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Method Validation Report for *Listeria monocytogenes* as per ISO 11290-1:2017

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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 ISO 11290-1:2017, 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.

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

INTRODUCTION

Circulation of zoonotic and Human pathogens within the biosphere is a major health issue. Agroecosystems may participate to the transmission of pathogens to the food chain through production of contaminated raw products. 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]

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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.

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]

Listeria monocytogenes is the agent of listeriosis, a food-borne illness. Health effects range from flu-like symptoms with vomiting and diarrhea in healthy adults to life-threatening diseases such as meningitis and septicaemia in vulnerable people and spontaneous abortion in pregnant women. In the light of these health hazards, this bacterium received much attention to understand the physiopathology of listeriosis. As a matter of fact, L. monocytogenes has become a paradigm for the study of intracellular pathogens. Similarly, the mechanisms that underlie its ability to persist in foodstuff and in the food manufacturing environment have been documented. However, the ecology of L. monocytogenes in outdoor environments is only partially understood. The objective of this review is to present the state of the art regarding extrinsic and intrinsic factors that shape the ecology of L. monocytogenes in the soil environment. [1, 5, 6]

The ISO 11290-1:2017 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 ISO 11290-1:2017 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

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.

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.

Method Validation

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

RESULTS

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

Linearity

Table 2 – Linearity

Samples / Dilution	Milk					Chicken Breast				
	Values		Mean	SD	% RSD	Values		Mean	SD	% RSD
10^{-5}	73	71	72	1.41	2.32	110	107	108.5	2.12	1.95
10^{-6}	8	7	7.5	0.70	6.73	11	12	11.5	0.70	6.15
10^{-7}	0	0	0	0	0	0	0	0	0	0

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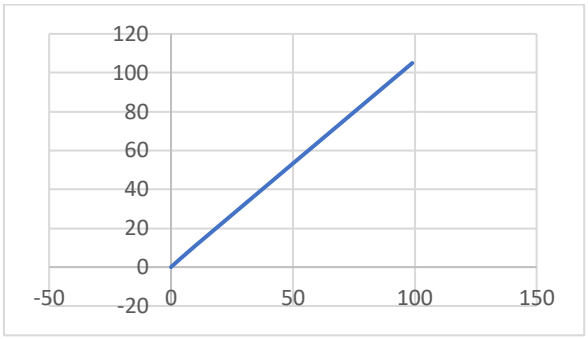
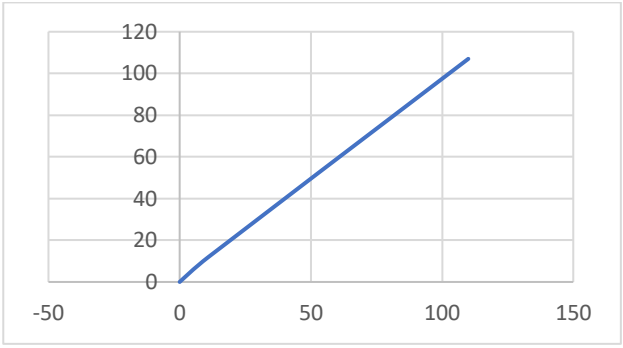
Graph 1a – Linearity – *Listeria monocytogenes* Chicken Breast Sample



Graph 1b - Linearity – *Listeria monocytogenes* Milk Sample



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}	111	119	115	115	110	116	114	119	110	119	114.8	3.5839147	3.1218769
10^{-6}	11	10	11	10	11	10	11	11	11	10	10.6	0.5163978	4.8716772

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}	113	119	117	110	115	110	115	114	113	118	114.4	3.0623158	2.6768494
10^{-6}	10	10	10	11	10	11	11	11	10	10	10.4	0.5163978	4.9653633

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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}	113	111	117	112	110	115	112	110	119	116	113.5	3.1001792	2.7314354
10^{-6}	10	11	10	10	11	11	11	10	11	10	10.5	0.5270463	5.0194883

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}	119	113	112	115	112	117	113	110	111	116	113.8	2.8596814	2.5129011
10^{-6}	8	10	10	11	10	10	10	9	10	11	9.9	0.875595	8.8443943

Table 3a – Repeatability for Milk 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	1.76	2.59
10^{-6}	6	6	7	6	6	6	6	7	6	7	6.3	0.48	7.67

Table 3b – Repeatability for Milk 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	1.79	2.63
10^{-6}	6	6	7	6	6	7	7	6	6	7	6.4	0.51	8.07

Table 3c – Repeatability for Milk 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	1.99	2.89
10^{-6}	7	7	7	6	6	6	6	7	7	7	6.6	0.52	7.82

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	69	67	65	70	69	65	69	70	66	67.6	2.01	2.97
10^{-6}	6	7	7	7	7	6	6	6	6	7	6.5	0.52	8.10

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Reproducibility.

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

Dilution	Reproducibility																			
	Analyst 1		Mean	SD	% RSD	Analyst 2		Mean	SD	% RSD	Analyst 3		Mean	SD	% RSD	Analyst 4		Mean	SD	% RSD
10-5	100	111	105.5	7.77	7.37	103	113	108	7.07	6.55	96	102	99	4.24	4.28	110	99	104.5	7.77	7.44
10-6	10	9	9.5	0.70	7.44	10	11	10.5	0.70	6.73	10	11	10.5	0.70	6.73	10	12	11	1.41	12.85

Table 4a- Reproducibility for Chicken Breast

Table 4a- Reproducibility for Milk

Dilution	Reproducibility																			
	Analyst 1		Mean	SD	% RSD	Analyst 2		Mean	SD	% RSD	Analyst 3		Mean	SD	% RSD	Analyst 4		Mean	SD	% RSD
10-5	71	67	69	2.83	4.09	68	70	69	1.41	2.05	70	67	68.5	2.12	3.09	68	71	69.5	2.12	3.05
10-6	7	6	6.5	0.71	10.88	7	7	7	0	0	6	7	6.5	0.70	10.87	7	7	7	0	0

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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 93.4%

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 ISO 11920-1:2017. Two different matrix of food sample was tested by the same 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 ISO 11920-1:2017 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 ISO 11920-1:2017 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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