DOI: http://dx.doi.org/10.33846/hn61202
http://heanoti.com/index.php/hn

RESEARCH ARTICLE
URL of this article: http://heanoti.com/index.php/hn/article/view/hn61202

# Statistical Test of Two Way Anova Block Design as a Decision Making Tool for Analyzing the Growth Results of *Staphylococcus aureus* Bacteria in Sorghum Media (*Sorghum bicolor L. Moench*)

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## ABSTRACT

Statistics has wide uses for making the right decisions in various areas of life, because statistics give a person the knowledge and ability to evaluate data. Through this research, researchers hope to create a new learning model from the results of the development of the Two Way Anova Design Block Statistical Test that can be used as a decision-making tool in the analysis of data on the growth results of *Staphylococcus aureus* bacteria on alternative media from sorghum media. The results obtained in this study, that in the variation of the sorghum period 4 grams, 5 grams, and 6 grams both in dilution with aqua distillation and meat broth the growth of *Staphylococcus aureus* bacteria in contrast to the growth in mannitol salt agar (positive control) media. Meanwhile, in the periodic variation of 7 grams of sorghum in both aqua distillation and meat broth, there is no difference in the growth in mannitol salt agar media. Two way Anova block design, used if the experimental material is grouped. So that each group aranges a replication for the treatment. The treatment was given randomly to each group. Based on statistical method approach block design, we find alternative mannitol salt agar media into sorghum.

**Keywords:** Anova Test Two Way block design; *Staphylococcus aureus*; mannitol salt agar; sorghum

## INTRODUCTION

### Background

Statistics in the narrow sense can be interpreted as data, but in a broad sense, statistics can be interpreted as a tool(1). Tools for analysis and tools for making decisions. Applied Statistics is an analytical tool in the form of a "Numerical Description" to describe each data obtained from the population and samples, for later estimates, forecasting, and decision making(2). Thus statistics is a science that studies statistics, that is, a science that studies how to collect data, simplify data, present data, and make conclusions based on information obtained from samples(3). The main function of statistics in education is in the field of research(4). Applied Statistics emphasizes how data processing uses SPSS, STATA, and other data processing software(5). SPSS is a statistical computer program that can process statistical data quickly and precisely, becoming various outputs desired by decision-makers(6).

Comparative research was made to be able to compare the presence of one or more variables in two or more different samples or at different times, in this study as an effort to make two way Anova statistical method approach block design to find alternative mannitol salt agar (MSA) media into sorghum to grow bacterial media(7).

MSA media is an example of a selective medium for the growth of *Staphylococcus aureus* bacteria(8). The bacterial growth media used in laboratories are mostly ready-to-use media produced by foreign companies, this can cause problems in the form of expensive components for the manufacture of MSA media(9). Therefore, the use of natural resources as an alternative medium is needed to provide solutions to these problems.

Many studies on alternative media have succeeded in using protein in animal and vegetable protein sources as a substitute for peptone in bacterial growth media(10). Indonesia has fairly diverse biodiversity, especially in agriculture(11). Several plants have not been used properly, one of which is sorghum (Sorghum bicolor (L.) Moench)

Sorghum protein (*Sorghum bicolor L. Moench*) has the potential to be a substitute for peptones in MSA alternative media (MSA)[12]. To prove it, this research is needed on sorghum protein (*Sorghum bicolor L. Moench*) can be a substitute for peptones for the growth of *Staphylococcus aureus* bacteria in alternative media MSA.

The manufacture of alternative media sorghum (*Sorghum bicolor L. Moench*) is carried out by equalizing the mass of peptone content in MSA media so that the protein content of sorghum (*Sorghum bicolor L. Moench*) is obtained which is equivalent to peptone in MSA media [13]. MSA media contains 10 grams of peptone in 1000 ml so that when converted into 100 ml, the peptone needed is 1 gram. According to Agricultural Technology Assessment Center, 2016, sorghum (*Sorghum bicolor L. Moench*) has a protein content of 10.11 grams at 100 grams[14].

**Purpose**

Based on the description above, researchers conducted a study to make Two Way Anova statistical method approach block design to find alternative MSA media into sorghum to grow media alternative media for the growth of *Staphylococcus aureus* bacteria, which are cheaper and can be made by students as practical material in the laboratory. For this reason, researchers conducted a two-way statistical test of the Anova design block as a scientific decision-making tool in looking for alternative media for the growth of *Staphylococcus aureus* bacteria with media from sorghum material diluted with aqua distillation and meat broth as a substitute for ready-to-use media (MSA media)

## METHODS

**Types of Research**

The type of research carried out is a laboratory experiment, which is a method that has the aim of determining the use of sorghum (*Sorghum bicolor L. Moench*) as a medium for modifying MSA for the growth of *Staphylococcus aureus* bacteria. This study was divided into two groups, namely control and treatment. The treatment was divided into four with variations in weight of sorghum using meat broth as an alternative medium for MSA. The results of bacterial growth were then analyzed by the statistical method of Two Way Anova block design. The data analysis technique of this research was carried out statistically using the Two Way Anova Block design test which was previously carried out by the Kolmogrov Smirnov test to find out the data was normally distributed, and the momogeneity of variances test was carried out to determine the homogeneity of the data. If the two conditions are met, then the Two Way Anova Block design test can be carried out, but if the two tests are not met, then a Non-Parametric test can be performed. If the results of data analysis have an effect, it can be continued with the Post Hoc Multiple Comparison test to determine the differences in the growth of Staphylococcus aureus bacterial colonies on modified sorghum (*Sorghum bicolor* L. Moench) media with aqua bides diluent and modified sorghum (*Sorghum bicolor* L. Moench) with meat broth diluent and on MSA (Mannitol Salt Agar) media as Gold Standard.

**Test Materials**

1. Pure Culture of Bacteria
   Pure culture of Staphylococcus aureus bacteria ATCC 25923 was obtained from the Health Laboratory Center (BBLK), Jalan Karang Menjangan Number 18, Surabaya City, East Java.
2. Sorghum (*Sorghum bicolor* L. Moench)
   Sorghum (*Sorghum bicolor L.* Moench) used in this study was sorghum (*Sorghum bicolor* L. Moench) in the form of flour obtained from the Indonesian Sorghum House, Jalan Langgar Waqf Number 20 Sawo, Babat, Lamongan, East Java.

**Variables**

The independent variables of this study was sorghum modification media (*Sorghum bicolor* L. Moench) made from sorghum processed into a flour form which is carried out a mass variation (4,5,6,7 gram). The dependent variables of this study was the growth of Staphylococcus aureus bacteria in sorghum media (*Sorghum bicolor* L. Moench) and MSA media as gold standards.

**Data Collection**

Data collection techniques were carried out by direct observation, namely observing growth, analyzing, and counting colonies of *Staphylococcus aureus* bacteria implanted in MSA media and in sorghum modification media (*Sorghum bicolor* L. Moench) using the ALT (Total Plate Number) method.

**Research Procedures**

1. Tools sterilization
   The tools and materials that will be used in this study will be sterilized first using an autoclave at a temperature of 121º C for 15 minutes.
2. MSA media manufacturing process
   Weigh 11.1 grams of MSA media, then dissolve using 100 mL of aqua distillation in the Erlenmeyer, then heat it on a hot plate while stirring until it dissolves but does not boil, cover the Erlenmeyer hole with a sterile cotton swab wrapped in aluminum foil or newspaper, and sterilization using autoclave at a temperature of 121ºC for 15 minutes. Sterile media solution is put into a sterile Petri dish ± 15 mL, and leave it until it solidifies, then the media is ready for use.
3. Sorghum Media Manufacture (*Sorghum bicolor* L. Moench)
   Weigh the sorghum flour (4, 5, 6, 7 gram) with a known mass variation. Dissolve the sorghum flour with 100 mL of meat broth into the Erlenmeyer, and add 7.5 grams of NaCl, 1 gram of mannitol, 0.025 grams of phenol red, 1.5 grams of bacto agar. Heat with a bunsen until it dissolves but does not boil. Adjust the pH of the solution to 7.5 ± 0.2. Sterilize the media using an autoclave at 121ºC for 15 minutes. Pour ± 15 mL of media on each sterile petridisk then let stand at room temperature until the media becomes completely solid.
4. *Staphylococcus aureus* bacterial Suspension (ATCC 25923)
   Preparing a suspension of Staphylococcus aureus bacteria (ATCC 25923), then put into 10 mL of 0.9% sterile NaCl solution using a nose loop, equivalent turbidity to Mac Farland 0.5 (0.5 mL BaCl2 1.175% + 9.95 mL H2SO4 1%). At Mac Farland standard 0.5 is equivalent to the approximate number of bacterial suspensions of 1.5 x 108 CFU/mL where the standard is the basis for experiments of bacterial culture results. The suspension of bacteria can then be thinned until a single colony is formed at a dilution to 1013. The purpose of dilution is that after incubation, a single colony is formed on the media in a calculatable amount, namely the range of 30 and 300 colonies. The suspension that has been equivalent can be pick picked as much as 0.1 mL on MSA media as Gold Standard and sorghum modification media (*Sorghum bicolor* L. Moench) with mass variations.
5. Spread Plate Method
   The spread plate method is a technique of growing microorganisms on the media by spreading bacterial cultures on the surface of the already dense media. The working principle of the spread plate is that bacteria that have been equated to the Mac Farland 0.5 standard are inoculated on MSA media as a positive control and sorghum modification media. Flatten the bacterial culture Spreader glass which has been previously fixed and soaked in 96% alcohol, and wait until the surface of the media dries. Incubation of the substrate using an incubator for 24 hours with a temperature of 37ºC.
6. ALT (Total Plate Number)
   Prepares dilution of pure cultures of *Staphylococcus aureus* bacteria already compared with Mc Farland 0.5 with a diluent solution. Plant bacterial cultures into the surface of the MSA media as a positive control and on the sorghum modification media (*Sorghum bicolor* L. Moench) with the spread plate method, namely dripping bacterial suspension on the media. Sterilize the spreader glass using alcohol and heat using a bunsen, then thoroughly flatten the bacterial suspension on the surface of the substrate. MSA media and sorghum modification media (*Sorghum bicolor* L. Moench) can be put into an incubator with a temperature of 37ºC for 24 hours. Count the number of colonies grown on sorghum modification medium.
7. (*Sorghum bicolor* L. Moench) and MSA media using the ALT (Total Plate Number) method, observe the characteristics of the colony directly such as the color, shape, and surface of the colony on the sorghum modification media and compare with the colony on the MSA media as a control, then can proceed to conduct biochemical tests.

## RESULTS

After conducting the research, on sorghum media of Staphylococcus aureus with the gold standard MSA, the following results were obtained (Table 1).

**Data Analysis Techniques**

The data analysis technique of this study was carried out statistically using the Two Way Anova Design Block test, which was previously carried out by the Kolmogorov Smirnov test to find out that the data were normally distributed

**Data Normality Kolmogorov-Smirnov test**

Based on the results of the data test, p-value of 0.132, so there was norm distributed data

**Two Way Anova Design Block Test**

Based on the results of the data test, p-value of p = 0.001, so there was differences between the groups (sorghum media diluted with aqua distillation and meat broth water)

Table 1. *Staphylococcus aureus* bacteria growth

| Replication | MSA | *Staphylococcus aureus* growth (CFU/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Weight sorghum media diluted with aqua distillation | | | | Weight sorghum medium diluted with meat broth water | | | |
| | | 4 g | 5 g | 6 g | 7 g | 4 g | 5 g | 6 g | 7 g |
| 1 | 107 | 52 | 64 | 82 | 105 | 62 | 66 | 92 | 102 |
| 2 | 104 | 55 | 62 | 81 | 94 | 60 | 67 | 89 | 103 |
| 3 | 101 | 51 | 61 | 79 | 96 | 59 | 64 | 87 | 99 |
| 4 | 98 | 53 | 59 | 84 | 101 | 61 | 65 | 90 | 105 |
| Average | 102.5 | 52.75 | 61.5 | 81.5 | 99 | 60.5 | 65.5 | 89.5 | 102.2 |

## DISCUSSION

The results obtained in this study, with a statistical test of two-way ANOVA design blocks, it was found that in variations in the sorghum period of 4 grams, 5 grams, and 6 grams both in dilution with aqua distillate and meat broth the growth of *Staphylococcus aureus* bacteria. In contrast to the growth in MSA (positive control) media. Meanwhile, in the periodic variation of 7 grams of sorghum in both aqua distillate and meat broth, there is no difference in the growth in MSA media. Based on the Anova Two Way Test, the design block, on the media Sorghum with a mass variation of 7 grams, both dilution with aqua distillate and meat broth can be used as an alternative medium for the growth of *Staphylococcus aureus* bacteria[15].

Based on the results of the study contained in table 1, the period variation of 7 grams of sorghum with a dilution of aqua distiilate obtained an average value of 99 x 1013 CFU / ml and in the periodic variation of 7 grams of sorghum with a dilution of meat, broth obtained an average value of 102.2 x 1013 CFU/ml. Statistically, it was found that the growth of *Staphylococcus aureus* bacteria in the period variation of 7 grams of sorghum was no different from positive control, namely MSA media where the average growth of *Staphylococcus aureus* bacteria was 102.5 x 1013 CFU / ml. However, descriptively dilution with meat broth, the growth of *Staphylococcus aureus* bacteria has almost the same average as MSA media, this is because meat broth contains high protein[16]. Protein is also present in sorghum, so when sorghum is diluted with meat broth it is a very suitable medium for the growth of *Staphylococcus aureus.* Both ingredients contain the same replacement protein according to those in the MSA media.

## CONCLUSION

Based on the results of the study entitled "Two Way Statistical Test of Anova Blok Design as a decision-making tool for analyzing the results of the growth of Staphylococcus aureus bacteria on sorghum media (*Sorghum bicolor* L. Moench) to make two way Anova statistical method approach block design to find alternative MSA media into sorghum to grow bacterial media it can be concluded that *Staphylococcus aureus* bacteria growth on Sorghum Modification media by dilution using aqua distillate and dilution using meat broth with a period variation of 4 grams, 5 grams, 6 grams Sorghum is different from the growth of *Staphylococcus aureus* bacteria on MSA media as a positive control. The results of the growth of *Staphylococcus aureus* bacteria on Sorghum Modification media by dilution using aqua distillate and dilution using meat broth with a variation in the period of 7 grams of sorghum are no different from the growth of *Staphylococcus aureus* bacteria on MSA media as a positive control. However, in dilution with meat broth, the growth of *Staphylococcus aureus* bacteria is almost the same as the growth of *Staphylococcus aureus* bacteria on MSA media. Based on the results of statistical tests using the Two Way Anova Blok Design Statistical Test, it can be concluded that sorghum media at a time variation of 7 grams can be used as an alternative medium to replace MSA.

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