



Method Validation Report for the Enumeration of *Escherichia Coli* Using 3M Petri-Film Medium

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Abstract

This study presents a comprehensive method validation report for the detection and enumeration of *Escherichia coli* using the 3M™ Petri-film™ *Escherichia coli*/Coliform Count Plate—a chromogenic, ready-to-use culture medium designed for food microbiological analysis. The primary objective was to evaluate the analytical performance of the method across a range of food matrices, including cheese, flour, lettuce, and raw chicken. These samples were deliberately inoculated with known concentrations of *Escherichia coli* to simulate controlled contamination and allow for precise assessment.

The validation protocol focused on key performance parameters such as linearity, repeatability, reproducibility, and the limit of detection. Each matrix was tested in duplicate by independent analysts to assess inter-analyst consistency and the method's robustness under routine laboratory conditions.

Results indicated that the 3M Petri-film *Escherichia coli* plates consistently supported the selective growth and reliable identification of *Escherichia coli* colonies, which were characterized by distinct blue coloration. The method exhibited a high degree of specificity, with negligible interference from non-target microorganisms, and demonstrated strong reproducibility across all tested matrices.

Quantitative outcomes closely matched expected colony-forming unit (CFU) counts, reinforcing the method's accuracy and consistency. Overall, the findings validate the 3M Petri-film *Escherichia coli*/Coliform Count Plate as a reliable, efficient, and user-friendly alternative to conventional culture-based methods for routine *Escherichia coli* enumeration in food safety testing laboratories.

Key words: *Escherichia coli* enumeration, Method validation, 3M™ Petri-film™ *Escherichia coli*/Coliform Count Plate, Food microbiology, Chromogenic culture plates, Food safety testing.



Introduction

Escherichia coli is a rod-shaped, Gram-negative, facultative anaerobic, non-sporulating bacterium belonging to the Enterobacteriaceae family. It forms a natural part of the gastrointestinal microbiota in humans and animals and is commonly found in environmental reservoirs such as soil and water (Denamur et al., 2021). Among microbial contaminants in food and water, *Escherichia coli* is one of the most frequently detected, and its presence whether pathogenic or non-pathogenic is widely recognized as an indicator of faecal contamination. As such, its detection is critical for assessing the hygienic status, potability, and overall safety of food and water samples (Carvalho et al., 2018).

Traditional culture-based detection methods, while well-established, are often labour-intensive, time-consuming, and less effective in identifying viable but non-culturable *Escherichia coli*. These limitations underscore the need for more rapid, accurate, and sensitive alternatives suitable for routine microbiological surveillance.

The 3M Petri-film *Escherichia coli*/Coliform Count Plate represents one such alternative is a chromogenic, self-contained system designed to streamline microbial enumeration. Comprising a cold-water-soluble gelling agent, nutrients, and chromogenic indicators, Petri-film plates are engineered to replicate the accuracy of conventional agar-based methods while offering greater ease of use. Previous comparative studies have demonstrated strong correlation ($R > 0.9$) between Petri-film and standard methods such as mTEC and Colilert in environmental testing (Heden et al., 2003), while Schraft and Watterworth (2005) reported minimal discrepancies in *Escherichia coli* counts between Petri-film and traditional agar in food matrices. Moreover, multi-centre validation studies have confirmed the method's equivalence to FDA BAM and ISO reference standards across diverse food categories, including pet foods (Bird et al., 2020).

Method Validation Overview: Validation of the 3M Petri-film *Escherichia coli*/Coliform Count Plate is critical to establish its reliability and applicability across a wide range of food matrices. In the current study, the method was evaluated in accordance with internationally recognized validation principles, with specific reference to performance indicators such as linearity, repeatability, and reproducibility. Four distinct food matrices cheese, flour, lettuce, and raw chicken were selected to represent a diverse set of sample types with varying physicochemical properties.



Artificial contamination of samples was performed using defined concentrations of *Escherichia coli*, and the method was applied by two independent analysts to ensure robustness under routine laboratory conditions. The 3M Petri-film method demonstrated consistent enumeration results across all matrices, with relative standard deviations for repeatability (%RSD) falling within acceptable limits. Notably, reproducibility assessments showed strong inter-analyst agreement, underscoring the method's precision and operator-independence.

These findings align with previous validation studies published in peer-reviewed literature, which confirm the equivalency of the 3M Petri-film system to ISO and FDA reference methods for microbial enumeration. Collectively, the results support the use of the 3M Petri-film *Escherichia coli*/Coliform Count Plate as a reliable, standardized, and efficient tool for routine microbiological analysis in food safety testing laboratories.

Current Validation Study: In the present study, the analytical performance of the 3M Petri-film *Escherichia coli*/Coliform Count Plate was evaluated across four distinct food matrices: cheese, flour, lettuce, and raw chicken. These samples were artificially inoculated with defined concentrations of *Escherichia coli* to simulate realistic contamination scenarios and ensure homogeneity. Validation parameters were assessed using test data generated independently by two trained analysts, and included the following:

Linearity: The method's capacity to produce results that are directly proportional to the concentration of *Escherichia coli* across a defined range. Linearity was assessed by calculating the uncertainty associated with any deviation from expected linear behaviour across serial dilutions.

Repeatability: Defined as the degree of agreement among replicate measurements performed under identical conditions by the same analyst. For this, each food matrix was tested ten times using identical inoculum levels, and results were analysed in terms of mean colony counts, standard deviation (SD), and percent relative standard deviation (%RSD).

Reproducibility: Defined as the ability of the method to generate consistent results when applied by different analysts under the same protocol. In this case, all four food matrices were tested independently by two analysts using identical sample preparations, and the data were compared using mean, SD, and %RSD values to assess inter-analyst variability.



Methodology

Preparation of the Stock Culture

One lyophilized pellet of *Escherichia coli* ATCC 8739, obtained from Microbiologics, was rehydrated using sterile phosphate buffered saline. The buffer was pre-incubated at thirty-five degrees Celsius for thirty minutes prior to inoculation. The resulting suspension was then incubated aerobically at thirty-five degrees Celsius for twenty-four hours to allow for optimal bacterial revival and proliferation. Following incubation, a serial dilution of the culture was performed to verify and quantify the concentration of viable bacterial cells.

Artificial Contamination of Food Samples

To simulate controlled contamination scenarios, four distinct food matrices cheese, flour, lettuce, and raw chicken were artificially inoculated with a standardized suspension of *Escherichia coli* prepared as described in the culture preparation protocol. Each matrix was spiked with varying concentrations of the bacterial suspension, ranging from 10^{-5} to 10^{-7} CFU/mL, to achieve defined inoculum levels suitable for validation studies. This approach ensured homogeneity and allowed for a robust evaluation of the method's quantitative capabilities across different sample types.

Method Validation Parameters

The analytical method was validated by evaluating the linearity, repeatability, reproducibility, accuracy, limit of detection.

Results

Table 1 presents the enumeration data for *Escherichia coli* stock culture across three serial dilutions: 10^{-6} , 10^{-7} , and 10^{-8} . Each dilution was plated in 10 replicates using sterile distilled water as the diluent. As expected, the colony-forming unit (CFU) counts decreased with increasing dilution. The results show high consistency and precision at higher concentrations, with increased variability observed at lower CFU levels. At the highest dilution, the counts were uniform across all replicates, indicating excellent repeatability of the method.



Table 01: Stock Culture – *Escherichia coli*

Dilution	Sterile Distilled Water										Mean	Standard Deviation	% Relative Standard
10 ⁻⁶	135	130	133	134	132	135	133	136	128	130	132.6	2.59058123	1.9536812
10 ⁻⁷	12	13	12	12	11	12	13	12	12	11	12	0.66666667	5.5555556
10 ⁻⁸	1	1	1	1	1	1	1	1	1	1	1	0	0

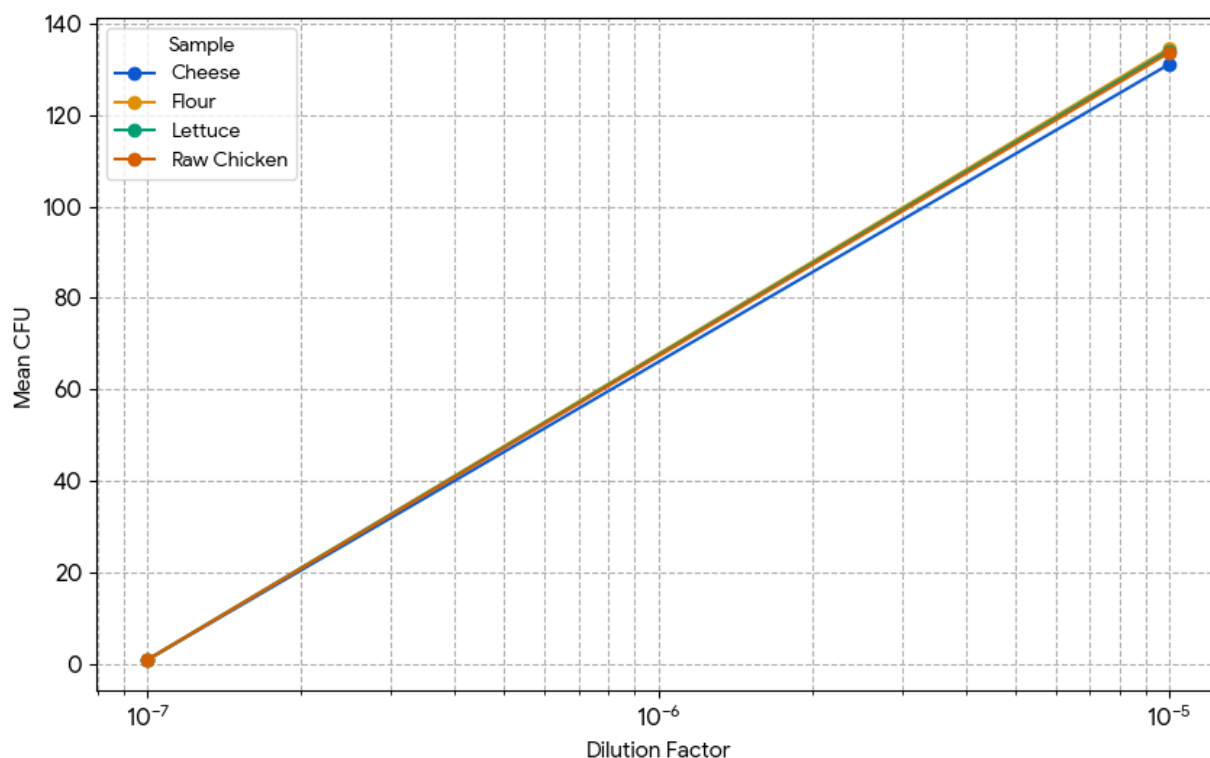
Linearity Results

Table 2 presents the evaluation of linearity for the enumeration of *Escherichia coli* across three serial dilutions (10⁻⁵ to 10⁻⁷) in four different food matrices: cheese, Flour, lettuce, and raw chicken.

Table 02: Linearity Results

Samples	Lettuce					Raw Chicken				
Dilutions	Values		Mean	SD	% RSD	Values		Mean	SD	% RSD
10 ⁻⁵	137	134	135.5	2.1213203	1.5655501	133	134	133.5	0.7071068	0.529668
10 ⁻⁶	14	12	13	1.4142136	10.878566	12	13	12.5	0.7071068	5.6568542
10 ⁻⁷	1	1	1	0	0	1	1	1	0	0

Samples	Cheese					Flour				
Dilutions	Values		Mean	SD	% RSD	Values		Mean	SD	% RSD
10 ⁻⁵	132	130	131	1.4142136	1.0795523	135	134	134.5	0.7071068	0.5257299
10 ⁻⁶	12	12	12	0	0	12	13	12.5	0.7071068	5.6568542
10 ⁻⁷	1	1	1	0	0	1	1	1	0	0



Graph 01 a: Linearity graph for cheese, flour, lettuce and raw chicken sample

Repeatability Results

Table 3 summarizes the repeatability results for the enumeration of *Escherichia coli* across different food matrices (cheese, flour, lettuce, and raw chicken) and two analysts. For each matrix, 10 replicate counts were recorded at two serial dilutions (10^{-5} and 10^{-6}). The mean colony counts, standard deviations (SD), and relative standard deviations (RSD%) were calculated to assess intra-laboratory precision.

Table 03 a: Repeatability for Cheese – Analyst 1

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	134	133	132	136	133	134	134	135	136	133	134	1.3333	0.99502
10-6	12	13	12	11	12	13	13	14	13	13	12.6	0.8432	6.69265



Table 03 b: Repeatability for Cheese – Analyst 2

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	133	135	133	132	136	133	134	135	135	132	133.8	1.3984	1.0451
10-6	12	12	13	14	13	13	11	13	12	14	12.7	0.9486	7.4699

Table 03 c: Repeatability for Flour – Analyst 1

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	133	135	128	133	137	132	130	134	134	135	133.1	2.6012	1.9543
10-6	13	13	12	12	13	14	12	11	12	13	12.5	0.8498	6.7986

Table 03 d: Repeatability for Flour – Analyst 2

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	133	135	134	128	130	134	135	132	136	130	132.7	2.6267	1.9794
10-6	12	13	13	13	14	13	12	15	12	11	12.8	1.1352	8.8694

Table 03 e: Repeatability for Lettuce – Analyst 1

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	129	127	130	132	127	135	134	129	133	136	131.2	3.2591	2.4841
10-6	11	11	12	12	13	13	14	12	13	14	12.5	1.0801	8.6409



Table 03 f: Repeatability for Lettuce – Analyst 2

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	133	134	128	136	137	132	134	127	135	129	132.5	3.4399	2.5961
10-6	11	12	13	13	12	11	14	12	13	13	12.4	0.9660	7.7910

Table 03 g: Repeatability for Raw chicken – Analyst 1

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	134	135	134	128	135	136	132	133	134	136	133.7	2.3593	1.7646
10-6	12	13	13	14	13	14	14	13	12	13	13.1	0.7378	5.6325

Table 03 h: Repeatability for Raw chicken – Analyst 2

Dilutions	Repeatability										Mean	SD	RSD
	1	2	3	4	5	6	7	8	9	10			
10-5	130	133	134	135	135	136	133	134	132	129	133.1	2.2335	1.6781
10-6	12	13	14	13	13	12	14	12	13	14	13	0.8164	6.2807

Reproducibility Results

Table 4 summarizes the reproducibility of the method between the two analysts for all sample types. Comparative values of mean, SD, and %RSD are provided for both dilution levels. The data reflect acceptable inter-analyst agreement, supporting the robustness of the method across different operators.



Table 04: Reproducibility

Limit of Detection

Dilution	Reproducibility- Cheese									
	Analyst 1		Mean	SD	%RSD	Analyst 2		Mean	SD	%RSD
10-5	132	135	133.5	2.1213	1.5890	134	133	133.5	0.7071	0.5296
10-6	12	14	13	1.4142	10.8785	133	128	130.5	3.5355	2.7092
Dilution	Reproducibility- Flour									
	Analyst 1		Mean	SD	%RSD	Analyst 2		Mean	SD	%RSD
10-5	133	136	134.5	2.1213	1.5771	135	132	133.5	2.1213	1.5890
10-6	13	14	13.5	0.7071	5.2378	12	14	13	1.4142	10.8785
Dilution	Reproducibility- Lettuce									
	Analyst 1		Mean	SD	%RSD	Analyst 2		Mean	SD	%RSD
10-5	134	136	135	1.4142	1.0475	134	135	134.5	0.7071	0.5257
10-6	12	14	13	1.4142	10.8785	13	13	13	0	0
Dilution	Reproducibility- Raw Chicken									
	Analyst 1		Mean	SD	%RSD	Analyst 2		Mean	SD	%RSD
10-5	137	135	136	1.4142	1.0398	135	134	134.5	0.7071	0.5257
10-6	12	13	12.5	0.7071	5.6568	13	13	13	0	0

The limit of detection (LOD) for the enumeration of *Escherichia coli* was determined to be 10 CFU/g, indicating the lowest concentration at which the organism can be reliably detected and enumerated under the test conditions

Discussion

This study presents a method validation of the 3M Petri-film *Escherichia coli* plate for the detection and enumeration of *Escherichia coli* across four diverse food matrices: cheese, flour, lettuce, and raw chicken. The method was evaluated using both low and high inoculum levels to simulate realistic contamination scenarios and assess performance parameters including sensitivity, selectivity, and dynamic range.

The 3M Petri-film *Escherichia coli* plates demonstrated effective selective growth of *Escherichia coli*, characterized by the development of typical blue colonies with associated gas bubbles, allowing for clear identification and accurate enumeration. Repeatability and reproducibility studies were conducted across multiple analysts and days. The calculated standard deviation (SD) and relative



standard deviation (%RSD) values for each food matrix were within acceptable limits, confirming the method's precision and robustness.

Overall, the 3M Petri-film *Escherichia coli* method proved to be a reliable, user-friendly, and efficient alternative to conventional culture methods. Its consistent performance across various food types, combined with ease of handling and rapid result interpretation, makes it well-suited for routine use in food safety laboratories, manufacturing environments, and on-site testing applications.

Conclusion

This study confirms the validity of the 3M petri-Film *Escherichia coli* method for the reliable detection and enumeration of *Escherichia coli* in food samples. The method demonstrated acceptable precision, accuracy, and sensitivity across cheese and flour matrices. With a clear detection limit of 10 colony forming units per gram, it is suitable for routine microbiological analysis in food safety laboratories and meets the necessary criteria for implementation in quality control and compliance monitoring.

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