

Research Article

## ***Escherichia coli* Bacteria Test on Polluted Meatballs with Several Variations of Positive Control Concentration**

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**Abstract.** *Escherichia coli* bacteria test on contaminated meat processed food products with several variations of positive control concentrations was carried out to provide additional information about determining the LOD value in the test method for the detection of *Escherichia coli* pathogenic bacteria. The purpose of this study was to detect and identify bacterial contamination of *Escherichia coli* ATCC 25922 in contaminated meat processed food products with variations in the concentration of positive control. The method used to identify is the enrichment method, using enrichment media to grow the suspected target bacterium *Escherichia coli* ATCC 25922 spiked in meat-processed food products, followed by isolation using selective media and ending with confirmation and affirmation tests. The samples used were 10 packages of processed meat food products. The samples were then spiked using various concentrations of positive control *Escherichia coli* 5, 10, 50, and 100 colonies/gr. The data from the research showed that all samples from the treatment of variations in the concentration of spike positive controls were detected and identified the presence of *Escherichia coli* ATCC 25922. Based on the results of this study, it can be concluded that all samples spiked with various variations in the concentration of positive control were detected and identified as *Escherichia coli* ATCC 25922.



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### **1. INTRODUCTION**

In various cases of food poisoning that occurred in Indonesia and internationally, it is sometimes associated with *Escherichia coli* contamination in food or water which is usually characterized by symptoms of diarrhea. According to Dewantoro *et al.* (2009), cases of poisoning caused by the prevalence of *Escherichia coli* bacteria in animal food products, especially beef and

chicken meat, were identified as quite high, but no pathogenic *Escherichia coli* species were identified. Contamination of pathogenic bacteria in food products can occur at all stages of the food chain including production, processing, distribution, retail marketing, and handling or preparation (Zao *et al.*, 2021). According to CSIRO (2002), *Escherichia coli* contamination in meat can come from the

room, equipment and table where the cuts are made, as well as the water used during the cutting process to processing. The results of a study conducted by [Prananda et al. \(2019\)](#), on grilled meatball samples, the results showed that most of the contamination came from *Escherichia coli*.

*Escherichia coli* is a type of pathogenic bacteria that has an impact on health, where if these bacteria enter the digestive tract they can cause illness or death if not treated, even for certain strains at very low doses. Taking into account the effects it causes, the government through the Ministry of Health of the Republic of Indonesia and the Food and Drug Supervisory Agency (NADFC/FDA Indonesia) makes regulations on the maximum level of pathogenic *Escherichia coli* contamination in food products, to ensure that the food consumed is completely safe from the threat of bacteria. Pathogenic *Escherichia coli*. Several studies on the detection of LOD in *E. coli* bacteria are known to be in the range of  $10^{-7}$  to  $10^{-8}$  which were detected using affinity-based methods with contactless atmospheric pressure ionization mass spectrometry ([Sari et al., 2022](#)), whereas using real-time PCR, the LOD of detection of *E. coli* was at Log 10 of the dilution series. Different results were shown in the research of [Yalcin et al. \(2017\)](#), where the LOD was detected at a concentration of 2 cfu/25 g, and [Liang et al. \(2019\)](#) with a LOD value of 102. Differences in test methods and tools used are also known to give different results in carrying out detection tests for pathogenic bacteria ([Sophian et al., 2020](#); [Sophian et al., 2021](#)).

One of the challenges of testing the detection of pathogenic bacteria using conventional testing techniques using selective media and biochemical

confirmation tests is the ability of these testing techniques to detect small concentrations of contaminants that contaminate the sample. Therefore, this study was conducted to provide information on the ability to detect small concentrations of contaminated samples using positive control of *Escherichia coli* with conventional testing techniques and biochemical confirmation tests. This is important to do so that it becomes a reference for the LOD (Limit of Detection) value in optimizing or measuring the sensitivity of the test method used when conducting a validation test or verifying the test method to be optimized.

## 2. METHODS

### 2.1. Materials

The materials used in this study were meatball samples, TSA media, TSB, McFarland Standard 1, Sterile Aquadest, ENDO, EMBA and a set of API 20 E kits.

### 2.2. Sample Preparation

The sample was weighed as much as 10 grams in a stomacher bag, then added 90 mL of TSB and after that, it was incubated at a temperature of 35-37 °C for 24 hours, after which the sample was then ready to follow a series of testing stages until the confirmation test.

### 2.3. Positive Control Spike

Positive control spikes carried out using standard microbes *Escherichia coli* ATCC 25922 were grown in TSA plate/sloping media which were incubated for  $\pm 24$  hours, then the microbes were suspended in sterile distilled water with a turbidity of 1 McFarland. The standard value of 1 McFarland used is equivalent to  $5 \times 10^8$  col/mL.

## 2.4. Positive Control Variations

The positive control variations used were 5 colonies/gr sample, 10 colonies/gr sample, 50 colonies/gr sample, and 100 colonies/gr sample.

## 2.5. Isolation on Selective Media

Isolation on selective media was carried out using two types of selective media, namely ENDO and Levine EMBA.

## 2.6. Confirmation Test

A confirmation test was carried out using biochemical techniques using rapid test kit API.

## 3. RESULT AND DISCUSSION

### 3.1. Test Results on Selective Media

Selective media used to isolate *Escherichia coli* bacteria were Levine EMBA and ENDO, the results of which were observed as presented in (Table 1). From the table, it can be seen that at all concentration levels, *Escherichia coli* was detected on Levine EMBA and ENDO media.

**Table 1.** Results of Observing Samples on Selective Media

Concentration Variation	Levine EMBA	ENDO	Number of Tests Confirmed Positive
5 cfu/gr	+	+	10
10 cfu/gr	+	+	10
50 cfu/gr	+	+	10
100 cfu/gr	+	+	10

### 3.2. Confirmation Test Results

The results of the confirmation test carried out using the API 20 E kit showed the results as presented in (Table 2). From

the table, it can be seen that at all concentration levels, *Escherichia coli* was detected positively in the API 20E kit.

**Table 2.** Biochemical Confirmation Test Results

Concentration Variation	API 20 E	Number of Tests Confirmed Positive
5 cfu/gr	+	10
10 cfu/gr	+	10
50 cfu/gr	+	10
100 cfu/gr	+	10

Based on the data presented in table (2) above, it can be seen that all treatments showed detectable results. This result is

different from several studies that have been conducted where the LOD in the E Coli detection test in a study conducted by

Yalcin *et al.* (2017), it is known that the LOD value of 2 cfu can still be detected for qualitative testing or identification testing. This difference can of course be caused by several things, which include: the use of different techniques, different tools, and the sample matrix used in the study (Sophian *et al.*, 2020; Sophian *et al.*, 2021).

On Levine-EMBA selective media, Colonies were metallic green. This color change is caused by a reaction between *Escherichia coli* bacteria and Methylene blue. Agar Eosin Methylene Blue (EMB) was originally designed by Holt-Harris and Teague (1916) and subsequently modified by Levine. The medium above is a combination of Levine and Holt-Harris and Teague formulas containing: peptone and phosphate as recommended by Levine and two carbohydrates as recommended by Holt-Harris and Teague.

Methylene blue and Eosin-Y inhibited Gram-positive bacteria to some degree. This dye serves as a differential indicator in response to carbohydrate fermentation. The ratio of eosin and methylene blue is adjusted to about 6:1. Sucrose is added to the medium as an alternative carbohydrate source to the usual lactose fermenting, Gram-negative bacilli, which sometimes do not ferment lactose or do so slowly. Coliform produces purplish-black colonies because it absorbs the methylene blue-eosin dye complex when the pH drops. The complex dye is absorbed into the colony. The non-fermenter may increase the pH of the surrounding medium by oxidative deamination of the protein, which dissolves the methylene blue-eosin complex resulting in colorless colonies.

Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue. Further tests are needed to confirm the

isolate. Peptones serve as a source of carbon, nitrogen, and other important growth nutrients. Lactose and sucrose are sources of energy by being fermentable carbohydrates. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffered media. The test sample can be directly etched on the medium plate. The inoculated plates should be incubated, and protected from light. However standard procedures must be followed to obtain isolated colonies. A non-selective medium must be inoculated in conjunction with EMB Agar. Further confirmatory tests should be performed for the identification of isolated colonies (Himedia, 2020).

According to Acumedia (2011), EMB Agar media is sufficient to inhibit several types of bacteria and fungi but *Staphylococci*, *Streptococci*, and yeasts can grow but their growth will appear as small pointed colonies. In this medium, the sterilization process carried out can reduce the methylene blue in the media and leave a medium orange color. The normal blue-violet color of the media can be recovered by gentle and homogeneous mixing. Endo Agar is a selective medium developed by Endo to distinguish gram-negative bacteria based on lactose fermentation, as well as inhibit Gram-positive bacteria (1904). The process of inhibiting Gram-positive bacteria was carried out by adding sodium sulfite and basic fuchsin. The resulting Endo Agar, also known as Fuchsin Sulphite and Infusion Agar is also used to isolate typhoid bacilli. Many types of selective media are principally modified from Endo media. This media is one of the media types recommended by APHA as an important medium in the microbiological examination of water and wastewater, dairy products and food (Braid *et al.*, 2015; Salfinger *et al.*, 2015; Wehr *et al.*, 2004).

The biochemical confirmation test was carried out using the API (Analytical Profile Index) kit. According to Carson *et al.* (2001), API is an economical confirmation technique and has a good level of accuracy. The API 20E strip is a biochemical test kit consisting of 20 tubes containing a dried substrate. The API strip contains several abbreviations which are dripped with a bacterial suspension in NaCl. The underlined abbreviations indicate that the bacterial suspension is dripped as much as half of the good height and added mineral oil until the well is full, namely ADH, LDC, ODC, H<sub>2</sub>S, and URE. The abbreviations in the box indicate that the bacterial suspension was dripped until the well was full, namely CIT, VP, and GEL, while the abbreviations that were neither underlined nor in the box were filled with a sample suspension of half the height of the well, namely ONPG, TDA, IND, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, and ARA (Biomerieux, 2002). Until now, many methods still use the API kit as a biochemical confirmation test on samples suspected of containing certain pathogenic bacteria that are the target of testing.

LOD test testing is part of a method that can be used to see the sensitivity of a method, where the smaller the LOD value that can be detected, the more sensitive the method so that the use of this method can guarantee better test quality. The value obtained from the research conducted, it is known that all tested samples were identified with the LOD value in the study being at 5 cfu/gr. Therefore, it is suggested for similar research, that the detection limit test should be carried out using a smaller concentration variation so that the smallest value that can be detected for testing the detection of *E. coli* can be ascertained.

Based on the data from the research conducted, it was found that on Levine EMBA and ENDO selective media, all samples at the spike concentration level of 5 cfu/gr - 100 cfu/gr showed positive results, as well as the results of the confirmation test on all test samples carried out. samples confirmed positive for *Escherichia coli*.

#### 4. CONCLUSION

Based on the results of this study, it can be concluded that all samples spiked with various variations in the concentration of positive control were detected and identified as *Escherichia coli* ATCC 25922.

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