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Coliform AND *Escherichia coli* TESTS IN THE AIR OF THE WELLS OF THE VILLAGE SIDO MAKMUR KUALA DISTRICT LANGKAT DISTRICT

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ABSTRACT

Dug well water is a source of clean water that is used by 99.39% of the residents of Sido Makmur Village, Kuala District, Langkat District to meet their daily needs. The majority of people who have cow pens around dug wells trigger the importance of conducting microbiological water quality research, given the existence of dug wells that are very vulnerable to cow manure contamination. Therefore the research sample was taken based on the distance of the dug well with a cattle pen that is less than 5 meters. Five samples from Inpres Hamlet, Petak Dua Hamlet, Handayani Hamlet, Sidorejo Hamlet and Mandailing Hamlet were tested using the Most Probable Number (MPN) method in the North Sumatra Province Health Laboratory, to determine the presence of Coliform and *Escherichia coli* through the estimation test, assertion test , and test the completeness and coloring of grams. Then a biochemical reaction test is performed to confirm the presence of *Escherichia coli*. The results showed that all dug well water samples with sample codes ASG 01, ASG 02, ASG 03, ASG 04 and ASG 05 were positively polluted by Coliform through assertion tests with total Coliform exceeding the 80% threshold and below the threshold of 20% based on Minister of Health Regulation No. 416 / MENKES / PER / IX / 1990 concerning Requirements and Supervision of Water Quality. Then there is also the content of *Escherichia coli* in the five samples which exceeds the established threshold, the positive results of E.coli have also been strengthened through the results of gram staining and biochemical reaction tests. Therefore the dug well water samples tested are not feasible or do not meet the microbiological quality requirements for clean water.

Introduction

Sidomakmur Village, Kuala District, Langkat Regency is an area with a population whose majority owns livestock land around the house. There are residents' houses that have beef cattle cages, goat cages and chicken coops. In general, the position of the cage that was built was close to the location of the dug well that the community in the village used as

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a source of water to meet their daily needs. According to Johnston (2019) the existence of cattle pens with dug wells that are close together will cause pollution to the soil, air and water.

The resident's pet beef cattle produce manure in the form of feces which is spread freely on the ground around the dug wells. Feces excreted through the digestive tract of beef cattle contains 108-109 Escherichia coli/gram (Schmithausen, 2018). The movement of Coliform bacteria including E.coli can be influenced by water absorption in the soil layer. Groundwater infiltration increases when it rains.

Dug wells are a means to tap and accommodate groundwater which is used as a source of raw water for clean water. Housing conditions that are not always around a clean water source make people use dug wells that can be made around their homes. Especially for areas that have not been reached by the Regional Drinking Water Company (PDAM) such as Sido Makmur Village, Kuala District, Langkat Regency.

One of the general requirements for the construction of dug wells is that they should not be built close to sources of pollution such as cattle pens, then the distance between the dug wells and the pollutant source has a minimum limit of 10 meters. Water pollution is caused by the entry of living things, substances or other components into the water so that the water quality drops to a certain level which causes the water to not function according to its designation (Anju, 2010; Singh, 2020).

Polluted water generally contains a lot of heterotrophic bacteria because it has high levels of organic matter. Heterotrophic microorganisms use organic matter to carry out metabolic processes. The most common microorganisms found are Coliform bacteria such as *E. coli* (Feng, 2002). At first E.coli was isolated by Escherich (1885) from the feces of infants, since the bodies were known to be distributed in all individuals, the bacteriological analysis of water was aimed at the presence of these bacteria. E.coli bacteria in a certain amount in the water can be used as an indicator of the presence of pathogenic bodies (Wen, 2020). Coliform bacteria are a group of microorganisms that are commonly used as indicators because they can be a signal to determine whether a water source has been contaminated by pathogens or not.

Coliform bacteria can produce ethionine which can cause cancer. In addition, it can also produce various kinds of toxins such as indole and skatole which can cause disease if the amount is excessive in the body. Coliform can be used as an indicator because its density is directly proportional to the level of water pollution. These bacteria can detect pathogens in water such as viruses, protozoa and parasites, also have high resistance than pathogens and are easier to isolate and grow (Rajapaksha, 2019). Meanwhile, E.coli bacteria can cause diarrhea if in excessive amounts in the digestive tract.

Diarrhea is characterized by loose or liquid bowel movements with a frequency that is more frequent than usual (three times a day) (Depkes RI, 2010). Diarrhea is an endemic disease in Indonesia that has the potential to become an extraordinary event (KLB) and cause death (Kemenkes RI, 2017). Outbreaks of diarrhea are still common with a large number of sufferers and deaths. Low coverage of hygiene, sanitation and low behavior are often risk factors for diarrhea outbreaks (Kemenkes RI, 2011). Outbreaks of diarrhea have occurred in 2013, namely 8 outbreaks of diarrhea spread across 6 provinces with 633 cases, in 2014 there were 6 outbreaks of diarrhea spread over 5 provinces with 2,549 cases, in 2015 there were 21 outbreaks of diarrhea spread in 12 provinces with 1,312 cases, in 2016 there were 3 outbreaks of diarrhea spread across 3 provinces with 198 cases, and in 2017 there were 21 outbreaks of diarrhea spread across 12 provinces with 1,725 cases (Kemenkes RI, 2013; Ministry of Health RI, 2014; Ministry of Health RI, 2015; Ministry of Health RI, 2016; Ministry of Health RI, 2017). Diarrhea sufferers in Indonesia have increased every year, it was recorded that in 2015 there were 4,017,861 people from the total estimated diarrhea in health facilities of 5,405,235 people, in 2016

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there were 2,544,084 people from the total estimated diarrhea in health facilities of 6,897,463 people and in 2017 there were 4,274,790 people from the total estimated diarrhea in health facilities of 7,077,299 people (Ministry of Health RI, 2015; Ministry of Health RI, 2016; Ministry of Health RI, 2017). Judging by age, diarrhea cases were more common in the 1-4 year age group, then the 20-44 year age group.

This is a health problem that needs attention, especially diarrhea, which generally affects children under five and is a contributor to death in toddlers. Hygiene and environmental sanitation factors, awareness of parents of toddlers to behave in a clean and healthy life and breastfeeding are important factors in reducing diarrhea morbidity in toddlers (Kemenkes RI, 2011). Langkat Regency, which is one of the regencies in North Sumatra, recorded 17,870 cases of diarrhea handled in 2017 with a target number of 66,629 people. While the data listed at the Kuala Health Center which is the only health center in Kuala Subdistrict, Langkat Regency, recorded the number of targets for finding diarrhea in 2017 as many as 2,245 people, with the number of patients being treated as many as 1,203 people. Then especially for Sido Makmur Village, cases of diarrhea handled in 2018 were 47 people. This condition shows that diarrheal disease is a threat to the community, therefore all factors that have the potential to spread diarrheal disease in the human body must be addressed immediately (Langkat District Health Office, 2017).

One of the conditions that have the potential to cause infectious diseases due to Coliform and E.coli in Sido Makmur Village is the condition of water sources in the form of dug wells adjacent to cattle pens, especially beef cattle pens. Beef cow dung that is in direct contact with the land in the yard of the house has the potential to contaminate the water sources of dug wells owned by residents. There are 80 families who have beef cattle pens around the house, while others are members of livestock groups with beef cattle pens positioned a bit far from the settlement. The only source of clean water for drinking, cooking, washing and other purposes in Sido Makmur Village is dug wells with a percentage of 99.39%, that is, there are 487 dug wells used by 490 families. A total of 78 heads of families who have dug well water sources and have cowsheds around the house. Based on the survey that has been carried out, there are dug wells that have a distance of under 5 meters with 5 wells for cattle, then there are dug wells that have a distance of below 10 meters with 8 wells for cattle and there are dug wells that have a distance of above. 10 meters with 70 wells for cattle. Based on this, microbiological examination of dug well water is very important because water is a substance that supports the life of microorganisms. Water quality checks are carried out to determine whether the water contains Coliform and *E.coli* bacteria that are harmful to humans. The number of these bacteria in the water indicates the low quality of the water it has. According to the Ministry of Health, the more *E.coli* and Coliform bacteria, the lower the water quality. Total Coliform levels in clean water for sanitation hygiene purposes should not exceed the threshold value of 50/100 mL and the maximum level of E.coli content is 0/100mL.

Coliform and *E.coli* tests were carried out to determine the quality of the dug well water to be analyzed. The method used is MPN (Most Probable Number). The water quality test consists of several stages, namely an estimator test, a reinforcement test and a complementary test. The MPN method can be used to calculate the number of bacteria that can ferment lactose to form gas, for example Coliform bacteria (Yusmaniar and Khairun, 2017). It proves that there is a strong relationship between the number of fecal coliforms in water samples from dug wells around pig farms. Research by Suwito (2014) also showed that all samples of dug well water around beef cattle farms tested were contaminated with E.coli bacteria. Then the results of Awuy's research (2018) showed positive E.coli results in all samples of dug well water which were located close to the pollutant source in the form of a septic tank.

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In addition, other studies also proved that samples of dug well water contaminated with household waste were positive for E.coli (Widiyanto, 2015). Even in Zega's research (2018) positive Coliform bacteria were found in water that is ready to drink, namely in depot water in the Medan Deli area.

In view of these conditions, it is important to carry out a Coliform and *Escherischia coli* content test in the dug well water of Sido Makmur Village, Kuala District, Langkat Regency, namely Inpres Hamlet, Petak Dua Hamlet, Handayani Hamlet, Sidorejo Hamlet and Mandailing Hamlet. The location of the cattle pens is around the house which is less than 5 meters from the dug well.

Materials and Methods

This research will be carried out at the Regional Health Laboratory of North Sumatra Province, precisely at the Microbiology Laboratory and Microbiology Laboratory, Universitas Negeri Medan.

The population of this study were all dug wells in Sido Makmur Village, Kuala District, Langkat Regency. While the sample of this research is dug well water taken from 5 hamlets of Sido Makmur Village, starting from Inpres Hamlet, Petak Dua Hamlet, Handayani Hamlet, Sidorejo Hamlet and Mandailing Hamlet. The sampling technique was carried out by purposive sampling, namely by taking well water samples according to the required criteria. In Sido Makmur Village, water samples were taken from dug wells with the criteria of dug wells that have a distance of less than 5 meters from the location of the beef cattle pen. From the 5 dug well water samples that have been obtained, bacteriological tests were carried out using the 3-3-3 fermentation tube series for 3 replications.

The tools used in this study were bottles, test tubes and tube racks, petri dishes, pipettes, volume pipettes, ose needles, Bunsen, measuring cups, Erlenmeyer tubes, spatulas, vortex, Durham tubes, light microscopes, slides, ice boxes, water bath, autoclave and laminar air flow. The materials used were dug well water samples, then distilled water, alcohol, spirit, cotton, label paper, tissue paper, aluminum foil, Nutrient Agar media, BGLB (Brilliant green lactose bile broth) media, LB (lactose broth) media, EMB media. (Eosin methylene blue), spirit, Lugol's solution, alcohol acetone solution, crystal violet, safranin solution, filter paper and water.

The research method uses the Most probable number (MPN) which passes through several stages of research, namely the estimation test, confirmation test and completeness test. And confirmed by Gram stain test and Biochemical Reaction to confirm the presence of E.coli.

Result and Discussion

Samples were obtained by conducting a survey of dug wells with a distance of less than 5 meters from the cattle pens. Then the samples were stored in sterile glass bottles that had been labeled on each bottle. The code given is as follows.

ASG 01 : Dusun Inpres
ASG 02 : Dusun Petak Dua
ASG 03 : Hamlet Handayani
ASG 04 : Dusun Sidorejo
ASG 05 : Dusun Mandailing

Prediction Test

The predictive test was carried out using a series of 3-3-3 fermentation tubes containing inverted Durham using Lactose Broth (LB) media. Each tube contains 5 ml of pre-sterilized LB media. In the first 3 tubes 10 mL of water samples were added, in the second 3 tubes 1 mL of water samples were added and in the next 3 tubes 0.1 mL of water samples were added. Then the incubation process was carried out for 24 hours at a temperature of 37^0C.

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Table 1. Bacterial Estimation Test

No	Sample Code	Treatment I			Treatment II			Treatment III		
		dilution			dilution			dilution		
		10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)
1	AGS 01	3	3	2	3	3	2	3	2	2
2	ASG 02	3	3	2	3	3	1	3	3	2
3	ASG 03	3	3	1	3	3	2	3	2	2
4	AGS 04	3	3	2	3	3	2	3	3	2
5	ASG 05	3	3	2	3	3	2	3	2	2

The presence of coliforms in water is used to determine the level of sanitation or water cleanliness, so that it can be seen if it is suitable for household use. Based on table 1, it can be seen that almost all tube series showed positive results from Coliform bacteria. The Coliform group consists of several genera from the Enterobacteriaceae family, including *Escherichia, Citrobacter, Enterobacter, Klabsiella, Salmonella,* and Shigella (APHA, 1992). The presence of Coliform bacteria which is a type of gram negative bacteria is indicated by the presence of gas (CO2) in the Durham tube. If the Durham tube shows the formation of gas bubbles and the presence of acid which is indicated by the crunch of LB media, it is most likely that the bacteria contained in it are Enterobacteriaceae bacteria, because they can ferment sugar through mixed fermentation of acid and butandiol (Muller, 2001; Widodo, 2015). Under aerobic conditions, bacteria are able to oxidize amino acids, while in the absence of oxygen, metabolism is fermentative and energy is produced by breaking down sugars into organic acids. Coli bacteria are capable of producing CO2, H2, 2,3 butanediol, formic acid, acetic acid, succinic acid and ethyl alcohol gas, with test results in the form of a medium color changing to a yellowish color and gas formation in the Durham tube (Widodo, T.S et al. 2015) .

Affirmation Test

Each tube contains 5 ml of BGLB media which has been previously sterilized. The positive tube in the presumptive test is continued in this confirmation test. For each positive tube in the presumptive test one needle was taken and then inserted into the BGLB media. Then the incubation process for 24-48 hours at a temperature of 370C to confirm the presence of Coliform and at a temperature of 440C to confirm the presence of faecal Coliform. The results of the estimation test carried out for 3 repetitions are presented in the table below.

Tabel 2. The results of the estimation test carried out 3 times

No	Sample Code	Treatment I			Treatment II			Treatment III		
		dilution			dilution			dilution		
		10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)
1	AGS 01	3	3	2	3	3	1	3	3	1
2	ASG 02	3	2	2	3	2	1	3	2	2
3	ASG 03	2	2	0	2	2	0	2	1	0
4	AGS 04	3	3	2	3	3	2	3	3	2
5	ASG 05	3	3	2	3	3	2	3	2	2

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Table 3. MPN index per 100mL through Confirmation Test of dug well water samples in Sido Makmur Village at a temperature of 37ºC.

No	Sample Code	Treatment I (Indeks MPN per 100mL)	Treatment II (Indeks MPN per 100mL)	Treatment III (Indeks MPN per 100mL)	Average	Below the threshold (≤ 50)	Above the threshold (> 50)
1.	AGS 01	1100	450	450	666,7	-	✓
2.	ASG 02	210	150	210	190	-	✓
3.	ASG 03	47	47	44	46	✓	-
4.	AGS 04	1100	1100	1100	1100	-	✓
5.	ASG 05	1100	1100	210	803,3	-	✓
Percentage						20%	80 %

Furthermore, to determine the MPN index value of faecal Coli/E. Coli, a confirmation test was carried out with BGBL media which was incubated at 440C for 24 hours. Positive results on the tube are also indicated by the presence of gas in the Durham tube and the change in the media becomes cloudy. The following is a table of confirmation test results in the 3-3-3 fermentation tube series.

Table 4. Confirmation test with BGLB media which was incubated at 44ºC for 24 hours

No	Sample Code	Treatment I Dilution			Treatment II (+) Dilution			Treatment III (+) Dilution		
		10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)	10 mL (Jtp)	1mL (Jtp)	0,1 mL (Jtp)
1	AGS 01	2	1	0	2	1	1	2	1	0
2	ASG 02	3	2	2	3	2	1	3	2	2
3	ASG 03	2	2	0	2	2	1	2	2	0
4	AGS 04	3	2	2	3	3	1	3	2	1
5	ASG 05	3	2	2	3	2	2	3	2	1

Through the positive test results in table 4.4, it is known that the MPN index value is adjusted according to the Hopkin table. So that the MPN Coli/E. coli index value is obtained as follows.

Tabel 5. MPN index value is adjusted according to the Hopkin table

No	Kode Sampel	Treatment I (Indeks MPN per 100mL)	Treatmen II (Indeks MPN per 100mL)	Treatment III (Indeks MPN per 100mL)	Average	Below the threshold (≤ 0)	Above the threshold (>0)
1.	AGS 01	15	20	15	16,7	-	✓
2.	ASG 02	210	150	210	190	-	✓
3.	ASG 03	21	28	21	23,4	-	✓
4.	AGS 04	210	150	150	170	-	✓
5.	ASG 05	210	210	150	190	-	✓
Percentation						0%	100%

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In the confirmation test the media used was Brilliat Green lactose Bile Broth (BGLB). This media is used because of the presence of brilliant green which can inhibit the growth of gram-positive and gram-negative bacteria other than Coliform, then the presence of bile salts can inhibit the growth of non-living bacteria in the human gastrointestinal tract. This content is what makes it different from LB media. Positive results in the confirmation test are also indicated by the formation of gas in the Durham tube because this media also contains lactose (Bambang, 2014; Radji, 2008).

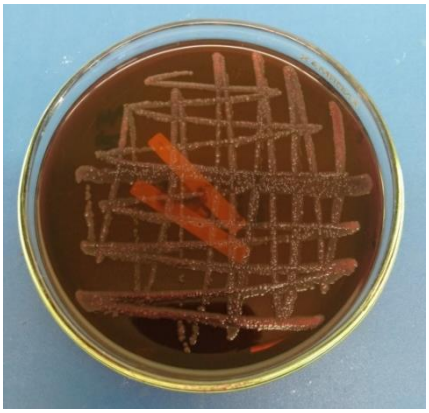
Then, to distinguish the total value of Coliform and faecal Coliform in the confirmation test, the positive result in the presumptive test was continued by making the test in duplicate, the first part to be incubated at 37^{o}C and the second part to be incubated at 440C for 24-48 hours. This difference in temperature treatment was carried out to determine the total value of Coliform and the value of faecal Coliform/*E.coli* (SNI, 1996).

The results of the confirmation test in table 4.2 show a positive result of Coliform in test tubes, then the number of positive results in test tubes has been adjusted according to the Hopkins table so that the MPN value is obtained in table 3, which shows 20% of the test samples are still below the threshold and 80% of the test samples were above the threshold for clean water requirements. However, this indicates that all dug well water samples tested positive for Coliform.

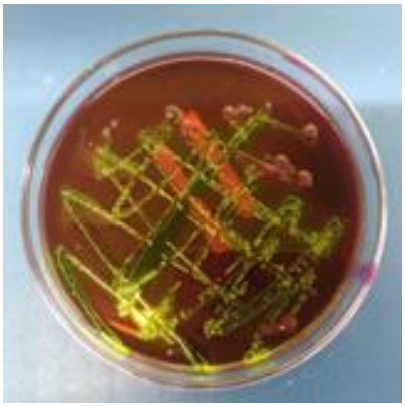
While the results of the confirmation test in table 4 show positive results from the fermentation tube incubated at 44^{o}C, then the positive results in the test tube are matched with the Hopkins table which has been presented in table 5. The table shows that all well water samples were contaminated with faecal Coliform bacteria/E.coli with MPN values greater than zero (> 0). So the *E.coli* content in all dug well water samples exceeded the permissible threshold as clean water. As in Suwito's research (2014), showed the presence of E.coli content in all samples of well water around cowsheds in the districts of Sleman, Bantul, and Kulon Progo.

Completeness Test

After being incubated at a temperature of 370 to confirm the presence of Coliform bacteria and a temperature of 440 to confirm the presence of faecal Colifom bacteria which is *E.coli*, there was a change in the EMB agar medium. Colonies of E. coli bacteria grew green with metallic flashes on media that were incubated at 440C, then pink colonies with mucus were the other Coliform groups on media that were incubated at 370C. Bacteria that ferment slowly will produce pink colonies in EMB media. Meanwhile, E. coli, which is a fermented bacterium, will produce metallic green colonies on EMB media (Dad, 2000).



(a)



(b)

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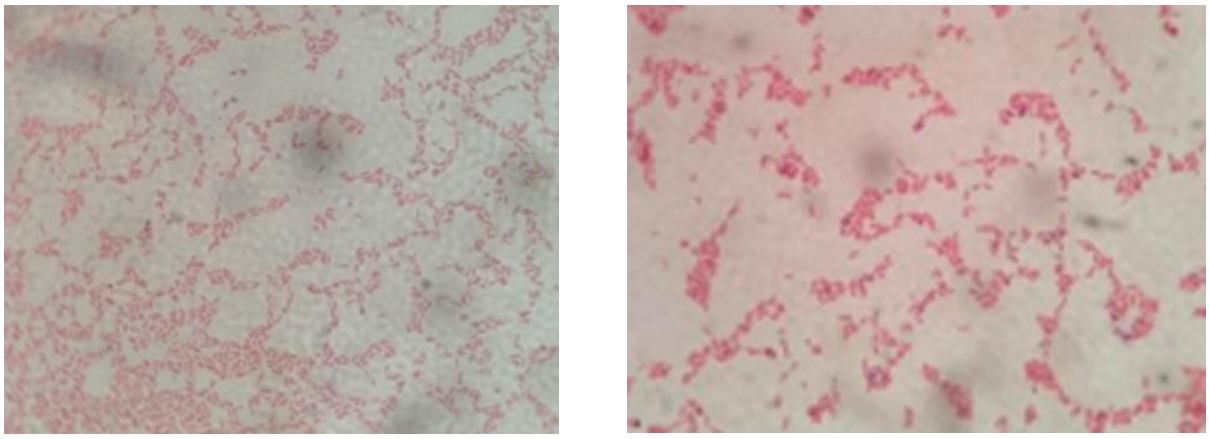
Figure 1. (a) Growth of bacterial colonies that have been incubated for 24 hours at 37^0C. (b) Growth of bacterial colonies that have been incubated for 24 hours at 44^0C.

In Figure 1 (a) it can be seen that there is the growth of Coliform bacteria colonies which are marked by pink colonies and in Figure 4.6 (b) it can be seen that the growth of E. coli bacteria colonies are marked by metallic green colonies. The metallic green or green color with a metallic luster is an indicator that lactose or sucrose has been fermented by faecal coliforms, namely E. coli (Leboffe and Pierce, 2010).

E.coli bacteria are bacteria that can ferment lactose quickly and produce a lot of acid so that it can produce metallic green colonies. Then Enterobacter aerogenes and Klebsiella sp bacteria can also ferment lactose but not as fast as E. coli, because Enterobacter aerogenes and Klebsiella sp bacteria produce weak acids so that the colonies formed are pink in accordance with the nature of the acid formed (Jawetz, 2009: Tankeshwar, 2015). So, based on the test results in table 4.6, it is increasingly convincing that all samples of dug well water contain Coliform and E.coli bacteria, because the completeness test using EMB media is entirely positive for Coliform and E.coli colonies.

Gram Staining

The positive complementary test results were followed by gram staining which was used to determine that the bacteria really belonged to the gram-negative bacteria group, and E.coli was a gram-negative bacteria so that the researchers were confident in the results obtained. Scratches of bacterial colonies were observed on a slide that had passed the gram staining procedure and then observed using a microscope with a magnification of 100x.



(a) (b)

Figure 2. (a) The results of gram staining on colonies that have been incubated at 44^0C with a magnification of 100x; (b) The results of gram staining on colonies that have been incubated at 37^0C with a magnification of 100x.

Bacteria inoculated on EMB media that produced colonies with a metallic green luster were *E. Coli* bacteria, while pink colonies were *Enterobacter aerogenes* or *Klebsiella* sp (Brooks, 2012; Tankeshwar, 2015). This can be observed in the completeness test results which show the color of the colonies on the EMB agar plate. Figure 2 (b) shows that observations at 100x magnification show the characteristics of *E. coli* bacteria. With the characteristics of coccobacil shaped bacteria, single arrangement, red color and gram negative.

Biochemical Reaction Test

Indotest

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Indole test is a test conducted to observe the ability of organisms to degrade the amino acid tryptophan and produce indole. *E. coli* is able to degrade amino acids so that the test results show a red layer when the Kovacs reagent is added (Hemraj et al, 2013). The results of the Indole test on 5 well water samples taken through the results of the previous incubation on EMBA media at a temperature of 44^0C for 24 hours. All samples showed positive results with a change in color on the surface of the media to red after being dropped with Kovacs reagent.

Simmons Citrate Test

Simmons Citrate media is one of the media used to test the ability of bacteria to use citrate as the only carbon source used. Bacteria can transport molecules into cells and convert them to pyruvate by their citrate-permease. Then pyruvate can be converted into various products, depending on the pH of the environment. This test shows positive results when Simmons Citrate media changes from green to blue (Chatim, et al, 2002). Simmons Citrate test results on 5 well water samples taken through the results of previous incubation on EMBA media at 44°C for 24 hours. All samples showed positive results with a green to blue color change on the agar medium.

Triple Sugar Iron Agar (TSIA) Test

The TSIA test will show that bacteria can ferment glucose, ferment lactose, ferment sucrose, and reduce sulfur. It contains phenol red which is an indicator and ferrous sulfate which is an indicator of hydrogen sulfide. The change in the media to yellow color was due to the glucose fermenter inoculated on the TSIA media so that it produced acid, this caused a decrease in pH and the media became yellow. The lifting of the media indicates that gas has formed which is the result of fermentation of sugars other than acids (Atlas, 2010; Jawetz et al., 2013).

The results of the TSIA test on 5 well water samples taken through the results of previous incubation on EMBA media at a temperature of 44^0C for 24 hours. All samples showed positive results with a red to yellow color change on the agar media and the agar media was lifted due to the presence of gas.

Conclusion

Samples of dug well water obtained from 5 hamlets namely Inpres Hamlet, Handayani Hamlet, Sidorejo Hamlet, Petak Dua Hamlet, and Mandailing Hamlet, Sido Makmur Village, Kuala District, Langkat Regency were positive for Coliform bacteria contamination, with a percentage of 80% of dug well water containing Coliform that passes through threshold requirements or unfit for use as a source of clean water and 20% of dug well water contains Coliform which is still below the required threshold based on Minister of Health Regulation no. 416/MENKES/PER/IX/1990.

Samples of dug well water were obtained from 5 hamlets, namely Inpres Hamlet, Handayani Hamlet, Sidorejo Hamlet, Petak Dua Hamlet, and Mandailing Hamlet, Sido Makmur Village, Kuala District, Langkat District, 100% positive for *Escherichia coli* contamination.

The microbiological quality of dug well water samples in Sido Makmur Village, Kuala District, Langkat Regency did not meet the requirements for clean water parameters.

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