [5]. Nine replicate samples were tested for each dilution. To ensure that the phage preparation did not interfere with the assay, the 3M™ Molecular Detection Matrix Control was used to analyze selected samples per manufacturer instructions [6]. The probability of detection was calculated in a similar way as described previously [7].

Table 1. Bacteriophage preparations for the study.

Lot code	Approximate concentration (PFU/mL)	Concentration from stock solution (%)
19M27-S	2.0×10^{11}	100.0
	2.5×10^{8}	0.125
	2.5×10^{7}	0.012
19N05-S	2.0×10^{11}	100.0
	2.5×10^{8}	0.125
	2.5×10^{7}	0.012
20A16-S	2.0×10^{11}	100.0
	2.5×10^{8}	0.125
	2.5×10^{7}	0.012
19L07a-S	1.0×10^{11}	50.00
	1.0×10^{10}	5.000
	2.0×10^{9}	1.000
	1.0×10^{9}	0.500
	2.0×10^{8}	0.100

Results and Discussion

Four commercial lots of anti-*Salmonella* bacteriophage were diluted and tested for the presence of *Salmonella* DNA. The number of positives at each phage concentration were compared to the total number of replicates to calculate the percentage (%) positives. The results of these replicates are shown in Table 2 and summarized in Table 3. Using the stock concentration, there was no interference with the LAMP-bioluminescent assay as determined with the 3M Molecular Detection Matrix Control. This result demonstrates that the phage preparation does not hinder the DNA amplification and bioluminescent reporter reactions.

All samples of PhageGuard S were positive for *Salmonella* DNA at 50% and 100% strength (1-2 x 10^{11} PFU/mL). At a 5% (1 x 10^{10} PFU/mL) PhageGuard S concentration, the positive detection rate for *Salmonella* DNA decreased to 50%. At 1% (2 x 10^{9} PFU/mL) concentration, the positive rate decreased further to 20%. A further decrease of positives was detected at 1.0 x10^{9} PFU/mL (0.5%), where only 3 out of 60 replicates (5%) were positive. At a PhageGuard S concentration of 2.5 x10^{8} PFU/mL (0.125%), out of 564 replicates across three lots, only 5 samples were positive (0.89%). At 2.0 x10^{8} PFU/mL (0.1%), none of the 60 replicates were positive, and at 2.5 x10^{7} PFU/mL (0.012%), only one sample was positive out of 564 replicates (0.18%). This last concentration is close to the recommended concentration for applications within a food processing facility. Figure 1 shows the probability of detection for the estimated limit of detection.

Table 2. Detection of *Salmonella* DNA from four lots of the anti-Salmonella bacteriophage preparation PhageGuard S with 3M Molecular Detection Assay 2- Salmonella.

Lot designation	Approximate concentration (PFU/mL)	Concentration from stock solution (%)	Replicates	Positives	% Positives
19M27-S	2.0x10^{11}	100.0	3	3	100.0
	2.5 x10^{8}	0.125	188	2	1.1
	2.5 x10^{7}	0.013	188	1	0.5
19N05-S	2.0x10^{11}	100.0	3	3	100.0
	2.5 x10^{8}	0.125	188	3	1.6
	2.5 x10^{7}	0.013	188	0	0.0
20A16-S-S	2.0x10^{11}	100.0	3	3	100.0
	2.5 x10^{8}	0.125	188	0	0.0
	2.5 x10^{7}	0.013	188	0	0.0
19L07a-S	1.0 x10^{11}	50.00	10	10	100.0
	1.0 x10^{10}	5.000	10	5	50.0
	2.0 x10^{9}	1.000	10	2	20.0
	1.0 x10^{9}	0.500	60	2	3.0
	2.0 x10^{8}	0.100	60	0	0.0

Table 3. Summary of Salmonella DNA detection rate from testing four lots of anti-Salmonella bacteriophage preparation PhageGuard S using 3M Molecular Detection Assay 2- Salmonella

Concentration from stock solution (%)	Approximate concentration (PFU/mL)	Replicates	Positives	% Positives
100.0%	$2.0x10^{11}$	9	9	100.0%
50.00%	$1.0\ x10^{11}$	10	10	100.0%
5.000%	$1.0\ x10^{10}$	10	5	50.0%
1.000%	$2.0\ x10^{9}$	10	2	20.0%
0.500%	$1.0\ x10^{9}$	60	2	3.0%
0.125%	$2.5\ x10^{8}$	564	5	0.89%
0.100%	$2.0\ x10^{8}$	60	0	0.00%
0.013%	$2.5\ x10^{7}$	564	1	0.18%

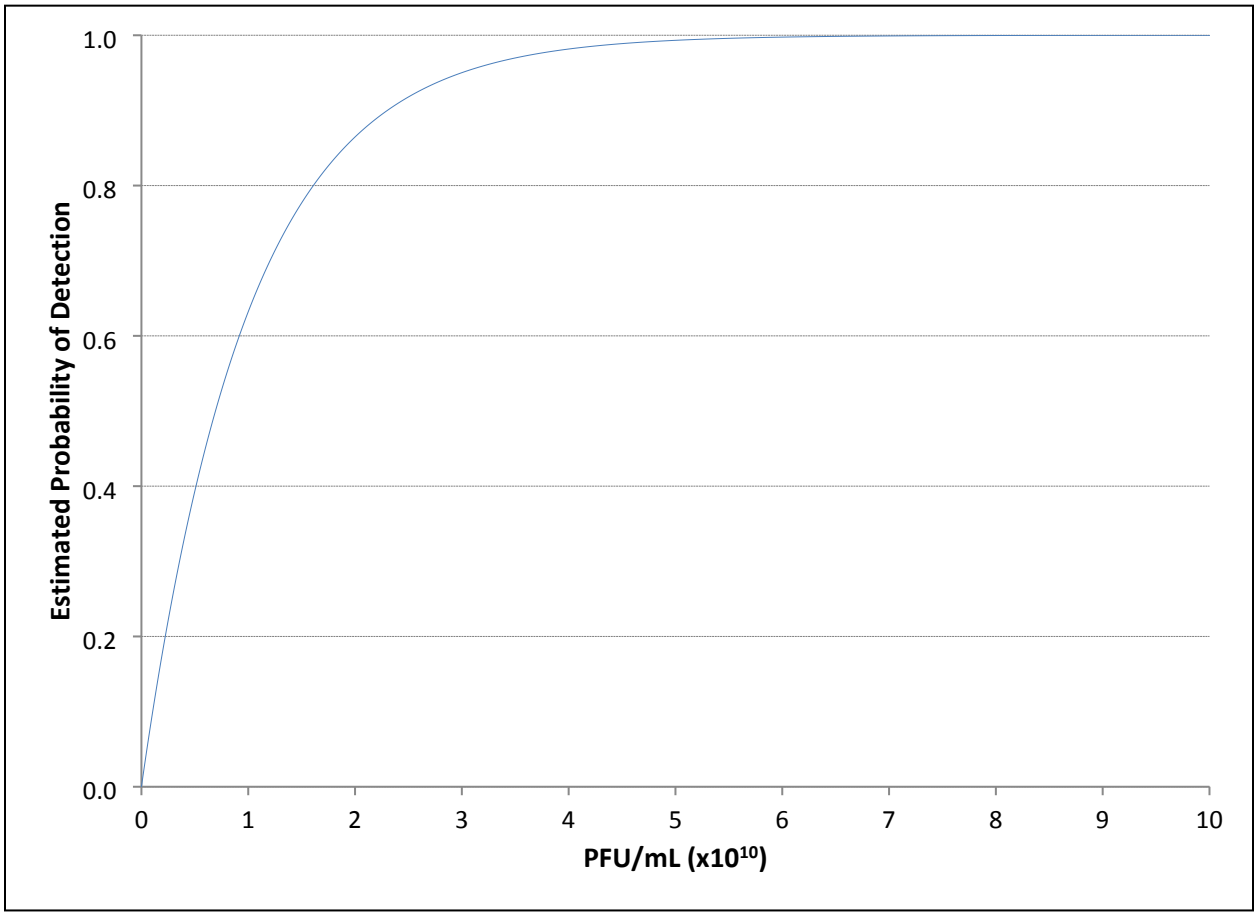

Figure 1. Probability of detection of Salmonella DNA in an anti-*Salmonella* bacteriophage preparation using a DNA-based detection system targeting *Salmonella* spp.

Conclusion

The maximum approved concentration of phage in a working solution for use in beef and poultry processing facilities is $1x10^8$ PFU/g. When considering the sample size and the enrichment process used for pathogen testing in foods, it can be calculated that the concentration of phage introduced into an enrichment solution would be approximately 2.5×10^7 PFU/mL for meat and approximately 1.7×10^7 PFU/mL for poultry. This is the result of a 1:4 sample to broth dilution ratio for enrichment. These levels are equal to or lower than the lowest concentrations tested in this study. Therefore, under the conditions tested, the expected rate of false positive detection due to application of PhageGuard S on a commercial food production facility is extremely low, approximately ≤ 0.2%.

References:

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