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Research Article

# Detection for Salmonella typhimurium ATCC 14028 on Sausage, Nugget, Meatballs, Otak-Otak, Tempura and Cilok Products Using the Kit Rapid Test

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### **ABSTRACT**

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Testing for Salmonella typhimurium ATCC 14028 using a rapid test kit was carried out in the microbiology and molecular biology testing laboratory of the Food and Drug Administration in Gorontalo. Research using a rapid test kit is a simple study that requires a relatively shorter time than using conventional techniques. The purpose of this study was to see the work of the rapid test kit in detecting Salmonella typhimurium ATCC 14028 spiked on several food processed products. The sample consisted of 12 replications with 6 types of food products including sausages, nuggets, meatballs, otakotak, tempura and cilok. Positive controls were made from Salmonella typhimurium ATCC 14028 phase 2 cultures, while negative controls used sterilized samples of sausage, nuggets, meatballs, otak-otak, tempura and cilok samples. The concentration of positive control used was taken from turbid work culture and equalized according to Macfarland standard 1. The data analysis was performed qualitatively based on the change in the colour of the kit in the sample which was compared to positive and negative controls. Based on the results of the study, it was found that all samples were detected Salmonella typhimurium ATCC 14028. This study concludes that the rapid test kit can be used to detect Salmonella typhimurium ATCC 14028 in sausage products, nuggets, meatballs, otak-otak, tempura and cilok

Keyword: Rapid, Test Kit, Salmonella typhimurium, Otak-otak.

### INTRODUCTION

Salmonella bacteria are gram-negative bacteria, in the form of forming rods, do not have spores and have characteristics as facultative anaerobic bacteria These bacteria can ferment glucose and belong to the Enterobacteriaceae family. Most strains are motile with peritrichous flagella and can reduce nitrates to nitrites (Grimont et al. 2000). Salmonella bacterial contamination in food processed materials can cause "food-borne disease", which is a disease that occurs due to the entry of pathogenic microorganisms into the body that is delivered by food. According to Jawetz et al. (2005), Salmonella can cause enteric fever, which is a fever caused by Salmonella typhi bacteria called typhoid fever, Septicemia, which is a fever caused by Salmonella choleraesuis bacteria, and Gastroenteritis, a digestive tract disease caused by food poisoning that contains *Salmonella typhimurium* bacteria. Therefore, the latest research on pathogenic bacteria detection methods using a rapid test kit is needed so that it can shorten the time of testing.

The Rapid Test kit is the most widely used method of a rapid test for field testing carried out outside the laboratory. This method is quite effective if used as an initial screening for bacterial contaminants in a product, but each type of kit has a different level of accuracy so that before using it, a preliminary test is needed to determine the level of detection in a product. This is important to do to avoid biased or dubious test results on the sample to be tested. Besides that, it could be that in its implementation, the test results and the interpretation of the results carried out during the preliminary test or confirmation will give an interpretation of the results that are different from the interpretation claims contained in the manual book used

BPOM Regulation No. 13 of 2019 concerning the Requirements for the Maximum Limit of Microbiological Contamination, regulates the limit of contamination of pathogenic bacteria in food products processed by sausages, nuggets, meatballs, otak-otak tempura and cilok which cannot contain Salmonella pathogenic bacteria. Salmonella bacteria is a type of pathogenic bacteria that causes foodborne diseases which are usually toxic or infectious. Bacterial contamination of food products can occur due to unhygienic production processes or during serving. Therefore, this research is very important to do as a reference in simple studies to detect pathogenic bacteria. Based on this, the research was conducted to develop a rapid detection method for detecting Salmonella in sausage products, nuggets, meatballs, otak-otak, tempura and cilok. Also, with this method, it is hoped that the level of decision making on sample control which has a short shelf life will be faster and better in the future.

### MATERIALS AND METHODS

#### Material

The materials in this study were processed food (sausage, nuggets, otak-otak, meatballs, tempura and cilok), Salmonella spp rapid test kit (SL-A06 / SRE01-706), Tryptone Soya Agar (TSA) Cat. CM0131, Buffered Peptone Water (BPW) paint. CM0509, Aquadest Steril and Standard 1 MacFarland..

#### Method

### Sample Preparation

Samples consisted of sausages, nuggets, meatballs, otak-otak, tempura and cilok weighed 25 grams each, then spiked with pure culture of *Salmonella typhimurium* ATCC 14028 phase 2 with a concentration equivalent to 1 MacFarland of 1 mL, then added with BPW solvent. 225 ml and homogenized and incubated in an incubator for 24 hours at a temperature of 35° – 37° C. *Salmonella typhimurium* ATCC 14028 phase 2 culture was made by sifting the F1 bacteria standard to produce a phase 2 working standard. 35° – 37° C. After that, take 1 ose and then scratch it onto the medium to tilt the TSA and incubate it in an incubator for 24 hours at 35° – 37° C. After the incubation period is complete, take 1 ose and dissolve it in a sterile distilled water solution until the turbidity level of the colony in a solution is equivalent to 1 MacFarland.

# Media Preparation

Buffered Peptone Water (BPW) is made by adding 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121 ° C for 15 minutes. The distilled water used must be of high quality with low mineral content/conductivity. Tryptone Soya Agar (TSA) is made by adding 40g to 1 litre of distilled water (purified as required). Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 ° C for 15 minutes, after that pour 10 mL into a sterile test tube then place it on an oblique position so that the TSA position in the tube will be tilted and let it cool and solidify.

#### Positive Control

The positive control used was the pure culture of *Salmonella yyphimurium* ATCC 14028 phase 2 with a concentration of 100-1000 cabbage / g, scratched on NA slant incubated for 24 hours at 35 °C - 37 °C. Colonies that grew on oblique TSA media were then made a

bacterial solution by taking one loop and then putting it in a tube containing 3 mL of sterile distilled water which was then clouded with a standard value of 1 Macfarland. Pipette 1 mL of the sample solution and put it in the Salmonella spp kit (SL-A06 / SRE01-706) and re-incubate it in the incubator for 24 hours at 35 °C - 37 °C. A positive result is indicated by a colour change from reddish-brown to yellow.

# Negative Control

The negative control used was a sample of processed food products (sausages, nuggets, meatballs, otak-otak, tempura and cilok) which were sterilized using autoclave at a temperature of 121°C for 15 minutes. After sterilization, the sample was then weighed 25 grams and added 225 mL of BPW solvent and incubated in an incubator for 24 hours at 35 °C - 37 °C. After that, pipette 1 mL of the sample solution and put it in the Salmonella spp kit (SL-A06 / SRE01-706) and re-incubate it in the incubator for 24 hours at 35 °C - 37 °C. A negative result is indicated by no change in the colour of the kit media solution which is reddish-brown in colour.

# Data Analysis

The data analysis was conducted qualitatively based on the change in the colour of the kit in the sample which was compared to positive and negative controls. As well as the manual book of the kit which is used as the main reference source in concluding the test results.

# **RESULTS**

### Rapid Test Kit Test

Results From the sample analysis using the rapid test kit, the results are as shown in (Table 1). From the table it can be seen, in all types of food processing, *Salmonella typhimurium* ATCC 14028 was detected positive.

Table 1. Data on Salmonella Detection Results using the Rapid Test KIT

Sample	Positive Detected	Negative Detected
Negative Control (-)	no	yes
Positive Control (+)	yes	no
sausages	yes	no

nuggets	yes	no
meatballs	yes	no
Otak-Otak	yes	no
Tempura	yes	no
Peek	yes	no

Based on the data presented in (Table 1) above, it can be seen that the entire sample was detected *Salmonella typhimurium* ATCC 14028 which was the same as the positive control. Positive results are indicated by the occurrence of a colour change from reddishbrown to yellow. While the negative control shows negative results which are shown by no change in the colour of the kit media solution which is reddish-brown. As seen in (Figure 1).



**Figure 1:** K + is a positive control, Sample is test sample which is spike Salmonella, K- is the negative control

When viewed from (Figure 1), it shows the colour change reaction of the sample so that it resembles a positive control and is concluded as positive. In a rapid test using a rapid test kit, the system used is a biochemical test system where this method uses biochemical principles to identify the species that are the research target.

# **DISCUSSION**

The detection of *Salmonella typhimurium* ATCC 14028 using a rapid test kit was carried out to see the performance of the rapid test kit used in detecting *Salmonella typhimurium* ATCC 14028 in several food processed products including sausages, nuggets, meatballs, otak-otak, tempura and peek. The sample is first crushed then sterile aqua dest is added and dissolved or homogenized using a stirring rod. This step is important to do to destroy the sample

matrix so that bacteria that may be trapped in the sample matrix can be identified during identification. The sample selection used in this study was adjusted to the grouping of similar mastery sample categories so that the research carried out could be duplicated for the type of sample with the same matrix.

In this study, the sample experienced a pre-enrichment stage through Buffered Peptone Water (BPW) media. This medium is composed of peptone, sodium chloride, disodium phosphate and potassium dihydrogen phosphate (BAM 2007). The success of this pre-enrichment stage is marked by a change in the colour of the BPW media to become more cloudy. The purpose of this pre-enrichment stage is to activate the target bacteria if it is dormant or to increase the number of bacteria present in the sample.

In the kit used, Salmonella bacteria work by changing the colour of the kit from reddish-brown to yellow. The manual kit itself does not explain in detail the detection system that is being carried out. In the manual kit only describes the detection rate (range: 0 ~> 106 CFU / g or ml, incubation Temp .: 37 ° C, Fastest detection: Less than 4 hours, Reading Result: Color change reaction, Semiquantitative). Due to the lack of information about the characteristics of the rapid test kit, optimization is needed to determine the level of accuracy before use. In addition, the preliminary test stage can also be used as a source of initial information to generate data about the kit used to prevent questionable results in decision making. When viewed from the manual book kit used, the test results are claimed to be visible after incubating for 4 hours, but in this study, the results of the test produced were only visible after 24 hours of incubation. This can be caused by the use of different bacterial standards or the level of concentration of positive samples or target bacteria that are the object of research is also different. Therefore, the preliminary test or confirmation stage before the kit is used is important to anticipate things like what happened in this research. This difference can also cause testers or laboratories that will use a rapid test kit to have a test protocol that will be adjusted to the results of the confirmation test or preliminary tests that have been carried out.

In general, the bacterial identification system using a rapid test system is based on a conventional miniature biochemical test. There are several types of rapid test bases, including nucleic acid-based, biosensor, immunological and modification of conventional techniques to shorten the testing time. However, this rapid test technique is a testing analysis technique that requires higher costs when compared to conventional techniques. In addition, the rapid test technique requires competent individual skills so that the accuracy of the test results can be recognized. Based on the results of the study, it was found that all samples were detected

Salmonella typhimurium ATCC 14028. The conclusion of this study is that the rapid test kit can be used to detect Salmonella typhimurium ATCC 14028 in sausage products, nuggets, meatballs, otak-otak, tempura and cilok. As a suggestion for further research, it is better to continue to the sensitivity and specificity stages, this is because it is necessary to see whether this kit has a good level of specificity or sensitivity. If it is needed again, in the future it is better to make a comparative analysis of the test results of the two identification techniques, namely conventional techniques using selective media, further biochemical and serological tests and rapid test kit techniques.

# **CONCLUSIONS**

This study concludes that the rapid test kit can be used to detect *Salmonella typhimurium* ATCC 14028 in sausage products, nuggets, meatballs, otak-otak, tempura and cilok.

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