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The Effect of Antibody Concentration on Pilator Sensitivity (Rapid *Salmonella* Detector) as a Colourimetric-Based *Salmonella* Detection Biosensor

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ABSTRACT

Food safety is one of the essential basics of human rights. *Food* should be the source of nourishment for *human beings* and not an open door for potentially pathogenic bacteria to avoid foodborne disease. One of the most pathogenic bacteria is Salmonella. This study aims to know the effect of antibody concentration on PILATOR (Rapid Salmonella Detector) uses the descriptive method. The results can detect Salmonella in 10 minutes, with the optimum concentration of primary and secondary antibodies being 10 µg ml-1 and 0.2 µg ml-1. Based on time detection (10 minutes) and 10^2 CFU/ml limit detection. PILATOR is categorized as a fast and accurate Salmonella detector.

KEY WORDS AND EXPRESSIONS: *Biosensor*; *Immunosensor*; *PILATOR; Salmonella; Antibody*.

INTRODUCTION

Food safety is one of the essential basics of human rights. *Food* should be the source of nourishment for *human beings* and not an open door for potentially pathogenic bacteria to avoid foodborne disease [1]. One of the most pathogenic bacteria is *Salmonella*.

The presence of *Salmonella* in food could be detected using conventional and modern techniques. Conventional testing is carried out by gram staining and biochemical tests [2]. Meanwhile, modern methods use ELISA and PCR [3]. Some of these methods are rapid detection which has been widely used. However, these methods require complicated equipment. One of the practical and fast test methods being developed at this time is the using biosensors.

Biosensor is a tool that can detect analyte compounds by utilizing biological elements and chemical reactions [4]. The biosensor has two components: the bioreceptor as a detector for the presence of analytes and the transducer as a signal converter generated by the bioreceptor. Of the many existing biosensors, colourimetric biosensors are the easiest to use because they can detect an analyte quickly, and the results can be seen directly with the naked eye [5]. One of the factors that affect the immunosensor is the concentration of antibodies used. Both primary and secondary antibody concentrations will affect the selectivity and sensitivity of a biosensor. In addition, if the concentration of antibodies and antigens used is excessive, it will inhibit the formation of existing complex bonds [6].

Several research results regarding antibody concentrations show that each antibody has a certain optimum point and can be influenced by several factors, including the antibody type, the antibody's origin and the substrate used to translate the signal [7]. According to research conducted by [8], the optimal antibody concentration for the detection of *Salmonella* typhimurium with monoclonal antibodies is 25 µg ml-1, while some concentrations of secondary antibodies that are recommended to be used are 500 µg ml-1, 250 µg ml -1 and 100 µg ml-1. After reaching this concentration, the sensitivity of the biosensor will decrease until it reaches a certain point. This study aims to design a *Salmonella* detection tool using colourimetric biosensor technology and test its performance using a quantitative descriptive method. PILATOR (Rapid *Salmonella* Detector) was created as an alternative to analyze the presence of *Salmonella*.

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METHOD

Salmonella typhimurium culture, *Salmonella* typhimurium antibody (BIOSS), Buffer Saline Phosphate (PBS) (Merck), secondary antibody -goat anti-rabbit igG-AP labelled (Chemicon), BCIP substrate (Thermo Scientific), Sodium Agar (Merch), BSA, glutaraldehyde, Tween 20, filter paper (Whatman), distilled water (Egra), 70% alcohol (Egra), Lactose Broth (Merck), tissue, gloves, sterilizing plastic, sterilizing paper, mask, aluminium foil, cotton, rubber bands, label paper.

Preparation Stage

The preparation stage was carried out by preparing various materials needed for testing purposes, including carrying out the sterilization process on the test paper to be used, namely Whatman #1 filter paper (121˚C, 60 minutes), making 1% PBST reagent (15µL Tween 20 in 1485µL Buffer Phospate Saline/BPS), 1% BSA (1 mg BSA in 100µL PBST), 5% glutaraldehyde (6µ in 194µl PBST, and 10µl in 190µl PBST), primary antibody 2; 4; 6; 8; 10µg/ml and secondary antibody 0.1; 0.125; 0.15; 0.175; 0.2 µg/ml [9].

Testing the Concentration of Primary Antibodies and Secondary Antibodies

Tests were conducted to determine the concentration of primary and secondary antibodies that cause the most optimal colour. Antibody concentration testing was carried out by making a PILATOR biosensor. As much as 4 µl of glutaraldehyde was dropped on the platform paper, after which the primary antibody was dripped with different concentrations, namely 2; 4; 6; 8; 10 µl. Platform paper was rinsed with PBST, added 4 µl BSA, 10 µl *Salmonella* culture, and 4 µl secondary antibody with different concentrations of 0.1; 0.125; 0.15; 0.175; 0.2 µg/ml. Rinsed again with PBST and added 5 µl of BCIP dye substrate. After that, the colour changes formed were observed within 30 minutes and scanned using a Canon LIDE 220® scanner. Next, the colour results on the platform paper are quantified using the image editing software Adobe Photoshop CS 6 Version 2013 to obtain RGB values. The resulting RGB value will indicate the intensity of the colour density on the biosensor [7].

Biosensor Fabrication (Zourob Modification, 2008)

4 µl immobilizing agent was dripped into the yield zone and left to dry (10 min, 25˚C). Then, drop 4 µl of primary antibody on the result zone and 4 µl of secondary antibody on the reaction zone and wait for it to dry (4 minutes, 25˚C). Rinse the test paper using 100 µl PBST to remove the immobilized primary antibody. Next, add 2µl BSA as a blocking agent to the yield zone to cover the area that does not bind to the primary antibody. Finally, carry out the incubation process (24 hours, 4˚C) before the biosensor is ready [10].

RESEARCH RESULTS AND ANALYSIS

A. The Principle of PILATOR

The working principle of this biosensor is to detect the presence of *Salmonella* in food which is indicated by a change in the colour of the yield zone to blue-purple, which the naked eye can see within 10 minutes. Meanwhile, a negative result is indicated by the absence of a colour change in the yield zone within 10 minutes. This colour change occurs due to a reaction between the antigen present in *Salmonella* and *Salmonella* typhimurium antibodies. In this biosensor, the primary antibody used is the *Salmonella* typhimurium antibody, while the secondary antibody is made from primary antibodies tagged with goat anti-rabbit igG-AP labelled. According to their role, primary antibodies (*Salmonella* typhimurium antibodies) will bind to antigens present in *Salmonella*. At the same time, the secondary antibody will bind to the antigen side so that it does not bind to the primary antibody that was previously immobilized.

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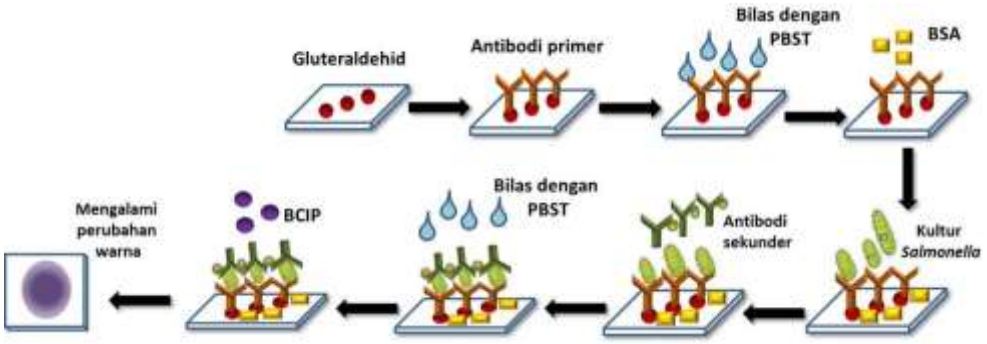

Figure 1. PILATOR Working Principle

A colour change in the result zone from white paper to purple is interpreted as a positive (+) result. The colour formation can occur within 10 minutes, with an optimal colour formation time of 30 minutes. While a negative result (-) is indicated by no colour change in the yield zone. The yield zone can be white or yellowish. The yellow colour is due to glutaraldehyde dripping on the platform paper. Negative results on this biosensor are interpreted as the absence of *Salmonella* bacteria at concentrations less than 102 CFU/ml (minimum limit of detection of PILATOR).

B. Primary Antibody Concentration Test Results

A primary antibody concentration test was carried out to know what is the most optimal primary antibody concentration to produce a colour change on the biosensor. In other words, this primary antibody concentration test shows how much the primary antibody has been immobilized, which binds to the *Salmonella* antigen. Immobilized primary antibodies have a role in binding to *Salmonella* antigens in the yield zone. The bond between the primary antibody and the antigen is stable and specific. The target of the analyte to be analyzed (*Salmonella* antigen) will be recognized by the indicator changing the colour of the biosensor to purple. It must be ensured that the target analyte can bind to the immobilized primary antibody. The test results for several primary antibody concentrations can be seen in **Figure 2.**

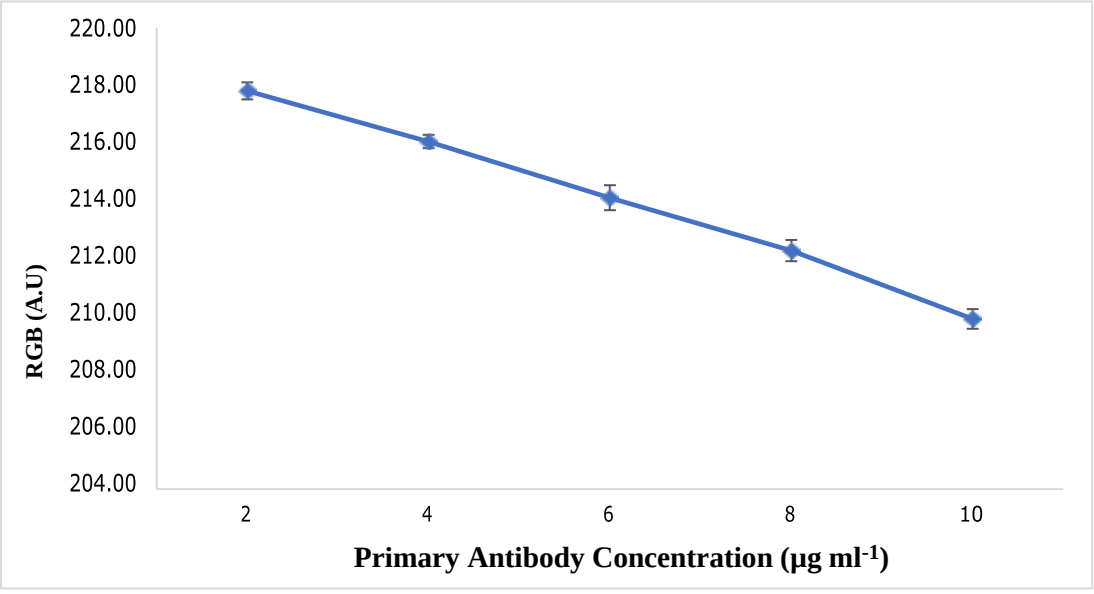

Figure 2. Effect of Primary Antibody Concentration on PILATOR Colour Intensity

In Figure 2, it can be seen that as the primary antibody concentration increases, the RGB value will decrease. This RGB value is correlated with the intensity of the colour on the biosensor. The smaller the RGB value generated, the intensity of the colour shown will be more intense. The darker colour of the biosensor indicates that the amount of *Salmonella* in the food will increase. The colour density of this biosensor also shows that more and more antibodies bind to *Salmonella* antigens. The average RGB value at a concentration of 2 µg ml-1 was 217.54, then decreased at a concentration of 4 µg ml-1 to 215.57.

Furthermore, at a concentration of 6 µg ml-1, the value will decrease to 213.38; at a concentration of 8 µg ml-1, it decreases To 211.31, and at a concentration of 10 µg ml-1, the resulting RGB value is even lower namely 208.65. Thus, it can be concluded that the intensity of the resulting purple colour will increase gradually with the higher concentration of the primary antibody used, where the intensity of the resulting colour is inversely proportional to the RGB value. The greater the RGB value, the more faded the resulting colour will be, and vice versa.

The optimal concentration of primary antibody from serum polyclonal for ELISA testing is 1-10 µg ml-1 [7]. RnD The most optimal primary antibody concentration in polyclonal capture ELISA is 0.2, 0.4 and 0.8 µg ml-1[11]. Meanwhile, based on research conducted by [8], the optimal antibody concentration for the detection of *Salmonella* typhimurium with monoclonal antibodies is 25 µg ml-1. /ml. However, the colour still tends to fade. The most optimal formation of purple colour is in the addition of antibodies with a concentration of 10 µg/ml. The most optimal antibody concentration required in this study differs from previous studies. Because in a study conducted by [8], it was carried out using monoclonal antibodies, whereas this study was carried out with polyclonal antibodies. Where polyclonal antibodies have better specificity than monoclonal antibodies because these antibodies are produced in large quantities by B cells, each of which produces antibodies at specific epitopes [6].

C. Secondary Antibody Concentration Test Results

A secondary antibody concentration test was carried out to determine the most optimal secondary antibody concentration to produce a colour change on the biosensor. In other words, this secondary antibody concentration test is based on the number of enzyme-tagged secondary antibodies that bind to the *Salmonella* antigen site. This test needs to be done because it will be used as a reference for the secondary antibody concentration that can cause changes in colour on the biosensor. The results of the secondary antibody concentration test on the PILATOR biosensor can be seen in Figure 3.

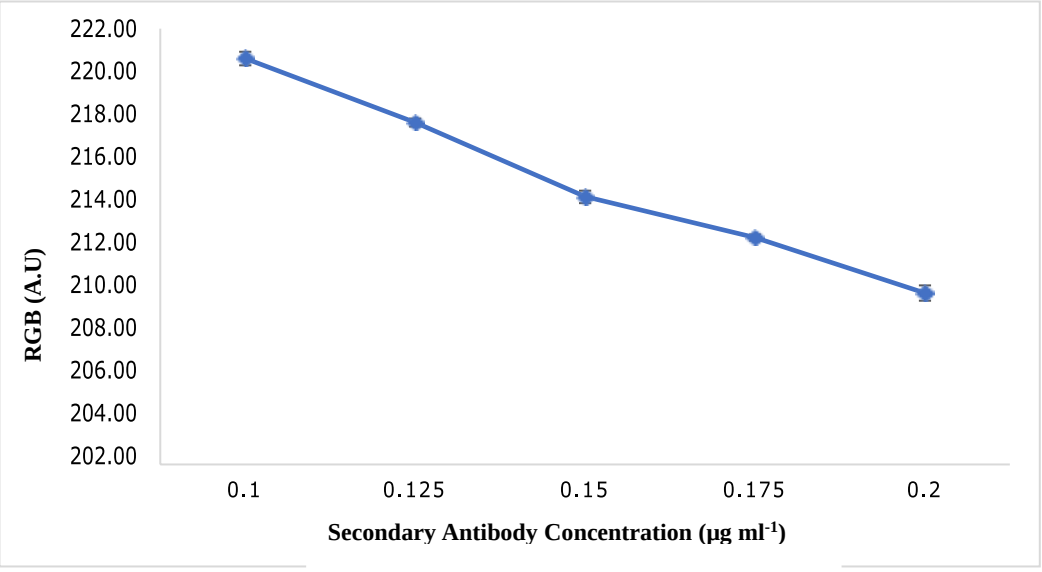

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Figure 3. Effect of Secondary Antibody Concentration on PILATOR Color Intensity

Figure 3. shows that as the secondary antibody concentration increases, the resulting RGB value continues to decrease, where the resulting RGB value is inversely proportional to the intensity of the existing colour. The darker the intensity of the resulting colour, the lower the RGB value. The RGB value at a concentration of 0.1 µg ml-1 was 220.49, then decreased at 0.125 µg ml-1 to 217.26. Furthermore, at a concentration of 0.15 µg ml-1, the value decreased to 213.51; at a concentration of 0.175 µg ml-1, it decreased to 211.5, and the smallest RGB value was obtained at a concentration of 0.2 µg ml-1. Thus, it can be concluded that the intensity of the purple colour is inversely proportional to the RGB value. The darker the purple colour on the biosensor, the smaller the resulting RGB value.

The recommended secondary antibody concentration for colourimetric ELISA testing using the AP enzyme (Alkaline phosphatase) is 100-200 ng/ml [7]. The optimal secondary antibody concentrations used for polyclonal detection are 50, 100, 200 and 400 ng/ml [11]. Meanwhile, according to research conducted by [8], several concentrations of secondary antibodies that can be recommended in electrochemical immunosensor are 500 µg ml-1, 250 µg ml-1 and 100 µg ml-1. This study found that the most optimal secondary antibody concentration in producing colour intensity on the biosensor was 200 ng/ml. The secondary antibody concentrations used in this study were within the optimal concentration range in previous studies.

CONCLUSION

PILATOR is a *Salmonella* detector that utilises the principle of a colourimetric immunosensor. *Salmonella* samples will flow into the reaction zone and react with a secondary antibody tagged with the enzyme and the *Salmonella* antigen in the sample. Then it will flow to the result zone, where a sandwich assay will occur between the enzyme-tagged antibody bound by the *Salmonella* antigen and the immobilized secondary antibody. The enzyme tag on the secondary antibody will react with the dye placed in the result zone to form a purplish blue colour indicating a positive test result (+).

A change in colour to purple indicates the presence of *Salmonella* in the food (+), while no colour change indicates a negative result (-). PILATOR can detect *Salmonella* in 10 minutes, with the optimum concentration of primary and secondary antibodies being 10 µg ml-1 and 0.2 µg ml-1. Based on time detection (10 minutes) and 102 CFU/ml limit detection. PILATOR is categorized as a fast and accurate *Salmonella* detector.

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