DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

Form Approved: OMB No. ; Expiration Date:
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: n/a

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Edith Chow	Research Scientist

Company *(if applicable)*: Gum Products International, Inc.

Mailing Address *(number and street)*: 1255 Journey's End Circle

City	State or Province	Zip Code/Postal Code	Country
Newmarket	Ontario	L3Y8T7	Canada

Telephone Number	Fax Number	E-Mail Address
905-853-8828	905-853-8886	edith.chow@gpiglobal.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position

Company *(if applicable)*:

Mailing Address *(number and street)*:

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

GPI Biotech VAM-S

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Paper

☒ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages 35

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN

☐ b) GRAS Affirmation Petition No. GRP

☐ c) Food Additive Petition No. FAP

☐ d) Food Master File No. FMF

☐ e) Other or Additional *(describe or enter information as above)*

6. Statutory basis for determination of GRAS status *(Check one)*

☒ Scientific Procedures *(21 CFR 170.30(b))* ☐ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

The intended use of GPI Biotech VAM-S is an antimicrobial on food to control *Salmonella* at an application rate of up to 2×10^8 PFU (plaque forming units) per gram of food.

Food categories include poultry, eggs, red meat, fruits, vegetables, fish, and shellfish.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☒ Yes ☐ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	
1	Bacteriophages	n/a	n/a		
2					
3					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s)),* and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

GPI Biotech VAM-S consists of a mixture of 3 bacteriophages (phages) that were isolated from farms in different geographical areas of Spain. All 3 phages are *Salmonella*-specific lytic phages that have the ability to lyse a wide range of *Salmonella enterica* serovars.

Phages have a low inherent toxicity due to their composition of only proteins and DNA. Numerous studies have demonstrated that consumption of phages is harmless to humans or animals (for sources please refer to attached GRAS notice).

3. Synonyms

Provide as available or relevant:

1	Bacteriophages (phages)
2	
3	

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- ☒ Any additional information about identity not covered in Part V of this form
- ☒ Method of Manufacture
- ☒ Specifications for food-grade material
- ☒ Information about dietary exposure
- ☒ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☒ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☒ Comprehensive discussion of the basis for the determination of GRAS status
- ☒ Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Edith Chow
(name of notifier)

has concluded that the intended use*(s)* of GPI Biotech VAM-S
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☒ Edith Chow *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Edith Chow *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

1255 Journey's End Circle, Newmarket, Ontario, Canada
(address of notifier or other location)

Edith Chow *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ----------
(GRAS Affirmation Petition No.)

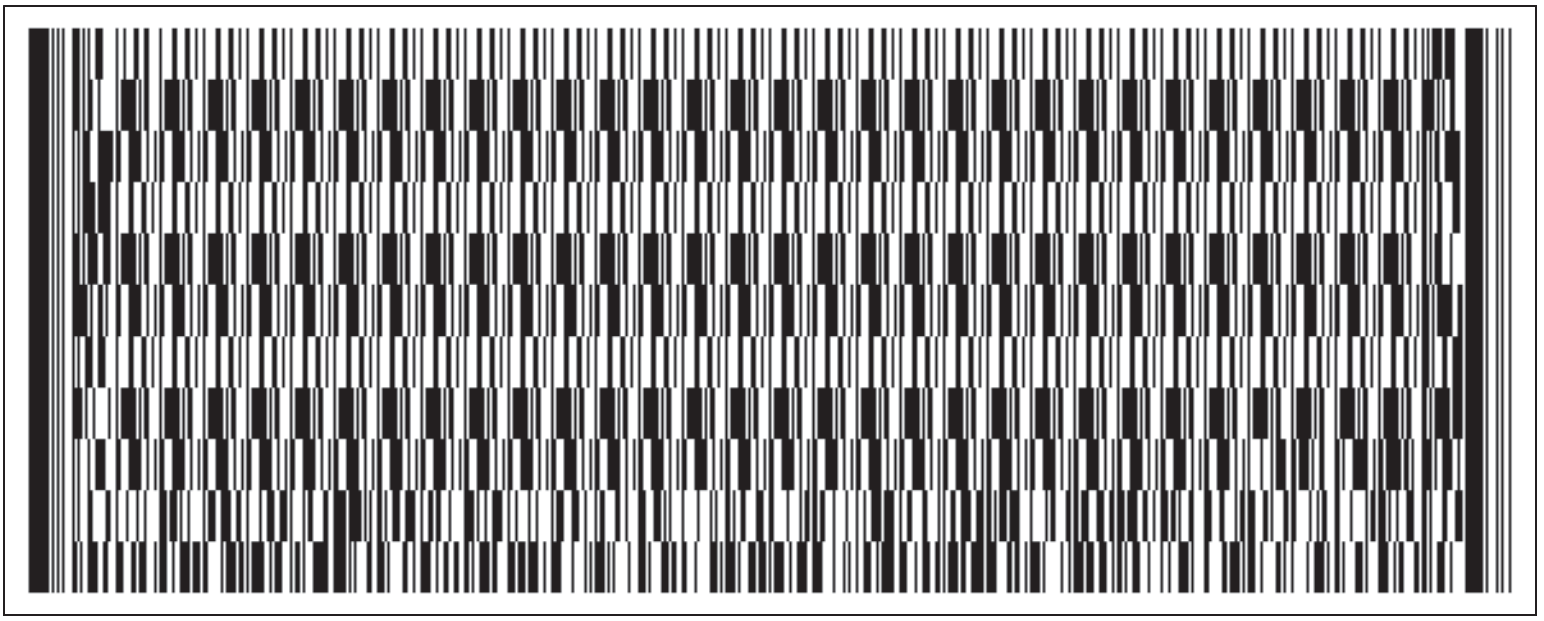
3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Edith Chow Digitally signed by Edith Chow Date: 2020.03.04 07:59:39 -05'00'	Edith Chow	03/04/2020

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	GRASNotice_GPIVAMS_2020-03-04.pdf	Submission (Page numbers - 35)

OMB Statement: Public reporting burden for this collection of information is estimated to average XX hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.



Gum Products International, Inc

GRAS NOTIFICATION:

GPI Biotech VAM-S

Table of Contents

PART 1 Signed Statements and Certification

1.1 Compliance with 21 CFR 170.225

Gum Products International (GPI), Inc. is hereby submitting a GRAS notice in accordance with 21 CFR 170.225.

1.2 Name and Address of Notifier

Gum Products International
1255 Journey's End Circle
Newmarket, Ontario L3Y 837
CANADA

1.3 Name of Notified Substance

GPI Inc. manufactures a *Salmonella*-specific bacteriophage cocktail under the commercial name GPI Biotech VAM-S.

1.4 Intended Use of the Notified Substance

The intended use of GPI Biotech VAM-S is an antimicrobial on food to control *Salmonella* at an application rate of up to 2×10^8 PFU (plaque forming units) per gram of food.

Food categories include poultry, eggs, red meat, fruits, vegetables, fish, and shellfish.

1.5 Basis for GRAS Determination

Pursuant to 21 CFR 170.30(a)(b), GPI Biotech has determined that GPI Biotech VAM-S is GRAS through scientific procedures.

1.6 Exemption from Premarket Approval

GPI Biotech VAM-S was determined by GPI to be GRAS and thus is exempt from premarket approval requirements when used under the intended use conditions described within this notification.

1.7 Availability of Information

All data and information that serve as basis for this GRAS determination is available for the Food and Drug Administration review or will be sent to the agency upon request, made to:

Henry Liu
henry.liu@gpiglobal.com
1255 Journey's End Circle
Newmarket, Ontario L3Y 8T7
CANADA
1 (905) 853-8828 (Work)
1 (416) 677-1888 (Mobile)

1.8 Freedom of Information Act

All information included can be disclosed under the Freedom of information Act, 5 U.S.C. 552.

1.9 Certification

To the best of our knowledge, this GRAS notice is complete, representative, and balanced that includes favorable and unfavorable information pertinent to the evaluation of the safety and GRAS status of the use of GPI Biotech VAM-S.

1.10 Signature

July 15, 2019

Henry Liu
Technical Director
Gum Products International

Date

1.11 FSIS Authorization

We request that a copy of this notification, including trade secrets, be shared with the Food Safety and Inspection Service (FSIS) of the United Sates Department of Agriculture (USDA) for determining the efficacy and suitability of VAM-S for use in meat, poultry, and egg products as a processing aid.

PART 2 Identity and Specifications of Manufacture

2.1 Identity

GPI Biotech VAM-S, henceforth referred to as VAM-S, consists of a mixture of 3 bacteriophages (phages) that were isolated from farms in different geographical areas of Spain. All 3 phages are *Salmonella*-specific lytic phages that have the ability to lyse a wide range of *Salmonella enterica* serovars.

VAM-S has the physical state of a liquid and is soluble in water. The 3 phages are mixed together and diluted in sterile water so that VAM-S has a minimal of total phage concentration of 1×10^{10} PFU/mL. VAM-S can then be applied at 0.5-2% v/w at the discretion of the food manufacturer, which equates to a maximum rate of 2×10^{8} PFU/g of food.

2.2 Phage Identity

The 3 phages were isolated from chicken cloacae and pig rectal swabs from farms in different geographical areas of Spain. The phages are named Phi_16, Phi_78, and Phi_87. They are a part of an academic lab collection and are not deposited in any other culture collection. Each phage is characterized by full-genome sequencing, electron microscopy, and lytic activity against a large number of *Salmonella* strains. Bioinformatic analysis of each phage genome sequence reveals that they are strictly lytic and lack any virulence, or undesired genes as identified in GenBank.

Phage: Phi_16
Order: Caudovirales
Family: *Siphoviridae*
Genome: dsDNA
Type: Lytic phage

Phage: Phi_78
Order: Caudovirales
Family: *Podoviridae*
Genome: dsDNA
Type: Lytic phage

Phage: Phi_87
Order: Caudovirales
Family: *Myoviridae*
Genome: dsDNA
Type: Lytic phage

The full genome sequence of Phi_78 and Phi_87 are publicly available through GenBank as GU595417.1 and NC_027360.1 respectively. Phi_16 has been fully sequenced but not yet deposited into GenBank at the time of writing.

2.3 Host Identity

All 3 phages are amplified in a non-virulent strain of *Salmonella* Typhimurium named LB5000. Derivation of LB5000 is from another strain of *S. typhimurium* named LT2[1]. The attenuated LT2 strain has been widely used in laboratories since its isolation. This strain is mutated in the *rpo*S gene, which is important for the bacteria to display an acid-tolerance response. Several studies have established that the *rpo*S mutation is the cause of the attenuation of the LT2 strain[2,3]. Virulence of the LT2 strain can be recovered by inserting a functional *rpo*S gene by genetic recombination.

The LB5000 strain was derived from the LT2 strain. It is developed to have all three *hsd* genes that regulates the restriction-modification system mutated. These mutations do not change the avirulent status of the LB5000 strain[1]. The LB5000 strain is commercially available through the *Salmonella* Genetic Stock Center at the University of Calgary.

The *Salmonella* production host LB5000 was tested for its sensitivity to antibiotics chloramphenicol, kanamycin, nalidixic acid, nitrofurantoin, penicillin, and tetracycline. LB5000 is sensitive to all the antibiotics tested.

2.4 Host Range

Host range studies were conducted by the Universitat Autonoma De Barcelona with 71 clonally unrelated strains of *S. enterica*. The tested *S. enterica* strains included strains of Enteritidis, Hadar, Infantis, Newport, Typhimurium, and Virchow. The lytic activity of Phi_16, Phi_78, and Phi_87 was demonstrated for 82%, 90%, and 93% of those 71 strains respectively.

2.5 VAM-S Properties

VAM-S is a clear translucent liquid and is composed of phages and sterile water.

Table 1 – Physical Properties

Physical Properties	VAM-S Lot # 1806251S	VAM-S Lot # 1806252S	VAM-S Lot # 1806253S
Color	Translucent	Translucent	Translucent
Odor	None	None	None
State	Liquid	Liquid	Liquid
Solubility	Soluble in water	Soluble in water	Soluble in water

2.6 Specifications

Each of the 3 phages are produced separately and then mixed to form a final cocktail in VAM-S. Therefore, quality control is completed after each individual phage production and after formation of the final cocktail.

Each phage production batch is quality controlled for its concentration, purity, endotoxin level, and sterility (Table 2). For phage concentration measurement, a standard protocol is used (see "Quantitative determination of *Salmonella* bacteriophage via plaque assay" in the Appendix).

For its sensitivity and specificity, the quantitative PCR (qPCR, also known as real-time PCR) is used to measure the purity of each production. During the process of industrial-scale production, it is possible for a phage other than the one being produced to be present in the reaction vessel and be amplified along with the intended phage. For example, there may be a presence of Phi_87 in the reaction vessel while Phi_16 is being produced. This will cause Phi_87 to be inadvertently produced, resulting in an impure Phi_16 production lot. Thus, the detection of contaminant phages, as in Phi_87 in the example above, is an essential tool to ensure an accurate final cocktail formulation. Henceforth, "contaminant phages" refers to the presence of 1 or 2 of the 3 *Salmonella* phages as identified in section 2.2 in a production lot designed for production of a different *Salmonella* phage. For a detailed protocol, see "Molecular detection of VAM-S bacteriophages" in the Appendix.

To measure the endotoxin content, a commercially available quantitative LAL-based assay was used. If the endotoxin content exceeds what is specified (>2500 EU/mL), the production lot can be washed again with buffer and retested. If the endotoxin content is below specification (<2500 EU/mL), the production lot will proceed to the next stage of blending of the 3 phages.

Bacterial sterility is tested by plating 100µL onto Luria-Bertani agar plates, which are then incubated at 37°C for 7 days. If no growth is detected after 7 days, then the production can proceed to the next stage of blending of the 3 phages. If there is bacterial growth detected, then the production lot can be re-filtered and retested.

Table 2 – Quality Control of Individual Phage Productions

Parameters	
Concentration	$>10^{11}$ PFU/mL
Purity	$<10^{5}$ DNA copies from contaminant phages
Endotoxin	<2500 EU/mL
Bacterial sterility	No growth detected after 7 days

After each individual production lot for the 3 phages have passed the specifications outlined in Table 2, they are mixed together and diluted in water to form VAM-S. This final formation is again tested for its

phage concentration, endotoxin content, and bacterial sterility (Table 3). Chemical analysis of VAM-S was also completed to ensure there are suitable levels of arsenic, lead, and mercury (Table 4).

Table 3 – Quality Control of VAM-S

Parameters	
Concentration	$>10^{10}$ PFU/mL
Endotoxin	<2500 EU/mL
Bacterial sterility	No growth detected >14 days

Table 4 – Chemical Analysis of VAM-S

	Units	Reportable Detection Limit	LOT #			Method Reference
			1806251S	1806252S	1806253S	
Arsenic	µg/g	0.01	0.01	0.01	0.01	EPA 6020B mod./200.3
Lead	µg/g	0.01	ND	ND	ND	EPA 6020B mod./200.3
Mercury	µg/g	0.005	ND	ND	ND	Health Canada Method
Kjeldahl Nitrogen	mg/L	20	820	820	790	OMOE E3516 m
Organic Carbon	mg/L	250	43000	44000	47000	SM 23 5310B m

ND = Not detected

Chemical analysis were conducted by Maxxam Analytics (Canada)

2.7 Method of Manufacture

The 3 phages are produced individually by aerobic fermentation into high concentrations. The broth media used is animal-product free. The non-pathogenic host bacteria is grown from a working stock that is derived from a low passage frozen stock. Once the growing culture reaches a pre-determined optical density (OD), a phage stock is then added at a pre-determined multiplicity of infection (MOI). The duration of this infection process was determined empirically for each phage to achieve the highest possible yield of phage amplification.

The culture is then clarified of the bacterial mass by micro-filtration and is followed by sterile filtration. Ultra-filtration is then used to wash the phages with phosphate-buffer saline (PBS), while the phages are

concentrated at the same time. During this process, endotoxins are expected to be removed during clarification and washing.

After qualification of each VAM-S lot as outlined in Table 3, it is then stored in a refrigerated (2-8°C) and dark environment. In this storage condition, stability tests have shown that the phage concentration and bacterial sterility is maintained for at least 1 year.

2.8 Food-grade material

All components used in the manufacturing of VAM-S are food grade and animal-product free.

PART 3 Dietary Exposure

3.1 Application Rates

For the dietary exposure estimation, the assumption is that VAM-S will be applied at the maximum rate of 2×10^8 PFU/g of food.

3.2 Dietary Intakes

VAM-S is expected to be used on the following foods:

- Poultry
- Eggs
- Red meat
- Fruits
- Vegetables
- Fish and shellfish

The USDA Food Availability (Per Capita) Data System was used to estimate the dietary exposure of VAM-S (https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/, accessed February 2, 2020). Values for the estimated average annual and daily consumptions were obtained from the Loss-Adjusted Food Availability database. At the time of access, the database was last updated on August 26, 2019, but no consumption estimates were given beyond 2017. Therefore, the food availability values for 2017 were used for the dietary intake estimates below. For this exercise, an assumption was made that all foods listed above have been treated with VAM-S at the maximum rate of 2×10^8 PFU/g of food. In addition, it is assumed that all the available food is consumed. Thus, the estimated dietary exposure of VAM-S calculated will be grossly over-estimated.

Table 5 – Average Food Consumption Data

	Average Annual Per Capita Consumption (lbs)	Average Daily Per Capita Consumption (g)
Poultry	60.4	75.1
Eggs	22	27.3
Red Meat	73.9	91.9
Fruits	113.8	141.5
Vegetables	172.3	214.1
Fish and Shellfish	6.6	8.1
Total	449.0	558.0

3.3 Estimated Dietary Exposure to VAM-S phages

If VAM-S consists of 1×10^{10} PFU/mL and is applied at the maximum rate of 2% v/w on foods, it equates to a maximum rate of 2×10^{8} PFU/g of food. The average daily per capita consumption was calculated to be 558.0g for all foods that can be possibly treated with VAM-S (Table 5).

$$(2\times10^{8}\ \text{PFU/g}) \times (558.0\text{g}) = 1.116\times10^{11}\ \text{PFU}$$

Each day, the average American daily consumption of phages in VAM-S will be 1.116×10^{11} PFU.

Each phage is calculated to have an average weight of 6.47×10^{-17} as calculated by:

	kilo-basepairs (k-bp)	weight of phage bp x660 (Da)	weight of phage (g)
Phi_16	45.52	30043200	4.99E-17
Phi_78	43.94	29000400	4.82E-17
Phi_87	87.6	57816000	9.60E-17
Average			**6.47E-17**

$$(1.116\times10^{11}\ \text{PFU}) \times (6.47\times10^{-17}\text{g/PFU}) = 7.22\times10^{-6}\ \text{g}$$

Therefore, the average American daily consumption of phages is 7.22×10^{-6} g.

3.4 Estimated Dietary Exposure to VAM-S

At the maximum rate of 2% VAM-S application, 1g of food is treated with 0.02mL of VAM-S. The average daily per capita consumption of all foods treated with VAM-S was calculated to be 558g (Table 5).

$$558.0\text{g} \times 0.02\text{mL} = 11.16\ \text{mL}$$

Therefore, the average daily consumption is 11.16mL of VAM-S.

3.5 Estimated Dietary Exposure to Phosphate-Buffered Saline

VAM-S is composed of 20% phages which is washed in PBS, while the remaining is composed of sterile water. If 11.16mL of VAM-S is consumed daily, the daily consumption of each component of PBS is:

	g/L	20% of 11.6mL (g)
Sodium chloride	8	0.01856
Potassium chloride	0.2	0.000464
Disodium hydrogen phosphate	1.15	0.002668
Potassium dihydrogen phosphate	0.2	0.000464
Total		**0.022156**

This amounts to a total of 0.022g of PBS consumed per day, which should be considered to be a very low amount.

3.6 Estimated Dietary Exposure to Endotoxins

A structural component of the Gram-negative bacteria cell wall is lipopolysaccharides (LPS), which are considered to be endotoxic. As there is an estimation of 1 kg of bacterial mass in the human colon, LPS are already found in the human digestive system. In addition, Gram-negative bacteria normally residing in human mouths produces endotoxin, and it is estimated that human saliva contains approximately 1mg of endotoxin per milliliter of saliva, equating to 1×10^6 EU/mL[4]. There is no scientific evidence that ingestion of LPS through foods results in any known side effects. In fact, some probiotic products contain viable *E. coli* bacteria. There are currently no regulations for a limit of endotoxins that can be found in foods.

The use of a Gram-negative bacteria to produce phages leads to the release of endotoxins. Through multiple filtration steps, most of the endotoxins are removed and does not significantly contribute to the daily intake of endotoxins. As specified in Table 3, endotoxin levels of less than 2500 EU/mL will be accepted for VAM-S to be released for use. Using this maximum allowed endotoxin level of 2500 EU/mL, the daily consumption of endotoxin consumed through all foods treated with VAM-S (section 3.4) will be:

$$2500 \text{ EU/mL} \times 11.16\text{mL} = 27900 \text{ EU}$$

Therefore, the daily consumption of endotoxin through foods treated with VAM-S is 2.79×10^4 EU. If human saliva contains about 1×10^6 EU/mL, and the average person is estimated to produce 0.4 to 2.8 liters of saliva per day, it amounts to at least 4×10^8 EU ingested per day through saliva alone. The endotoxin contribution from VAM-S is less than 0.01% of the endotoxin load from saliva. This level is insignificant and thus should be considered safe.

PART 4 Self-Limiting Levels of Use

The functional properties of VAM-S are phage-targeted killing of *Salmonella enterica*. Therefore, the proposed use of VAM-S is an antimicrobial processing aid for foods that has a risk to be contaminated with *Salmonella*.

The amount of VAM-S that can be added to food is limited by the following factors:

1) The manufacturer will likely use the minimum application rate required to achieve the desired level of *Salmonella* reduction for financial reasons.

2) Once the *Salmonella* targets are depleted on foods, the phages will stop replicating.

3) Phages are susceptible to degradation from various environmental factors, such as temperature, acidity, and salinity [5]. Phage inactivation can also occur by chemicals and enzyme breaking down the virion [6].

PART 5 Experience Based on Common Use in Food Before 1958

This section is not applicable to this GRAS notification.

PART 6 Narrative

The basis of GPI's determination of VAM-S as GRAS is provided below.

6.1 Background on *Salmonella*-Related Illnesses

Foodborne illnesses remain a public health problem in both industrialized and developing countries[7]. According to the Centers for Disease Control and Prevention (CDC), non-typhoidal *Salmonella* is estimated to cause over 1 million illnesses and 450 deaths annually in the United States alone. Infection by *Salmonella* can cause mild to severe gastroenteritis, resulting in abdominal cramps, diarrhea, and fever. Invasive infections can also occur where the *Salmonella* infection spreads from the intestines to the blood stream, leading to a life-threatening illness.

Salmonella can be found in various foods, such as meats, fruits, vegetables, eggs, and processed foods. In addition, contamination can occur anywhere between pre-processing in the farm, to the site of consumption. For these reasons, *Salmonella* infection causes more hospitalizations and deaths compared to other food-borne pathogens[8].

The use of antibiotics have played a vital role in agriculture to prevent or control pathogenic bacterial infections such as *Salmonella* to improve growth and production in the past 60 years[9]. However, there is mounting evidence that the use of antibiotic use in food-producing animals is concomitant to the increase of antibiotic resistance in bacteria that cause human infections. Antibiotic-resistant bacteria can be transmitted to humans by the consumption of infected foods treated with antibiotics, transmitting resistant bacteria to the human chain. This can lead to illnesses that are difficult to treat, thereby increasing morbidity and mortality rates. In addition, fecal waste from animals treated with antibiotics can be composted and spread as fertilizer, allowing antibiotic resistant bacteria to spread into the environment[9]. Therefore, there should be a progression away from utilizing antibiotics to control bacterial infections, including in the food chain.

6.2 The Use of Phages to Control *Salmonella*

Phages are natural predators of bacteria in nature, found anywhere bacteria are present[10,11]. Each phage often targets only specific species of bacteria, while having no effect on other bacteria, human, animal, or plant cells. In natural environments, phages and their bacterial hosts are involved in continuous cycles of co-evolution. The bacteria can adopt various changes biochemically or structurally to resist phage infection, but unlike antibiotics, phages also have mechanisms to counter bacterial resistance[12,13].

Since its discovery, phages have been used to treat human diseases such as dysentery, food poisoning, typhoid fever, and various other infections in the former Soviet Union, Poland, France, and Georgia[14]. Therefore, the utilization of phages as a natural solution to reduce harmful bacteria that can be found in

foods can be considered. By directly applying phages in the food, the level of target pathogenic bacteria present can be significantly reduced, making the food safer to consume[15–19].

Lytic (or virulent) phages can be an effective tool to control pathogens due to their rapid and specific killing nature. Lysis of the bacterial host cell can occur by either the enzymatic actions of the phage to release newly created progenies, or by the loss of membrane potential due to the attachment of a large number of phage particles onto the cell wall[20]. The latter method leads to a quicker bacterial cell death, as it does not involve the internalization of the phage genome and completion of the phage replication cycle. In contrast, the other class of phages called temperate phages enter the lysogenic cycle where their genetic information is injected into the host cell, which can exist either as a plasmid or inserted into the host genome, staying in this quiescent state until an induction event that triggers it into the lytic cycle. Therefore, temperate phages should not be used as antimicrobial agents due to the possibility of horizontal gene transfer of toxic or antibiotic resistance genes[21].

The use of lytic phages as a bio-control agent of *Salmonella* is demonstrated with various food products. Typically, the effectiveness of *Salmonella*-specific phage or phage cocktail is tested on experimentally contaminated foods. The reduction of *S. enterica* was demonstrated on poultry in numerous studies[22–25]. In one of these studies[24], 2 of the 3 phages named in this notification was used as a cocktail and evaluated for bio-control of *S.* Typhimurium and *S.* Enteritidis in various food matrices – pig skin, chicken breasts, fresh eggs, and lettuce. It was demonstrated that the reduction was highest in pig skin, chicken breasts, and lettuce ranging from 1.9 to 2.5 log reduction. The *Salmonella* reduction in fresh eggs was observed to be only 0.9 logs. This suggests that different food matrices affect the efficacy of phages, and thus, the method and the rate of phage application should be determined for each type of food to achieve the highest rate of *Salmonella* reduction.

The use of phages to reduce *Salmonella* on fresh fruits has also been studied[26]. The authors demonstrated the survival of *S.* Enteritidis on melons and apples at refrigerated temperatures, and the increase of the *S.* Enteritidis population as storage temperature increases. A *Salmonella* phage cocktail from Intralytix Inc. demonstrated a 2.5 to 3.5 log *Salmonella* reduction on melon slices depending on the incubation temperature. However, there was no significant *Salmonella* reduction on apple slices, which may be attributed to the higher acidity environment.

Application of phages on other foods, such as mung bean sprouts, mung bean seeds, cheese, chocolate milk, hot dogs, seafoods, and pet foods[27–30] have also been evaluated. The measured *Salmonella* reduction varies, which may likely be due to the use of different phages and the different food matrices which can either promote or hinder phage distribution. However, the trend of *Salmonella* reduction in the presence of phages is common throughout these studies.

6.3 Other Phage Products Used as Antimicrobials

Several commercially available phages specific for various bacterial pathogens have been approved as GRAS or cleared by other regulatory agencies:

SalmoFresh™ – a phage product for control of *S. enterica* on poultry, fish and shellfish, and fresh and processed fruits and vegetables (GRN No. 435).

SalmoPro® - a phage product for control of *Salmonella* on poultry products (GRN No. 603).

Salmonelex™ - a phage product for control of *Salmonella* in pork and poultry products (GRN No. 000468).

EcoShield™ – a phage product for control of *E. coli* O157:H7 in red meat parts and trims as a processing aid with no labeling requiredments (FSIS Directive 7120.1).

ListShield™ – a phage product for control of *L. monocytogenes* in fish and shellfish, fresh and processed fruits and vegetables, and dairy products (GRN No. 528)

Listex™ -a phage product for control of *L. monocytogenes* in poultry products (GRN No. 218)

6.4 Lytic Phages are Inherently GRAS

Lytic phages are obligate intracellular parasites that target only specific species of bacteria, while having no effect on other bacteria, human, animal, or plant cells[31,32]. Phages have low toxicity due to their composition of only proteins and DNA. Numerous studies have demonstrated that consumption of phages is harmless to humans or animals. In a study involving human volunteers, there were no significant effects on subjects who consumed *E. coli* phages[33]. Rats fed high doses of *Listeria* phages also had no measurable effects compared to those that were not fed any phages[34].

Phages are the most ubiquitous and abundant biological entity on earth. As they are nature's counterbalance to bacteria, they can be found anywhere where bacteria exists, including the soil, ocean, water supply, various foods, human mouths, and human stomachs[35–39]. As phages exist everywhere, humans are not only in contact with them, but consume them constantly. Thus, phages should be considered safe to consume and be readily considered to have the GRAS status.

6.5 GRAS Status of Starting Material

The growth medium for producing VAM-S contains only GRAS affirmed ingredients. The components of the medium are peptones (23 CFR 184.1553), yeast extracts (21 CFR 184.1983), dextrose (21 CFR 168.110), sodium chloride (21 CFR 182.70), and phosphates (21 CFR 182.1778). Sodium hydroxide (21 CFR 582.1763) is used to adjust pH of the medium during fermentation. These components are mostly washed away during down-stream processing with PBS.

The host strain of *Salmonella* used for amplification of phages is non-virulent. They are also removed post-fermentation by filtration and is verified to be devoid of it during quality control as specified in section 2.6.

6.6 Undesirable Host-Derived Components

The non-virulent *Salmonella* strain used for phage amplification is a Gram-negative bacteria, which have an outer membrane containing lipopolysaccharides (LPS) and may also produce other endotoxins. During manufacture in the filtration phase, the culture media is washed with PBS to remove most of the endotoxins. Each phage production lot is measured for its endotoxin level and only released for final blending if it is below the threshold level set out in the specification (Table 2).

6.7 Efficacy Data at the Intended Level of Use

Challenge studies were designed to evaluate the potential of VAM-S to reduce various strains of *S. enterica*. Three different *Salmonella* serovars were mixed equally and used to experimentally contaminant meats. VAM-S was sprayed onto the meats to promote even distribution so that the 1×10^8 PFU/g of phages was applied. It is shown that VAM-S reduced *Salmonella* at 2.2 to 2.5 logs for whole muscles and at 1.7 to 2.1 logs for ground chicken (Appendix).

6.8 Summary and Basis for GRAS

VAM-S consists of 3 naturally occurring lytic phages that have specificity to lyse various serovars of *Salmonella enterica*. It is shown here that all 3 phages are strictly lytic and do not contain any virulence or undesired genes. Each phage production is also required to pass specifications to ensure the safety of the final product. Other bacteriophage products for pathogen reduction have previously been GRAS-approved, and VAM-S is equivalent to these products.

Based on genetic, biological, and chemical analysis, VAM-S is considered safe as they are strictly lytic phages absent of undesirable genes, have low endotoxin levels, and devoid of bacterial contamination. Through experimental challenges, VAM-S is demonstrated to be effective in reducing *Salmonella* in different food matrices and has the potential to be effective in other food matrices not yet tested.

GPI has reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

PART 7 LIST OF SUPPORTING DATA AND INFORMATION

7.1 Appendix (Not Generally Available)

Challenge studies

Standard Operating Procedures:

1) Quantitative Determination of *Salmonella* Bacteriophage via Plaque Assay
2) Molecular detection of VAM-S bacteriophages
3) Bacteriophage In-vivo Challenge

7.2 References (Generally Available)

APPENDIX

Study 1: Determine the effectiveness of VAM-S on skin-on chicken experimentally contaminated with *S. enterica* serovars Tyhpimurium, Heidelberg, and Newport.

Objective: Compare the levels of *Salmonella* between untreated or VAM-S treated chicken

Materials:

- skin-on chicken drumsticks
- LB broth
- Buffered peptone water (BPW)
- XLD agar
- electrostatic sprayer
- VAM-S
- *Salmonella* cocktail (1:1:1 ratio) consisting of *Salmonella enterica* subsp. enterica serovars Typhimurium, Heidelberg, and Newport

General procedure:

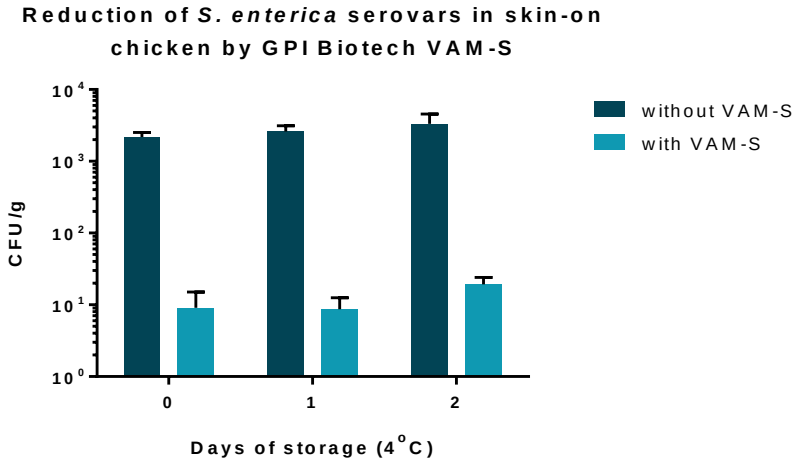
1. *Salmonella* cocktail was diluted to 10^5 CFU/mL, and 2mL was applied on the chicken drumstick surface evenly. For non-inoculated chicken drumstick, 2mL of PBS was applied instead.
2. Chicken drumsticks were left for 15 min to allow for bacterial attachment.
3. A sprayer was used to apply BPW or VAM-S onto chicken drumsticks.
4. After a 5 min incubation, the surface of the chicken drumstick was washed with BPW by massaging, followed by 1 min in the stomacher.
5. Viable *Salmonella* was determined by standard plating the appropriate dilutions on XLD agar plates.

Results:

Table R1: Level of *S. enterica* in experimentally contaminated chicken drumsticks not-treated or treated with VAM-S. Triplicate samples were stored for 0, 1, or 2 days before surface bacterial extraction.

Day(s) of incubation at 4°C	Concentration of *S. enterica* (CFU/g)	After VAM-S treatment (CFU/g)	Log reduction
0	2.17E+03	9.06E+00	2.38
1	2.61E+03	8.64E+00	2.48
2	3.33E+03	1.92E+01	2.24

Figure R1: Graphical representation of Table 3. Error bars indicate the SEM of 3 samples.



Conclusions:

There was an initial *Salmonella* reduction of 2.38 logs in chicken drumsticks treated with VAM-S compared to those that were not treated. This level of reduction was similarly seen after 1 and 2 days of storage at refrigeration temperature, which suggests that the initial *Salmonella* reduction was a result of irreversible killing by VAM-S. The data shown here demonstrates that 5 minutes of contact time with VAM-S is effective in reducing viable *Salmonella* in skin-on chicken drumsticks.

Study 2: Determine the effectiveness of VAM-S on ground chicken experimentally contaminated with *S. enterica* serovars Tyhpimurium, Heidelberg, and Newport.

Objective: Compare the levels of *Salmonella* between untreated or VAM-S treated ground chicken

Materials:

- skinless chicken breast
- LB broth
- Buffered peptone water (BPW)
- XLD agar
- sprayer
- VAM-S
- meat grinder
- *Salmonella* cocktail (1:1:1 ratio) consisting of *Salmonella* enterica subsp. enterica serovars Typhimurium, Heidelberg, and Newport

General procedure:

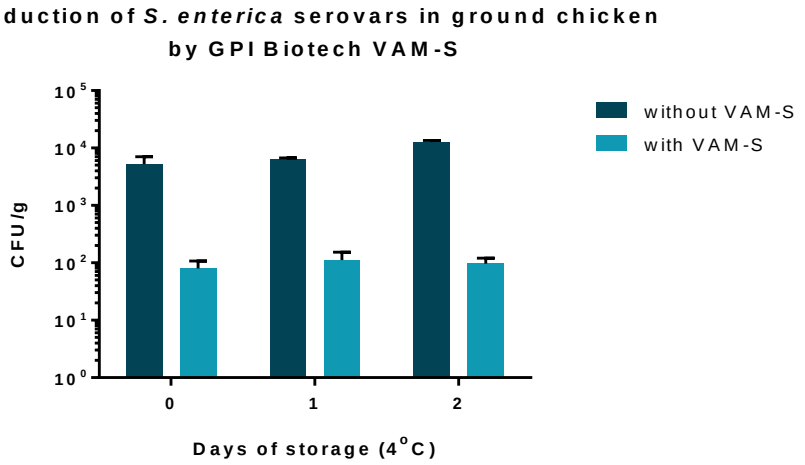
1. Skinless chicken breasts were aseptically cut into 100g pieces.
2. The *Salmonella* cocktail was diluted to 10^5 CFU/mL, and 2mL was applied onto the chicken surface evenly. For non-inoculated chicken, 2mL of BPW was applied instead.
3. Chicken breast pieces were left for 15 min to allow for bacterial attachment.
4. An electrostatic sprayer was used to apply BPW or VAM-S onto chicken breast pieces.
5. After a 5 min incubation, chicken breast pieces were grounded with a meat grinder. Grinder equipment parts that were in contact with the meat were cleaned thoroughly between samples, and separate parts were used for untreated and VAM-S treated samples to minimize cross-contamination.
6. 10g of ground chicken breast was put into a sterile stomacher bag with filter.
7. 90mL of BPW was added into the stomacher bag, and homogenized for 1 min.
8. Viable *Salmonella* was determined by standard plating the appropriate dilutions of the homogenate on XLD agar plates.

Results:

Table R2: Level of *S. enterica* in experimentally contaminated ground chicken breast not-treated or treated with VAM-S. Triplicate samples were stored for 0, 1, or 2 days before bacterial extraction.

Day(s) of incubation at 4°C	Concentration of *S. enterica* (CFU/g)	After VAM-S treatment (CFU/g)	Log reduction
0	5.21E+03	8.00E+01	1.81
1	6.26E+03	1.12E+02	1.75
2	1.26E+04	9.63E+01	2.12

Figure R2: Graphical representation of Table 4. Error bars indicate the SEM of 3 samples.



Conclusions:

The reduction of *Salmonella* in experimentally contaminated ground chicken breast was assessed. VAM-S was applied on the chicken breast trim prior to grinding, leading to a reduction of 1.7 to 2 logs reduction throughout 2 days of storage at refrigerated temperatures. In addition, there was no increase in the level of *Salmonella* in VAM-S treated samples during the storage time, which suggests that the initial *Salmonella* reduction at "day 0" was a result of irreversible killing by the phage cocktail. These results suggest that it is possible to apply VAM-S on meat trims prior to grinding to reduce the *Salmonella* load in the grounded product.

Prepared by: Edith Chow	**Standard Operating Procedure**	Revised by: Edith Chow
Effective Date: November 4, 2014	**Quantitative Determination of *Salmonella* Bacteriophage via Plaque Assay**	Rev. No. 4
Reference No.:		Revision Date: January 7, 2018
Approved by: Henry Liu, Technical Director		DEPARTMENT: R&D

Objective: To grow isolated plaques of *Salmonella* phage particles and determine sample phage titre.

Equipment:
Heat plate or water bath
Heated shaking incubator
Spectrophotometer
p200 and p1000 pipettes
Biosafety cabinet

Apparatus:
500mL Erlenmeyer flask
Inoculating loop
1.5mL Eppendorf tubes
15mL conical tubes

Materials:
10mM $MgSO_4$
50mL sterilized LB media
Host strain bacteria
3-5mL of soft agar in 15mL conical tubes
LB Agar plates

Recipes:

Prepare LB broth and LB agar by following manufacturer's directions.

To prepare soft agar, mix the following with 1L of ddH_2O and sterilize at 121°C for 30 min. After sterilization, aliquot 5mL into 15mL conical tubes.

	grams
NaCl	5
Tryptone	10
Agar	6

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Procedures:

1. Prepare LB agar plates.
2. Aliquot 30-50mL of sterile LB broth into a 500mL Erlenmeyer flask.
3. Inoculate host bacteria into the liquid broth.
4. Incubate in a 37°C shaking incubator @140rpm.
5. Cease incubation once OD_{600} of approximately 1 has reached.
6. Dilute phage sample 10-fold nine-times in 10mM $MgSO_4$.
7. Heat conical tubes containing soft agar until it is fully melted. (e.g. put in boiling water until soft agar is in full solution phase)
8. With the soft agar in full solution, and cooled below 49°C, add 200µL of host bacteria and 100µL of the highest phage dilution.
9. Vortex to mix, then pour onto an agar plate immediately.
10. Repeat steps 8 to 9 with the next two highest phage dilutions.
11. Once the soft agar has solidified, incubate agar plates upside-down in an incubator set at 37°C for 16-24 hours.
12. Count plaques and determine phage titre with the following equation:

$$\mathrm{PFU/mL} = \frac{\#\ plaques}{(100\mu L)(dilution\ factor)}$$

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Prepared by: Edith Chow	**Standard Operating Procedure**	Revised by: Edith Chow
Effective Date: July 26, 2016	**Molecular detection of VAM-S bacteriophages**	Rev. No. 1
Reference No.:		Revision Date:
Approved by: Henry Liu, Technical Director	DEPARTMENT: R&D	

Objective: To detect for any bacteriophage (phage) impurity in a volume of amplified and purified phages.

Equipment: Real-time PCR system
p2, p20, p200, and p1000 pipettes

Materials: Norgen Biotek Corp. Phage DNA Isolation Kit or equivalent
Nanodrop or equivalent to measure DNA concentration
PowerUP SYBR Green Master Mix
Aerosol-resistant pipette tips
Nuclease-free microcentrifuge tubes
Nuclease-free water

Procedures:

1. Following phage production and purification, take 3 random 1mL samples from the production lot.
2. Extract DNA following manufacturer's protocol.
3. Measure concentration of DNA extracted and normalize all samples to 12.5pg/µL.
4. Plan for 9 reactions per sample: triplicate replications for each of the 3 primers specific to the 3 different phages.
5. Include a no-template control (NTC) for each primer mix as a reference for negative signals.
6. Prepare a reaction mix without DNA template according to Table R3 for the number of samples and control reactions as required, plus 50% coverage.
7. Mix thoroughly and distribute 12µL to each reaction well.
8. Add 8µL of DNA template or nuclease-free water (NTC) into the appropriate well.
9. Seal the plate and centrifuge briefly to bring the contents to the bottom.
10. Program the thermal cycler as indicated in Figure R3.
11. Load the plate into the thermal cycler. Run the thermal cycler program and collect real-time amplification data.

12. If production lots are composed purely of the intended phage amplified, C_T values should be high (17-26 cycles) for each sample with its corresponding primer (E.g. Phi_16 sample would have a high C_T value in reaction wells with the Phi_16 primer). C_T values should be comparable to the NTC signal for the other 2 primers (32 to 40 cycles).

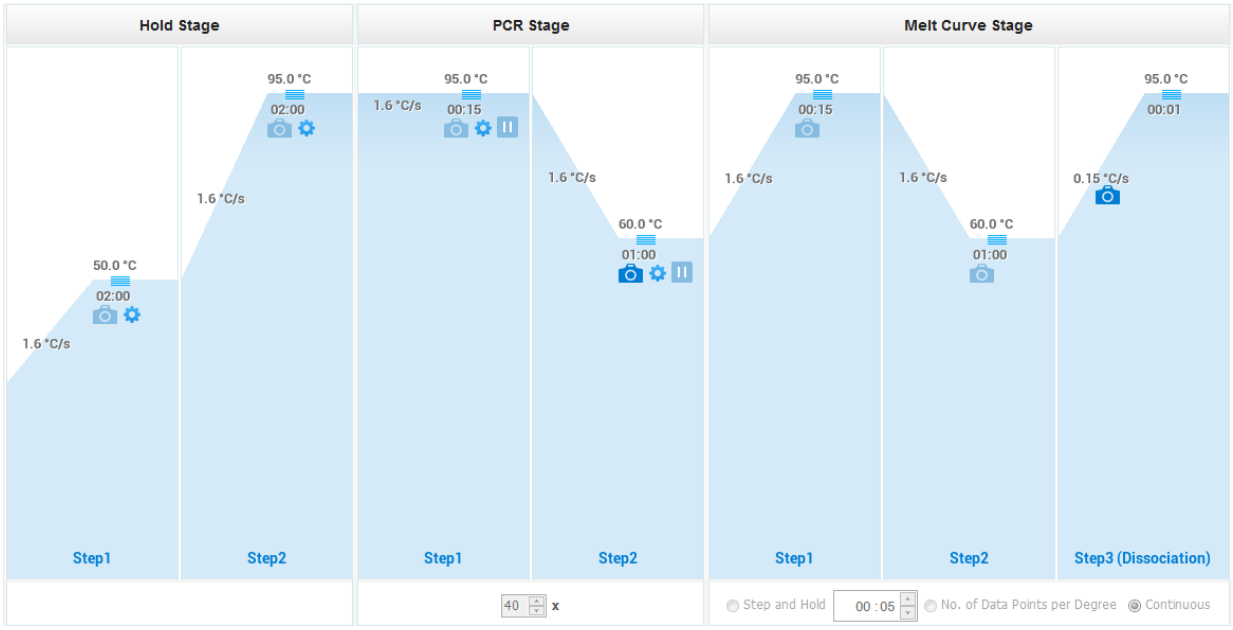
13. If C_T values are found to be significantly higher than NTC (< 30 cycles) for one or both of the non-corresponding primer(s), consult historical data to determine the amount of copy number that correlate with the C_T value. If the contaminant level is greater than 1×10^5 copies, which equates to 1×10^5 phages, then the lot will be rejected for final blending.

Table R3

PowerUp SYBR Master Mix	10 µL
Primer mix (10uM ea)	1 µL
H2O	1 µL
Template	8 µL
Total	20 µL

Prepared by: Edith Chow	**Standard Operating Procedure**	Revised by: Edith Chow
Effective Date: July 26, 2016	**Molecular detection of VAM-S bacteriophages**	Rev. No. 1
Reference No.:		Revision Date:
Approved by: Henry Liu, Technical Director	DEPARTMENT: R&D	

Figure R3



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Prepared by: Edith Chow	**Standard Operating Procedure**	Revised by:
Effective Date: December 12, 2018	**Bacteriophage *In-vivo* Challenge**	Rev. No.
Reference No.: RD-SOP-xxx		Revision Date:
Approved by: Henry Liu, Technical Director	DEPARTMENT: R&D	

Objective: To evaluate the effectivity of bacteriophage on reducing specific bacteria on food products

Equipment:
Plate spreader
Aluminum paper
15mL and 50mL conical tubes
Microtubes
Sprayer

Materials:
Target bacterial strain
Purified bacteriophages
Buffered peptone water (BPW)

Procedures:

Contamination of food product

1. Dilute bacterial culture to an appropriate concentration
2. Prepare food product as necessary (e.g. cutting into target weigh)
3. Contaminant food product by either spot inoculation, spreading onto the surface with a plate spreader, or by submersion.
4. Allow food product to air-dry after inoculation to allow bacterial attachment.

Bacteriophage treatment

1. Transfer appropriate samples to a tray lined with a clean foil paper.
2. Determine the amount of bacteriophage to be applied onto each food sample.
3. Spray VAM-S onto the surface of the food product.
4. Allow a 5 min incubation period after bacteriophage application.
5. If necessary, turn the sample over to spray bacteriophage.
6. If applicable, place sample into the tumbler and set to tumble for 15 min.

Further processing

1. If applicable, use the meat grinder to ground meat samples.

Extraction of bacteria from samples

Prepared by: Edith Chow	**Standard Operating Procedure**	Revised by:
Effective Date: December 12, 2018	**Bacteriophage *In-vivo* Challenge**	Rev. No.
Reference No.: RD-SOP-xxx		Revision Date:
Approved by: Henry Liu, Technical Director	DEPARTMENT: R&D	

1. Place samples individually into stomacher bags with filter.
2. Add a pre-determined amount of BPW into the stomacher bag.
3. Place the stomacher bag into the stomacher and homogenize for 1 min.
4. Extract BPW through the filter so to exclude food particles into a 50mL conical tube.

Enumeration of target bacteria

1. From the extracted BPW, make appropriate dilutions as necessary.
2. Spread-plate 100uL of the appropriate dilution on a selective agar.
3. Incubate plates at the appropriate temperature for 24 h.
4. Count colonies and calculate the concentration of bacteria present in each sample.

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39. Batinovic S, Wassef F, Knowler SA, et al. Bacteriophages in Natural and Artificial Environments. *Pathog Basel Switz*. 2019;8(3). doi:10.3390/pathogens8030100

From: Edith Chow
To: Hice, Stephanie
Subject: RE: GRN 000917 - Questions for Notifier
Date: Thursday, June 25, 2020 8:06:49 AM
Attachments: image002.png
2020-06-16-GRN 917 Responses_FDA.PDF
2020-06-16-GRN 917 Responses_FSIS.PDF

Hello Dr. Hice,

Please see the attached 2 files for the responses to the FDA and FSIS questions in your email on June 16, '20. Each response immediately follows each question.

Please let me know if further clarifications are needed.

Regards,
Edith

Edith Chow, Ph.D. | Research Scientist | edith.chow@gpiglobal.com| **Gum Products International** |
1255 Journey's End Circle | Newmarket | Ontario | CANADA | L3Y 8T7 |
1.905.853.8828 x 329 (O) | 1.905.853.8886 (F) |1.416.723.1907 (M)

From: Hice, Stephanie [mailto:Stephanie.Hice@fda.hhs.gov]
Sent: June 18, 2020 1:42 PM
To: Edith Chow
Subject: RE: GRN 000917 - Questions for Notifier

Dear Dr. Chow,

Thank you for your email. Please find our responses to your questions in BLACK, and FSIS's responses in RED.

Question 1 - Should we date it for March 4, 2020, or the current date? I also assume I will change it in the original GRAS submission document and that should be resent to you in addition to the document for the responses?

- The date for Part 1.10, Signature (page 5), should reflect the date that the notice was submitted to FDA, which was March 4, 2020. Please do not revise or re-submit the GRAS notice itself. The amendment will be included in the administrative record for GRN 000917 and posted with the original GRAS notice to our online GRAS inventory. To recapitulate, please organize your responses in two separate PDF documents: one with responses to FDA's questions and one with responses to USDA FSIS's questions. Please do not include any confidential information in your responses.

Question 5 - In regards to internally-developed methods, is this only referring to the qPCR method to detect contaminant phages?

- Question 5 refers to any internally-developed methods of analysis used for specification parameters. We note that Tables 2-4 list specification parameters. For part one of question 5, a statement to the effect that the methods are validated and suitable for the purpose is appropriate. With regards to part two of question 5, for each of the standard methods employed, we ask that you please provide complete and appropriate citations for each method.

In addition, I just noticed a typo regarding the EU limit - it should be 25000 instead of 2500. I believe I started with one typo and copied this number throughout the document. Would it be possible to note this typo in the response and have it corrected in the updated GRAS document?

- In your amendment to GRN 000917, you may make a clarifying statement that corrects this reference. Please do not revise or re-submit the GRAS notice itself. To recapitulate, the amendment will be included in the administrative record for GRN 000917 and posted with the original GRAS notice to our online GRAS inventory.

I also would like a clarification regarding one of the FSIS questions, and I included it below in case you can clarify as well. Otherwise, would you be able to share an appropriate contact person from FSIS who can help me?

Question 3 - In regards to lasting functional or technical effect to be labeled as a processing aid, I believe I have included scientific-backed evidence in section 4 (Self-limiting levels of use). I would also add that once the *Salmonella* targets are depleted on foods, the phages will not only stop replicating but also gradually decrease in numbers by degradation. Previous GRN documents (GRN 672, 827, 834) approved have similarly presented evidence to support the determination that their phage products have no lasting functional or technical effect to be labeled as a processing aid. My question is: Is the evidence presented in section 4 sufficient? If not, does the FSIS require actual experimental data? As I mentioned, previously approved GRN documents do not provide any such experimental data.

- In order for this product to be used as a processing aide (and not be on the label) it must have no lasting technical or functional effect. We understand the principles of why a phage shouldn't have a lasting or functional effect. What we are asking for is that data supporting those principles, i.e. data that shows there is no lasting or functional effect. They need to support their product has no lasting technical or functional effect we don't extrapolate from other similar GRNs.

- If they have already included that data, just point us to it because we don't see it.

- For example, often folks have data that shows the initial reduction of the target organism by the application of their substance but then over time, the target organism numbers go back up, this is one way to show no lasting functional or technical effect.

We recommend that you review similar GRAS notices posted in our online GRAS inventory; the

search feature is quite robust. Please note, in the inventory, amendments are posted with the original notice and are included at the end of the PDF document.

Should you have any additional questions, please do not hesitate to let me know.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

From: Edith Chow <Edith.Chow@gpiglobal.com>
Sent: Wednesday, June 17, 2020 12:11 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: RE: GRN 000917 - Questions for Notifier

Hello Dr. Hice,

Thank you for the review. I do have some questions before I submit an official response. I hope you are able to clarify for me:

Question 1 - Should we date it for March 4, 2020, or the current date? I also assume I will change it in the original GRAS submission document and that should be resent to you in addition to the document for the responses?

Question 5 - In regards to internally-developed methods, is this only referring to the qPCR method to detect contaminant phages?

In addition, I just noticed a typo regarding the EU limit - it should be 25000 instead of 2500. I believe I started with one typo and copied this number througout the document. Would it be possible to note this typo in the response and have it corrected in the updated GRAS document?

I also would like a clarification regarding one of the FSIS questions, and I included it below in case

you can clarify as well. Otherwise, would you be able to share an appropriate contact person from FSIS who can help me?

Question 3 - In regards to lasting functional or technical effect to be labeled as a processing aid, I believe I have included scientific-backed evidence in section 4 (Self-limiting levels of use). I would also add that once the *Salmonella* targets are depleted on foods, the phages will not only stop replicating but also gradually decrease in numbers by degradation. Previous GRN documents (GRN 672, 827, 834) approved have similarly presented evidence to support the determination that their phage products have no lasting functional or technical effect to be labeled as a processing aid. My question is: Is the evidence presented in section 4 sufficient? If not, does the FSIS require actual experimental data? As I mentioned, previously approved GRN documents do not provide any such experimental data.

Thank you in advance and I would appreciate any assistance you can offer.

Regards,
Edith

Edith Chow, Ph.D. | Research Scientist | edith.chow@gpiglobal.com| **Gum Products International** |
1255 Journey's End Circle | Newmarket | Ontario | CANADA | L3Y 8T7 |
1.905.853.8828 x 329 (O) | 1.905.853.8886 (F) |1.416.723.1907 (M)

From: Hice, Stephanie [mailto:Stephanie.Hice@fda.hhs.gov]
Sent: June 16, 2020 11:38 AM
To: Edith Chow
Subject: GRN 000917 - Questions for Notifier

Dear Dr. Chow,

During our review of GRAS Notice No. 000917, we noted further questions that need to be addressed and are attached to this email.

Additionally, please find questions from the US Department of Agriculture (USDA), Food Safety Inspection Service (FSIS) attached to this email. Please organize your responses in two separate PDF documents: one with responses to FDA's questions and one with responses to USDA FSIS's questions.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Questions/Comments Regarding GRN 000917 (FDA):

1. Please provide an updated signature and date for Part 1.10, Signature (page 5). We note, that the notice was submitted on March 4, 2020 and the provided signature and date does not reflect this.

GPI response – Part 1.10 is re-signed and dated correctly below.

1.10 Signature

X 

Henry Liu

Technical Director, Gum Products International

March 4, 2020

Date

2. References to "*Salmonella typhimurium*" on page 7 should read *Salmonella* Typhimurium. Please make a statement that corrects this reference.

GPI response – *Salmonella typhimurium* references on page 7 should be read as *Salmonella* Typhimurium. This was a typographical error.

3. Please specify how the purity of the host culture is ensured.

GPI response – The host bacteria was originally purchased from the University of Calgary. Once received, frozen stocks were created by multiple sequential passages of a single colony. This ensures the purity of the frozen stocks, which are then propagated for each production lot to appropriate volumes and optical density. So, the host bacteria inoculum used for each production is always derived from the original master bank. In addition, its genetic fingerprint was analyzed by the standard PFGE protocol for bacteria[1], which served as a reference pattern for comparison to any future PFGE analysis of the bacterial culture. If the host culture was contaminated, the PFGE analysis would reveal a different pattern, indicating impurity of the host culture. Further characterization includes sensitivity analysis with a panel of antibiotics as stated in section 2.3. Impurity of the host culture may also cause the antibiotic resistance profile to be different.

4. Please state whether any of the raw materials used in the fermentation are major allergens or derived from major allergens. If any of the raw materials used are major allergens or derived from major allergens, please discuss why these materials do not pose a safety concern.

GPI response – The only component of the growth medium for producing VAM-S that is derived from an allergen is the soy-based peptone. During growth of the host bacteria, the media components are hydrolyzed, leading to protein breakdown into amino acids. In addition, the media are mostly removed during downstream filtration and washing with phosphate-buffered saline. Finally, the same 3 lots listed in Table 4 were analyzed by an independent third party (Bureau Veritas Laboratories) for its soy level, and it was undetectable in all 3 lots. The assay had a detectable limit of 2.5ppm.

5. Please clarify if internally-developed methods of analysis used for specification parameters have been validated for that particular purpose. If using standard methods, please provide complete and appropriate citations.

GPI response – The double agar overlay plaque assay[2] was used to measure the concentration of phages. Bacterial sterility was tested by standard plating[3]. A commercially available kit that utilizes an endpoint amebocyte lysate assay was used to detect and quantify endotoxin levels[4,5]. An internally developed qPCR method was validated to detect and measure the level of contaminant phages in a production lot. Chemical analysis were conducted by Bureau Veritas Laboratories (formerly Maxxam Analytics), an accredited laboratory by the Standard Council of Canada and conforms with the requirements of ISO/IEC 17025:2017. Arsenic and lead were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)[6]. Mercury levels were measured by the cold vapour method[7]. Kjeldahl nitrogen was measured by colorimetric determination[8]. Organic carbon levels were measured by high temperature combustion and infrared detection (ASTM D7573 method[9]).

6. Please provide results from three non-consecutive batches to demonstrate that the manufacturing can meet the provided specifications. Please also include results for the three parameters (concentration, endotoxin, bacterial sterility) listed in Table 3 (page 9).

GPI response:

	VAM-S Lot #1806251S	VAM-S Lot #1812051S	VAM-S Lot #1912231S
Concentration (PFU/mL)	6.3×10^{10}	4.5×10^{10}	5.9×10^{10}
Endotoxin (EU/mL)	10,595	9,288	11,075
Bacterial Sterility	No growth detected	No growth detected	No growth detected

	Units	Reportable Detection Limit	VAM-S LOT #			Method Reference
			1806251S	1812051S	1912231S	
Arsenic	µg/g	0.01	0.01	0.01	0.01	EPA 6020B mod./200.3
Lead	µg/g	0.01	ND	ND	ND	EPA 6020B mod./200.3
Mercury	µg/g	0.005	ND	ND	ND	Health Canada Method
Kjeldahl Nitrogen	mg/L	20	820	800	820	OMOE E3516 m
Organic Carbon	mg/L	250	43000	45000	42000	SM 23 5310B m

ND = Not detected

Chemical analysis was conducted by Bureau Veritas Laboratories (formerly Maxxam Analytics), Canada.

Additional Amendment:

The specification of endotoxin limit noted in Table 2 and 3 should read as 25,000 EU/mL. Calculations on the estimated dietary exposure to endotoxins (part 3.6) based on 25,000 EU/mL consumption through VAM-S will still amount to an insignificant level compared the amount of endotoxin ingested through saliva per day.

References

1. Neoh H-M, Tan X-E, Sapri HF, Tan TL. Pulsed-field gel electrophoresis (PFGE): A review of the “gold standard” for bacteria typing and current alternatives. *Infect Genet Evol*. 2019;74:103935. doi:10.1016/j.meegid.2019.103935

2. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. In: Clokie MRJ, Kropinski AM, eds. *Bacteriophages*. Vol 501. Humana Press; 2009:69-76. doi:10.1007/978-1-60327-164-6_7

3. Harrigan, W.F. M ME. *Laboratory Methods in Microbiology*. Elsevier; 1966. doi:10.1016/C2013-0-12452-X

4. Cooper JF, Levin J, Wagner HN. Quantitative comparison of in vitro and in vivo methods for the detection of endotoxin. *J Lab Clin Med*. 1971;78(1):138-148.

5. Lindsay GK, Roslansky PF, Novitsky TJ. Single-step, chromogenic Limulus amebocyte lysate assay for endotoxin. *J Clin Microbiol*. 1989;27(5):947-951.

6. Choi SH, Kim JY, Choi EM, et al. Heavy Metal Determination by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Direct Mercury Analysis (DMA) and Arsenic Mapping by Femtosecond (fs) – Laser Ablation (LA) ICP-MS in Cereals. *Analytical Letters*. 2019;52(3):496-510. doi:10.1080/00032719.2018.1471484

7. Kopp JF, Longbottom MC, Lobring LB. “Cold Vapor” Method for Determining Mercury. *Journal (American Water Works Association)*. 1972;64(1):20-25.

8. Willis RB, Montgomery ME, Allen PR. Improved Method for Manual, Colorimetric Determination of Total Kjeldahl Nitrogen Using Salicylate. *J Agric Food Chem*. 1996;44(7):1804-1807. doi:10.1021/jf950522b

9. ASTM D5173-15, Standard Guide for On-Line Monitoring of Total Organic Carbon in Water by Oxidation and Detection of Resulting Carbon Dioxide, ASTM International, West Conshohocken, PA, 2015, www.astm.org.

June 16, 2020

Questions/Comments Regarding GRN 000917 from USDA FSIS:

1. Clarification of the intended use of GRN 000917 on products other than chicken (red meat, fish, and/or eggs products);

GPI response – The intended use of GRN 917 is on poultry, eggs, red meat, fruits, vegetables, fish excluding fish of the order Siluriformes, and shellfish.

2. Data supporting the suitability of GRN 000917 for the products identified in 1 above; and

GPI response – Efficacy studies on eggs, red meat, apples, salmon, and shrimps are appended in this document. A summary of the results is followed.

Eggs – Experimentally contaminated eggs were left untreated or treated with VAM-S at a rate of 2×10^8 PFU/g. After 5 minutes of incubation, there was a 2.08 log reduction of *Salmonella* in samples that were treated with VAM-S compared to untreated samples. Full results are described in the attached report.

Red meat – Experimentally contaminated beef was left untreated or treated with VAM-S at a rate of 2×10^8 PFU/g. The beef was then grounded and stored at 4°C. There was a 1.75 log, 1.74 log, and 1.68 log reduction of *Salmonella* at 0, 1, and 2 days of incubation respectively. Full results are described in the attached report.

Apples – Experimentally contaminated apple slices were left untreated or treated with VAM-S at a rate of 2×10^8 PFU/g. After 5 minutes of incubation, there was a 1.7 log reduction of *Salmonella* in samples that were treated with VAM-S compared to untreated samples. Full results are described in the attached report.

Salmon – Experimentally contaminated salmon were left untreated or treated with VAM-S at a rate of 2×10^8 PFU/g. After 5 minutes of incubation, there was a 1.97 log reduction of *Salmonella* in samples that were treated with VAM-S compared to untreated samples. Full results are described in the attached report.

Shelled shrimps - Experimentally contaminated shrimps were left untreated or treated with VAM-S at a rate of 2×10^8 PFU/g. After 5 minutes of incubation, there was a 3.0 log reduction of *Salmonella* in samples that were treated with VAM-S compared to untreated samples. Full results are described in the attached report.

3. Data supporting that GRN 000917 has no lasting functional or technical effect and is acceptable to be labeled as a processing aid.

GPI response – In Study 1 (page 22 of GRAS notification), the effectiveness of VAM-S on experimentally contaminated skin-on chicken was examined. The level of *S. enterica* was measured over the course of 3 days in both untreated and VAM-S treated chickens. The efficacy of VAM-S was clearly demonstrated as there was a 2.38, 2.48, and 2.24 log reduction with VAM-S treatment on day 0, 1, and 2 respectively. The one-tailed *p* value

were all less than 0.005, which is considered to be very significant. If VAM-S exerts a lasting functional or technical effect, a further reduction of *S. enterica* after the initial measurement (day 0) would be expected. In this case, there would be a statistically significant difference between VAM-S treated chickens between day 0 and day 1, and between day 1 and day 2. However, the one-tailed *p* value was 0.4775 and 0.1269 respectively, indicating no significant difference. The level of *S. enterica* in untreated chickens between each day of incubation also revealed no significant differences. Therefore, after the initial reduction of *S. enterica* by VAM-S, there were no further reduction during additional days of storage, indicating an absence of a lasting functional or technical effect.

A similar statistical analysis was carried out for Study 2 (page 24 of GRAS notification). The efficacy of VAM-S on each day of storage was demonstrated as there was a 1.81, 1.75, and 2.12 log reduction on day 0, 1, and 2 respectively. The one-tailed *p* value were all less than 0.005, which is considered to be very significant. However, there was no statistical difference between VAM-S treated ground chicken between day 0 and day 1, and between day 1 and day 2, as the one-tailed *p* value was 0.2785 and 0.3806 respectively. Therefore, there were no significant changes in the level of *S. enterica* after the initial reduction by VAM-S. Again, this indicates an absence of a lasting functional or technical effect by VAM-S.

Finally, the red meat study included in this document (page 5) was analysed for any lasting functional or technical effect by VAM-S. There were no statistical differences in VAM-S treated samples between days 0 and 1, and between days 1 and 2 as the one-tailed *p* value was 0.2825 and 0.3547 respectively. This indicates that there were no significant changes in the level of *S. enterica* after the initial reduction by VAM-S.

The evidence provided above demonstrates that VAM-S does not have a continued technical effect. Further reduction of *S. enterica* during subsequent days of incubation would otherwise be seen. In the above examples, incubation between days of testing was at 4°C, so no significant re-growth of *S. enterica* was seen in either untreated or VAM-S treated samples.

1255 Journey's End Circle, Newmarket ON L3Y 8T7 Tel: (905) 853 – 8828 Fax: (905) 853 – 8886

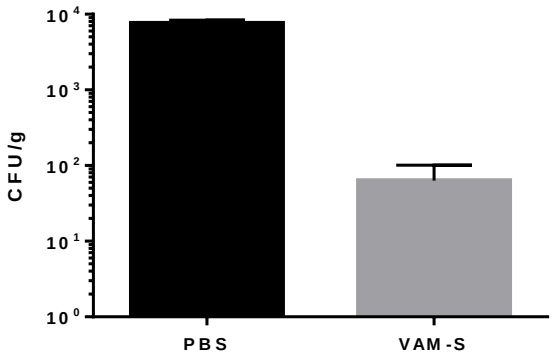
GPI Biotech VAM-S **Dec. 6, 2019**
Summary Data Report

Reduction of *Salmonella* Typhimurium on Eggs

Purpose

The purpose of this study is to examine the potential of GPI Biotech VAM-S to reduce *Salmonella* on eggs at an application rate of 2×10^8 PFU/g.

Summary Results



Error bars represent SEM of 3 independent samples

Reduction of *Salmonella* Typhimurium on experimentally contaminated eggs was demonstrated. The *Salmonella* level on untreated apple slices (PBS) was approximately 7.6×10^3 CFU/g, while VAM-S treated apple slices was approximately 64 CFU/g. Therefore, VAM-S can significantly reduce viable *Salmonella* levels on experimentally contaminated eggs by 2 logs in 5 min of treatment time.

Method

1. Grown *Salmonella* Typhimurium culture in LB broth to approximately OD_{600}=1.
2. Place egg in sterile stomacher bag.
3. Add 0.1mL of *Salmonella* culture into the stomacher bag.

4. Shake the bag to coat the apple slice for 1 min. and allow to stand for 15 min. for bacterial attachment.
5. Remove the apple slice and place in a new sterile stomacher bag.
6. Add 0.1mL of PBS or VAM-S into the bag.
7. Shake the bag for 1 min. and allow to stand for 4 min.
8. Remove the apple slice and place in a new sterile stomacher bag.
9. Add 10mL of buffered peptone water (BPW) into the bag and shake the bag for 1 min. to rinse the surface.
10. Extract BPW and dilute as necessary.
11. Plate appropriate dilutions onto XLD agar plates.
12. Include samples without *Salmonella* inoculation to assess background flora on the apple slices.

Data

	(CFU/g)				*Sal.* Reduction		
	Replicate 1	**Replicate 2**	**Replicate 3**	**AVG**	**Log**	**%**	**P-Value**
no inoculation	0.00E+00	0.00E+00	0.00E+00	0.00E+00			
PBS	8.48E+03	7.25E+03	7.12E+03	7.61E+03			
VAM-S	1.96E+01	8.31E+01	8.77E+01	6.35E+01	2.08	99.2%	<0.001

1255 Journey's End Circle, Newmarket ON L3Y 8T7 Tel: (905) 853 – 8828 Fax: (905) 853 – 8886

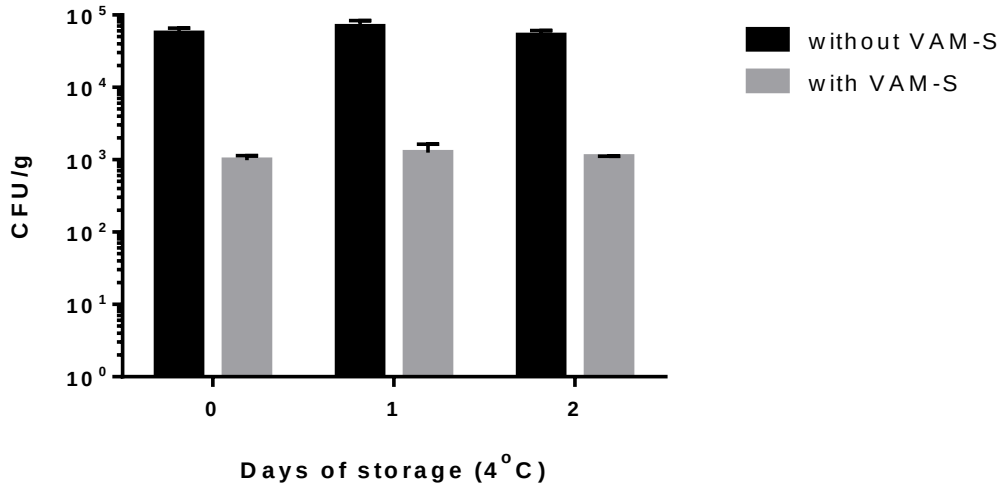
GPI Biotech VAM-S **Feb 26, 2020**
Summary Data Report

Reduction of *Salmonella* on Ground Red Meat

Purpose

The purpose of this study is to examine the potential of GPI Biotech VAM-S to reduce *Salmonella* on ground red meat at an application rate of 2×10^8 PFU/g.

Results Summary



Error bars represent SEM of 3 triplicate samples.

VAM-S can effectively reduce *Salmonella* on experimentally contaminated ground red meat. VAM-S irreversibly reduces the level of *Salmonella* and maintains the reduction for at least 2 days. These results suggest that it is possible to use VAM-S to treat whole contaminated meat trims prior to grounding to reduce the *Salmonella* load in the grounded product.

Method

1. Grow *Salmonella* Typhimurium, Newport, and Heidelberg strains in LB broth to approximately OD_{600}=1. Mix all 3 strains equally and dilute to 10^5 CFU/mL.

2. Place boneless inside round steak pieces cut to approximately 100g into a sterile stomacher bag.
3. Add 2 mL of *Salmonella* culture into the stomacher bag.
4. Shake the bag to coat the sample for 2 min. and allow to stand for 15 min. for bacterial attachment.
5. Remove sample from the stomacher bag and place on a flat surface.
6. Apply PBS or VAM-S by spray.
7. Allow sample to stand for 5 min.
8. Ground the inside round steaks.
9. Place grounded sample in a new sterile stomacher bag and store at 4°C.
10. Repeat steps 2 to 8 in triplicate for each treatment (PBS and VAM-S) 3 times for each day of storage (9 times total for each treatment).
11. Measure out 10g of grounded sample and add 40mL of buffered peptone water (BPW) into a sterile stomacher bag with a filter.
12. Homogenize for 1 min.
13. Extract BPW and dilute as necessary.
14. Plate appropriate dilutions onto XLD agar plates.

Data

Days of incubation (4°C)	Concentration of *Salmonella* (CFU/g)	After VAM-S treatment (CFU/g)	Log reduction	P-value
0	5.57E+04	9.90E+02	1.75	<0.005
1	6.90E+04	1.25E+03	1.74	<0.005
2	5.23E+04	1.10E+03	1.68	<0.005

1255 Journey's End Circle, Newmarket ON L3Y 8T7 Tel: (905) 853 – 8828 Fax: (905) 853 – 8886

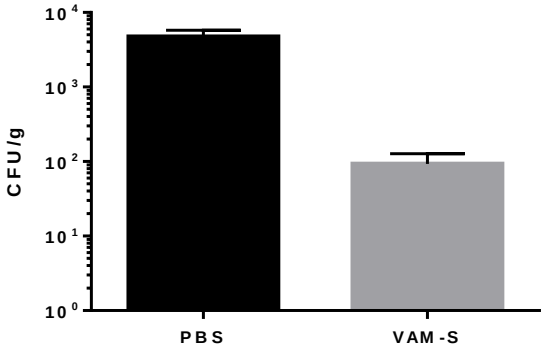
GPI Biotech VAM-S **Dec. 4, 2019**
Summary Data report

Reduction of *Salmonella* Typhimurium on Pre-Sliced Apples

Purpose

The purpose of this study is to examine the potential of GPI Biotech VAM-S to reduce *Salmonella* on fruits at an application rate of 2×10^8 PFU/g.

Summary Results



Error bars represent SEM of 3 independent samples

Reduction of *Salmonella* Typhimurium on experimentally contaminated apple slices was demonstrated. The *Salmonella* level on untreated apple slices (PBS) was approximately 5×10^3 CFU/g, while VAM-S treated apple slices was approximately 100 CFU/g, equating to 1.7 log of *Salmonella* reduction. This demonstrates the potential of VAM-S to reduce *Salmonella* in slightly acidic environments.

Method

1. Grown *Salmonella* Typhimurium culture in LB broth to approximately OD_{600}=1, which equates to approximately 10^9 CFU/mL.
2. Cut apples to approximately 10g pieces and place in a sterile stomacher bag.
3. Add 0.1mL of *Salmonella* culture into stomacher bag with apple slice.

4. Shake the bag to coat the apple slice for 1 min. and allow to stand for 15 min. for bacterial attachment.
5. Remove the apple slice and place in a new sterile stomacher bag.
6. Add 0.1mL of PBS or VAM-S into the bag.
7. Shake the bag for 1 min. and allow to stand for 4 min.
8. Remove the apple slice and place in a new sterile stomacher bag.
9. Add 10mL of buffered peptone water (BPW) into the bag and shake the bag for 1 min. to rinse the surface.
10. Extract BPW and dilute as necessary.
11. Plate appropriate dilutions onto XLD agar plates.
12. Include samples without *Salmonella* inoculation to assess background flora on the apple slices.

Data

	(CFU/g)				*Sal.* Reduction		
	Replicate 1	**Replicate 2**	**Replicate 3**	**AVG**	**Log**	**%**	**P-Value**
no inoculation	0.00E+00	0.00E+00	0.00E+00	0.00E+00			
PBS	4.10E+03	6.00E+03	4.80E+03	4.97E+03			
VAM-S	9.75E+01	6.25E+01	1.38E+02	9.92E+01	1.70	98.0%	<0.001

The pH of the apples used in this study was found to be approximately 3.6.

1255 Journey's End Circle, Newmarket ON L3Y 8T7 Tel: (905) 853 – 8828 Fax: (905) 853 – 8886

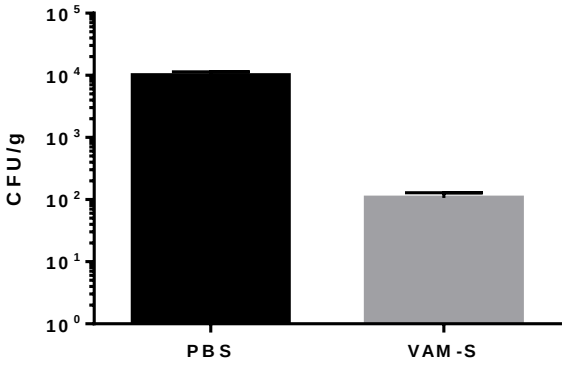
GPI Biotech VAM-S **Dec. 10, 2019**
Summary Data Report

Reduction of *Salmonella* Typhimurium on Salmon

Purpose

The purpose of this study is to examine the potential of GPI Biotech VAM-S to reduce *Salmonella* on salmon at an application rate of 2×10^8 PFU/g.

Summary Results



Error bars represent SEM of 3 independent samples

Reduction of *Salmonella* Typhimurium on experimentally contaminated salmon was demonstrated. The *Salmonella* level on untreated salmon pieces (PBS) was approximately 9.9×10^3 CFU/g, while VAM-S treated salmon pieces was approximately 107 CFU/g. Therefore, VAM-S can significantly reduce viable *Salmonella* levels on experimentally contaminated salmon by 2 logs in 5 min of treatment time.

Method

1. Grown *Salmonella* Typhimurium culture in LB broth to approximately OD_{600}=1.
2. Place an approximately 10g salmon sample in a sterile stomacher bag.
3. Add 0.1mL of *Salmonella* culture into the stomacher bag.

4. Shake the bag to coat the salmon piece for 1 min. and allow to stand for 15 min. for bacterial attachment.
5. Remove the salmon piece and place in a new sterile stomacher bag.
6. Add 0.1mL of PBS or VAM-S into the bag.
7. Shake the bag for 1 min. and allow to stand for 4 min.
8. Remove the salmon piece and place in a new sterile stomacher bag.
9. Add 10mL of buffered peptone water (BPW) into the bag and shake the bag for 1 min. to rinse the surface.
10. Extract BPW and dilute as necessary.
11. Plate appropriate dilutions onto XLD agar plates.
12. Include samples without *Salmonella* inoculation to assess background flora on the salmon pieces.

Data

	(CFU/g)				*Sal.* Reduction		
	Replicate 1	**Replicate 2**	**Replicate 3**	**AVG**	**Log**	**%**	**P-Value**
no inoculation	0.00E+00	0.00E+00	0.00E+00	0.00E+00			
PBS	8.26E+03	1.06E+04	1.09E+04	9.93E+03			
VAM-S	1.30E+02	1.04E+02	8.62E+01	1.07E+02	1.97	98.9%	<0.001

1255 Journey's End Circle, Newmarket ON L3Y 8T7 Tel: (905) 853 – 8828 Fax: (905) 853 – 8886

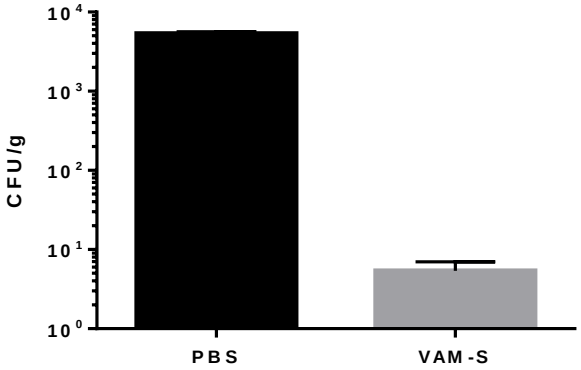
GPI Biotech VAM-S **Dec. 16, 2019**
Summary Data Report

Reduction of *Salmonella* Typhimurium on Shelled Shrimps

Purpose

The purpose of this study is to examine the potential of GPI Biotech VAM-S to reduce *Salmonella* on shelled shrimps at an application rate of 2×10^8 PFU/g.

Summary Results



Error bars represent SEM of 3 independent samples

Reduction of *Salmonella* Typhimurium on experimentally contaminated shrimps was demonstrated. The *Salmonella* level on untreated shrimps (PBS) was approximately 5.4×10^3 CFU/g, while VAM-S treated shrimps was approximately 5 CFU/g. Therefore, VAM-S can significantly reduce viable *Salmonella* levels on experimentally contaminated shrimps by 3 logs in 5 min of treatment time.

Method

1. Grown *Salmonella* Typhimurium culture in LB broth to approximately OD_{600}=1.
2. Place shrimp in a sterile stomacher bag.
3. Add 0.1mL of *Salmonella* culture into the stomacher bag.

4. Shake the bag to coat the shrimp for 1 min. and allow to stand for 15 min. for bacterial attachment.
5. Remove the shrimp and place in a new sterile stomacher bag.
6. Add 0.1mL of PBS or VAM-S into the bag.
7. Shake the bag for 1 min. and allow to stand for 4 min.
8. Remove the shrimp and place in a new sterile stomacher bag.
9. Add 10mL of buffered peptone water (BPW) into the bag and shake the bag for 1 min. to rinse the surface.
10. Extract BPW and dilute as necessary.
11. Plate appropriate dilutions onto XLD agar plates.
12. Include samples without *Salmonella* inoculation to assess background flora on the shrimps.

Data

	(CFU/g)				*Sal.* Reduction		
	Replicate 1	**Replicate 2**	**Replicate 3**	**AVG**	**Log**	**%**	**P-Value**
no inoculation	0.00E+00	0.00E+00	0.00E+00	0.00E+00			
PBS	5.19E+03	5.21E+03	5.68E+03	5.36E+03			
VAM-S	6.61E+00	6.00E+00	3.51E+00	5.37E+00	3.00	99.9%	<0.001

From:	Edith Chow
To:	Hice, Stephanie
Subject:	RE: GRN 000917 - Questions for Notifier
Date:	Wednesday, July 15, 2020 9:47:17 AM
Attachments:	image003.png 2020-07-15-GRN 917 Responses_FSIS.PDF

Dr. Hice,

Please see the attached in response to the question from the FSIS.

Thank you.

Edith

Edith Chow, Ph.D. | Research Scientist | edith.chow@gpiglobal.com| **Gum Products International** |
1255 Journey's End Circle | Newmarket | Ontario | CANADA | L3Y 8T7 |
1.905.853.8828 x 329 (O) | 1.905.853.8886 (F) |1.416.723.1907 (M)

From: Hice, Stephanie [mailto:Stephanie.Hice@fda.hhs.gov]
Sent: July 15, 2020 8:13 AM
To: Edith Chow
Subject: RE: GRN 000917 - Questions for Notifier

Dear Dr. Chow,

During review of GRAS Notice No. 000917, USDA FSIS noted an additional question that needs to be addressed and is below:

- How many replicates were performed for the suitability studies?

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety

U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

July 15, 2020

Question Regarding GRN 000917 from USDA FSIS:

How many replicates were performed for the suitability studies?

GPI response – 3 replicates were performed for each suitability study.

From:	Edith Chow
To:	Hice, Stephanie
Subject:	RE: GRN 000917 - Questions for Notifier
Date:	Tuesday, July 28, 2020 3:09:38 PM
Attachments:	image001.png 2020-07-28-GRN 917 Responses_FDA.pdf

Dr. Hice,

Please see the attached response to the question.

Thank you.

Regards,
Edith

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From: Hice, Stephanie [mailto:Stephanie.Hice@fda.hhs.gov]
Sent: July 28, 2020 1:33 PM
To: Edith Chow
Subject: RE: GRN 000917 - Questions for Notifier

Dear Dr. Chow,

During review of GRAS Notice No. 000917, we noted an additional question that needs to be addressed and is below:

- In the June 25, 2020 amendment to the notice, the notifier includes a December 6, 2019 study report titled, "Reduction of *Salmonella* Typhimurium on Eggs" (p. 3-4). We note, page 4 of the report references a protocol for apple slices. For the administrative record, please clarify this discrepancy.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

July 28, 2020

Questions/Comments Regarding GRN 000917:

In the June 25, 2020 amendment to the notice, the notifier includes a December 6, 2019 study report titled, "Reduction of *Salmonella* Typhimurium on Eggs" (p. 3-4). We note, page 4 of the report references a protocol for apple slices. For the administrative record, please clarify this discrepancy.

GPI response – Apple slice or slices referenced in the protocol on page 4 should be read as egg or eggs. We apologize for the typographical errors.