

**ANALYTICAL METHOD VALIDATION REPORT FOR THE DETERMINATION OF ASSAY OF LACTIC ACID BACILLUS BY MICROBIOLOGICAL ASSAY METHOD****1. Summary**

Microbiological Assay Method for the determination of Assay of Lactic Acid Bacillus has been carried out using Microbiological Assay Method. Microbiological Assay Method for the determination of Assay of Lactic Acid Bacillus has been validated. The method is found to be precise, accurate & rugged for the intended studies and therefore suitable for use in determining the assay of Lactic Acid Bacillus.

TABLE OF CONTENT**1.1 Summary Table**

The method is studied for following parameters for Lactic Acid Bacillus.

Precision			
Content	Observation		Acceptance Criteria
Method Precision	Result (Spores Count in Millions)	% Assay	- Relative Standard deviation (%RSD) for Assay of six different sample preparations: Not more than - 10.0%
	6205.87	103.43	
	6208.20	103.47	
	6158.45	102.64	
	6245.66	104.09	
	6223.82	103.73	
	6278.65	104.64	
Mean	6220.11	103.67	
% RSD	0.65	0.65	
Intermediate Precision (Ruggedness)			
Method Precision	Result (mg)	% Assay	-Relative Standard deviation (%RSD) for Assay of six different sample preparations: Not more than - 10.0%
	6114.50	101.91	
	6278.03	104.63	
	6213.90	103.57	
	6191.95	103.20	
	6193.99	103.23	
	6250.62	104.18	
Mean	6207.16	103.45	
% RSD	0.91	0.91	



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Summary for overall 12 Assay	Overall Relative Standard deviation (%RSD) of assay of Lactic Acid Bacillus with 12 determinations is 0.76%	-Relative Standard deviation (%RSD) for Assay of 12 different sample preparations: Not more than - 10.0%	
Accuracy as Recovery	Recovery for assay from the sample obtained with triplicate test preparation at each level (i.e. about 80%, 100%, 120% of specification level) is in the limit of 95-105%.	-Recovery for assay from the sample obtained with triplicate test preparation at each level (i.e. about 80%, 100%, 120 % of specification level) should be between 95 and 105 %	
Prep. No	80%	100%	120%
1	103.47	100.36	103.15
2	100.26	104.85	104.03
3	100.75	99.71	100.77

1. Introduction:

This report describes the validation of test procedure used for the determination of assay of Lactic Acid Bacillus. The analytical methodology used for Microbiological Assay Method. The method was validated as per validation protocol.

2. Objective:

The objective of this analytical method is to demonstrate that it is suitable for its intended purpose. The overall purpose of the validation is to provide documented evidence of precision and accuracy for the method with the help of the following parameters.

- Precision
- Method Precision
- Intermediate precision (Ruggedness)
- Accuracy as recovery

Detail of method, each experiment, and observations during the performance and results are reported below.



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3. Raw Material used:

Raw Material

Name : Lactic Acid Bacillus

Batch no. :

REAGENTS & PRERQUISITIES:

- 90mm Petriplates
- Glucose Yeast Extract Agar
- 0.9% Saline
- Distilled water
- 25x 150mm Test Tubes
- 100 ml Volumetric Flask
- Vortex Mixer
- Sodium chloride

4.0 EQUIPMENTS:

- Vortex Mixer
- Calibrated Weighing Balance
- Water Bath at 75°C
- Micropipette

5.0 PREPARATIONS PROCEDURE:

Method for the determination of Assay of Lactic Acid Bacillus:

Weight accurately powder of 1gm of Lactic Acid Bacillus and add 100ml of sterile normal saline solution and homogenize at 12,000 to 15,000 rpm for 7-10 minutes. Mix well the test solution by hand shaking and dilute it further step wise through a series of test tubes (size 25mm X 150mm), containing 9ml of sterile normal saline solution by an appropriate decimal dilution method.

The final dilution is estimated to produce 30-300 colonies per plates after incubation (Recommended final dilution should 100×10^{-6}). The tube containing the final dilution is allowed to stand in water bath at 75° for 30 minutes. Cool the tube immediately to about 45°C and disperse 1ml aliquot in each of 5 sterile petri plates.



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Add 15ml of pre sterilised and held at 50°C glucose yeast extract agar medium previously sterilized, melted and cooled 45-50°C to each of the 5 sterile petri plates containing the test solution. Incubate the plates at 37°C for 72 hours after incubation count the number of colonies in each plate.

After incubation count the number of colonies in each plate.

Take average number of colonies and calculate the spore count of lactic acid bacillus.

Calculation:

Take average number of colonies x by the dilution number (dilution factor) represent the viable lactobacillus spores count per gm powder.

6.0 PRECISION:

6.1 Precision:

Precision was performed by Microbiological assay in six replicates of sample preparations of Lactic Acid Bacillus at 100 % specification level and results are shown in the Table – 1.

Table –1: Precision

Lactic Acid Bacillus		
Sample Preparation	Result (Spores Count in Millions)	% Assay
1	6205.87	103.43
2	6208.20	103.47
3	6158.45	102.64
4	6245.66	104.09
5	6223.82	103.73
6	6278.65	104.64
Mean	6220.11	103.67
Stdev	40.60	0.68
RSD (%)	0.65	0.65

Conclusion: Relative standard deviation (%RSD) for Lactic Acid Bacillus with six replicates sample preparation is less than 10.0%.

Conclusion: The method is found to be precise.

**ANALYTICAL METHOD VALIDATION REPORT FOR THE DETERMINATION OF ASSAY OF LACTIC ACID BACILLUS BY MICROBIOLOGICAL ASSAY METHOD****6.2 Intermediate precision (Ruggedness)**

This study shall be carried out as per method precision by a different analyst, different day by using different set of sample solution.

Intermediate Precision of the method was demonstrated by calculating the assay with six different sample preparations prepared Results found of Lactic Acid Bacillus is 0.91% of 6 assays RSD results are shown in the Table – 2.

Table –2: Precision (Lactic Acid Bacillus)

Sample Preparation	Result (Spores Count in Millions)	%Assay
1	6114.50	101.91
2	6278.03	104.63
3	6213.90	103.57
4	6191.95	103.20
5	6193.99	103.23
6	6250.62	104.18
Mean	6207.16	103.45
RSD (%)	0.91	0.91

% RSD of assay for 12 sample preparations between different analysts

Table 3:

Sample Preparation	Lactic Acid Bacillus Assay (%)
1	103.43
2	103.47
3	102.64
4	104.09
5	103.73
6	104.64
7	101.91
8	104.63
9	103.57



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Sample Preparation	Lactic Acid Bacillus Assay (%)
10	103.20
11	103.23
12	104.18
Mean	103.56
R.S.D. (%)	0.76

CONCLUSION:

The % RSD for the overall assay of the twelve sample preparations is within the limits.

7.0 Accuracy as recovery

The accuracy of the method was demonstrated at three different concentration levels by calculating recovery (about 80 %, 100 %, and 120 % of specification level). The method is found to be accurate. Results are shown in table-4.

Table –4: % Recovery

% Level	Sample Preparation	Lactic Acid Bacillus
80 %	1	103.47
	2	100.26
	3	100.75
100 %	1	100.36
	2	104.85
	3	99.71
120 %	1	103.15
	2	104.03
	3	100.77

CONCLUSION:

% Recovery complies with specified acceptance criteria, hence the method is found to be accurate in the range of 80 to 120%.



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Conclusion of overall Study for Lactic Acid Bacillus Analytical Method Validation:

The assay by Microbiological Assay Method adopted for Lactic Acid Bacillus is validated, found to be precise and accurate; it is also proved to be rugged, so this method can be used for routine analysis and stability studies.

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