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Method Validation Report for *Listeria Monocytogenes* as per FDA BAM Chapter Number 10

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ABSTRACT

The bacteria constituting the species Listeria monocytogenes are commonly found in the intestinal flora of man and animals. Listeria monocytogenes food poisoning ranks among the most common gastrointestinal diseases in developed countries. The method for the enumeration of Listeria monocytogenes in food
samples as per FDA BAM Chapter Number 10, was validated for a project involving the use of the standard test method in commercial laboratories across different regions. The study was performed to attend precision in terms of linearity, repeatability, reproducibility, accuracy, and limit of detection. Sampled were artificially contaminated (spiked) to achieve desired concentration
of bacterial cells for validating the method. The precision depended on the type of the samples being analysed.

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Keywords- Listeria monocytogenes, Method Validation, Linearity, Spike.

INTRODUCTION

One serious threat to public health in both developed and developing countries is the microbial contamination of food. This problem poses a great challenge and consequently has economic implications. Causes of microbial contamination are diverse and these maybe natural, environmental, or technological. The microbiological quality of most ready to- eat
foods is of great significance to human health because they require minimal or no processing when consumed. [6,7,9]

Today, many methods exist that are used to assess the microbiological safety and quality of raw materials and finished food products and to monitor the microbiological status of manufacturing processes. The developers, end-users and public health authorities need a reliable common protocol for the validation of such alternative methods. [2, 3, 5, 10]

A test method must be shown to be fit for purpose so that a facility's customers can have confidence in the results produced by its application. Method validation provides objective evidence that a method is fit for purpose, meaning that the requirements for a specific intended use are fulfilled.

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Validation is always a balance between costs, risks, and technical possibilities. The extent of validation required will depend on the status of the method under consideration and the needs relating to its intended application. [2, 3, 7]

The bacteria constituting the species Escherichia coli are commonly found in the intestinal flora of man and animals, and were until late 1950s recognized as non-pathogenic normal cohabitants. However, certain strains might induce disease, and Listeria monocytogenes should therefore be regarded as a potential pathogenic organism. The pathogenic strains can cause distinct disease syndrome as different diarrheal diseases, wound infections meningitis, septicaemia, atherosclerosis, haemolytic uremic syndrome and immunological diseases such as reactive and rheumatoid arthritis. Several different groups of diarrhea-inducing strains are known. [1, 5, 6]

The FDA BAM Chapter Number 10 International Standard describes a method for the enumeration of viable Listeria monocytogenes. This test method is applicable to products intended for human consumption and the feeding of animals, and environmental samples in food production and food handling. [1]

For the validation the method was challenged with two different food matrices. For this study raw chicken breast and Milk sample were used. In addition, a reference material was used to identify any serious errors in any of the participant's performance and to determine the maximum precision possible as the FDA BAM Chapter Number 10 is quantitative method. The food samples were artificially inoculated to achieve desired inoculum levels and homogeneity. These materials were tested extensively by four different trained analyst's and the data from the testing was used to calculate the following in relation with the food type and inoculum level -

1. Linearity- which is the property of a mathematical relationship or function that can be graphically represented as a straight line. Therefore, linearity uncertainty would the uncertainty associated with non-linear behaviour observed across the range of an assumed linear function.

2. Repeatability is defined as the ability of the analytical procedure to obtain test results within a given range of accuracy and precision when the same sample is repeated in accordance with the same method protocol. Chicken breast and Milk samples were spiked with known concentration of culture suspension and the same sample was repeated 10 times, to calculate the Mean, SD and %RSD.

3. Reproducibility is defined as the ability of the analytical procedure to obtain test results within a given range of accuracy and precision when the same sample is performed by different analyst in accordance with the same method protocol. Chicken Breast and Milk samples were spiked with known concentration of bacterial cell suspension and the same sample was repeated by four different analysts', to calculate the Mean, SD and %RSD.

Accuracy is the closeness of agreement between a test result and accepted reference value. Accuracy of the tested sample was determined by using the following formulae –

(Test Result/Expected Value) *100

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EXPERIMENTAL METHODS

2.1. Preparation of Stock Culture.

1 lyophilized pellet of Listeria monocytogenes ATCC 19115 cultures provided by Microbiologic' s is added to sterile phosphate buffer saline which was pre-incubated at 35oC for 30 minutes. This suspension is incubated at 35oC for 24 hrs under anaerobic conditions. Prepare a serial dilution of the culture to verify the known concentration.

2.2 Spiking of samples.

Sterile chicken breast and sterile milk samples were spiked with the cell suspension which was prepared in 2.1. The samples were spiked with different concentration of cell suspension ranging from 10-5 to 10-7 CFU/ml.

2.3 Method Validation

To perform the method validation different parameters were conducted which were-Linearity, Repeatability, Reproducibility, Accuracy and Limit of Detection.

RESULTS

3.1 Stock Culture.

Table 1- Stock Culture- Listeria monocytogenes ATCC 19115

Dilution	Sterile Distilled Water										Mean	SD	% RSD
10-5	710	690	680	700	695	700	685	690	680	710	694.0	11.01	0.1585742
10-6	70	71	69	65	66	70	68	66	71	69	68.5	2.173	0.3172361
10-7	6	6	7	7	7	6	6	7	8	6	6.6	0.699	1.0594029
10-8	0	0	0	0	0	0	0	0	0	0	0	0	0

3.2 Linearity

Table 2 – Linearity

Samples / Dilution	Milk					Chicken Breast				
	Values		Mean	SD	% RSD	Values		Mean	SD	% RSD
10-5	73	71	72	1.4142136	2.3183829	69	70	69.5	0.7071068	1.0174198
10-6	8	7	7.5	0.7071068	6.7343503	7	8	7.5	0.7071068	9.4280904
10-6	0	0	0	0	0	0	0	0	0	0

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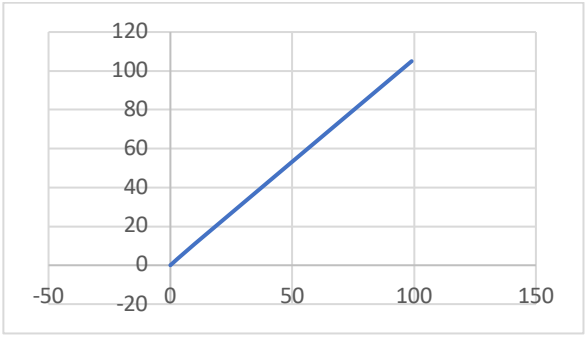
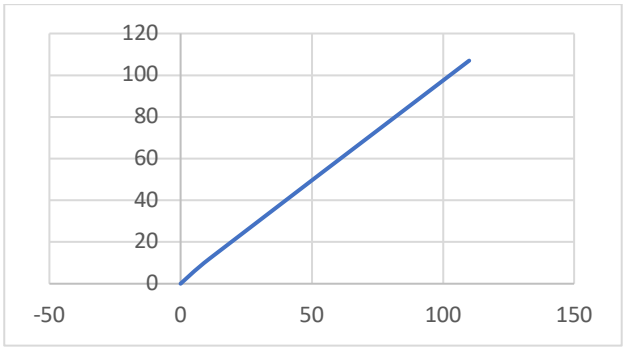
Graph 1a – Linearity – Listeria monocytogenes Chicken Breast Sample



Graph 1b - Linearity –Listeria monocytogenes Milk Sample



3.3 Repeatability

The results for the analysis are demonstrated in the tables which are given below.

Table 3a – Repeatability for Chicken Breast Analyst 1

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	69	68	65	66	70	69	66	70	69	68	68	1.7638342	2.5938738
10-6	6	6	7	6	6	6	6	7	6	7	6.3	0.4830459	7.6673951

Table 3b – Repeatability for Chicken Breast Analyst 2

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	66	69	70	65	69	70	66	68	67	69	67.9	1.7919573	2.6391124
10-6	6	6	7	6	6	7	7	6	6	7	6.4	0.5163978	8.0687153

Table 3c – Repeatability for Chicken Breast Analyst 3

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	70	71	69	70	68	65	66	69	71	69	68.8	1.9888579	2.8907818
10-6	7	7	7	6	6	6	6	7	7	7	6.6	0.5163978	7.8242088

Table 3d – Repeatability for Chicken Breast Analyst 4

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	66	69	67	65	70	69	65	69	70	66	67.6	2.0110804	2.974971
10-6	6	7	7	7	7	6	6	6	6	7	6.5	0.5270463	8.1084043

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Table 3a – Repeatability for Milk Analyst 1

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	68	69	71	69	65	66	69	70	69	67	68.3	1.8287822	2.6775728
10-6	7	7	6	6	6	7	7	7	7	6	6.6	0.5163978	7.8242088

Table 3b – Repeatability for Milk Analyst 2

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	71	70	69	67	66	68	70	69	68	66	68.4	1.7126977	2.503944
10-6	7	6	6	7	7	6	6	7	6	7	6.5	0.5270463	8.1084043

Table 3c – Repeatability for Milk Analyst 3

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	69	67	66	69	66	65	68	71	69	66	67.6	1.8973666	2.8067553
10-6	6	6	6	6	7	7	6	6	7	7	6.4	0.5163978	8.0687153

Table 3d – Repeatability for Milk Analyst 4

Dilution	Repeatability										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	66	70	71	69	66	69	65	69	65	71	68.1	2.3781412	3.492131
10-6	7	7	6	6	6	7	7	7	6	6	6.5	0.5270463	8.1084043

Reproducibility.

Dilution	Reproducibility														
	Analyst 1	Mean	SD	% RSD	Analyst 2	Mean	SD	% RSD	Analyst 3	Mean	SD	% RSD	Analyst 4	Mean	SD	% RSD

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10-5	71	67	69	2.82	4.09	68	70	69	1.41	2.04	70	67	68.5	2.12	3.09	68	71	69.5	2.12	3.05
10-6	7	6	6.5	0.70	10.87	7	7	7	0	0	6	7	6.5	0.70	10.87	7	7	7	0	0

The results for the analysis are demonstrated in the table below-

Table 4a- Reproducibility for Chicken Breast

Dilution	Reproducibility																			
	Analyst 1		Mean	SD	%RSD	Analyst 2		Mean	SD	%RSD	Analyst 3		Mean	SD	%RSD	Analyst 4		Mean	SD	%RSD
10-5	70	67	68.5	2.12	3.09	68	69	68.5	0.70	1.03	66	70	68	2.82	4.15	69	71	70	1.41	2.02
10-6	7	7	7	0	0	7	7	7	0	0	6	7	6.5	0.70	10.87	7	7	7	0	0

Table 4b- Reproducibility for Milk

Accuracy

Accuracy of the given test method was analysed from the data obtained after the samples spiked with known concentration of 10-5 and 10-6 of Listeria monocytogenes was tested.

The known concentration of the culture suspension is obtained by referring to the mean values of the culture suspension in Table 1

Accuracy is calculated as 88.69%

Limit of Detection

Limit of Detection for enumeration of Listeria monocytogenes was found to be 10 cfu/g

DISCUSSION

In the present study, the test parameter Listeria monocytogenes was validated by using two different food matrices by the test method FDA BAM Chapter Number 10. Two different matrix of food sample was tested by the same

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analysts under same laboratory conditions. Raw chicken and Milk sample were used for the entire study and the same spiked sample was used by the analysts to prevent more uncertainty in the testing. During the study the SD (standard deviation) and %RSD (Relative Standard Deviation) for the repeatability and reproducibility among the analysts was between the acceptable range. The analysis was performed by using an inoculum with low and high concentration of bacterial cells; and during the validation study it was observed that the test method FDA BAM Chapter Number 10 was able to enumerate the Listeria monocytogenes when spiked in the food samples.

CONCLUSION

In the present study Enumeration of Listeria monocytogenes in food sample by test method FDA BAM Chapter Number 10 was validated. The study shows that low and high concentration of Listeria monocytogenes cells can be detected and enumerated, hence it is an efficient method which can be used for analysis of food samples.

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