

# Determination of *Coliform* bacteria contamination on household ice cube in Bukittinggi

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## Abstract

**Aim:** To evaluate the quality of water used in the manufacture of household ice cubes in market of Bukittinggi. **Material and Methods:** Household ice cube samples were tested Most Probable Number (MPN) and Total Plate Count (TPC) method to determine MPN of *Coliform* and *Coli fecal* bacteria. **Results and Discussion:** The result is Most Probable Number (MPN) value of *Coliform* and *coli fecal* on Ice Cube A equal to Ice Cube B about 1100 *Coliform*/100 ml, meanwhile seller C has greater amount compared to the two previous seller, amounting to >2400 *Coliform*/100mL. For Total Plate Count (TPC), The number of sellers A =  $181 \times 10^4$  *Coliform*/g, seller B =  $20,7 \times 10^4$  *Coliform*/g, and seller C =  $1920 \times 10^4$  *Coliform*/g. **Conclusion:** The household ice cubes used by the drink seller in market of Bukittinggi are not suitable for consumption.

**Key words:** *Coliform* bacterial, contamination, household ice cube

## INTRODUCTION

Thirst is a sign when our body needs water, where humans will try to find drinking water or beverages, both packaged and fresh drinks, which are healthy and safe for we consume.<sup>[1]</sup> Water can be found in various forms, whether in liquid or solid form. Water in solid form is usually called ice cubes. Ice cubes are complementary product which is usually used for mixed juice, tea, and other fresh drinks.<sup>[2]</sup> In general, community assumed that ice cubes are safe for their daily consumption. Ice cubes are known as frozen water, and this freezing occurs when water is cooled below 0°C in the refrigerator. In the process to make household ice cubes, the material water must fulfill hygienic and sanitary standards which set by the regulation.<sup>[3]</sup>

The presence of pollutant bacteria leads to a low quality of household ice cubes, which may come from things such as raw materials (water), tools used in ice making, and poor level of hygiene which was had by ice cubes producer.<sup>[4]</sup> Enterobacteriaceae class bacteria are bacteria which often contaminate water. This bacteria family consists of several genera including *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Proteus*.<sup>[5]</sup> Health problems that occur because of these

bacteria can be in the form of disorders of the digestive with symptoms of nausea, stomachache, vomiting, and diarrhea.<sup>[6]</sup>

*Coliform* are bacteria group that produces gas in lactose medium, has basil form, and belongs to Gram-negative group bacterial. This bacteria can be used to measure clean environment indicator and caused diarrhea disease and fever in bad sanitary.<sup>[7]</sup> The number and type of bacteria are varied and differ according to the place and conditions that affect it.

Drinking water is healthy if it has the physical properties such as colorless, tasteless, lower temperature than air; the microbiological property that is contain less than 4 *Escherichia coli* bacteria for every 100 cc water; and the chemical property that should not contains ion compound or metals exceeding specified amounts, and radioactive requirements contained in mandatory and additional parameters.<sup>[8,9]</sup>

To ensure the quality of drinking water, the government issued a regulation through the Minister of Health No.907/Menkes/

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SK/VII/2002 about the standards and requirement of drinking water quality control, in which the maximum allowable for *E. coli* in drinking water is  $<3/100$  ml.<sup>[6]</sup> The total number of ice cubes allowed by the Indonesian National Standard (SNI) number 7388: 2009 is 1 colony/g.<sup>[10,11]</sup> With the supervision of water quality used in making ice cubes, it is expected to improve community's health level, to prevent the occurrence of health problems. Therefore, the quality of water used in the manufacture of ice cubes in the household must meet health requirements.

This study aims to determine whether there is a contamination of coliform bacteria on ice cubes in Aur market in Bukittinggi and determine whether the ice cubes are worth to be consumed.

## MATERIALS AND METHODS

### Tools

Autoclave, reaction tube, Erlenmeyer, becker glass, Spiritus light, reaction tube shelf, cotton, parchment paper, matches, funnel, incubator, stirrer bar, wire basket, sterile plastic, colony counter, Durham tube, Petridish, ose needle, water bath incubator, and icebox were used.

### Materials

Ice cubes taken at the Aur market were picked by simple random sampling, lactose broth (LB), Bractant Green Lactose Bile Broth, Endo agar, Aquadest, and ethanol 70%.

### Sampling

The samples used were taken from three beverage vending places in Aur market. Samples were taken using sterile plastic and put into an icebox. Let the ice cubes melt.

### Experimental Procedure

#### Sterilization

The reaction tube, Durham tube, Petridish, and becker glass are first washed and dried before we use for testing. Tools are wrapped first with parchment paper separately. Then, the sterilization was done using autoclave At 121°C for 15 min.

The ose needle is sterilized by means of a spray using a spirit light. The aseptic cupboard is sterilized by cleaning the table from dust and spraying 70% alcohol.

#### Testing Most Probable Number (MPN)

For testing, the water quality can be used the MPN method, which is also called the closest approximation by counting

**Table 1:** The maximum limit of bacterial contamination on ice cube<sup>[12]</sup>

No	Type of microbial contamination	Maximum limit
1	ALT (30°C, 72 h)	1×10 <sup>4</sup> colony/g
2	<i>Salmonella</i> sp.	Negative/25 g

**Table 2:** The research result of estimated test of MPN

Sample	Volume			MPN/100 ml
	10	1	0,1	
Ice cube A	3	3	2	1100
Ice cube B	3	3	2	1100
Ice cube C	3	3	3	>2400

MPN: Most probable number

**Table 3:** Research result test of affirmation number MPN

Temperature	Sample	Sample volume (ml)		
		10 ml	1 ml	0.1 ml
37°C	A	+++	+++	++
	B	+++	+++	++
	C	+++	+++	+++
44°C	A	+++	+++	++
	B	+++	+++	++
	C	+++	+++	+++

In Samples A, B, and C, there are gas bubbles in Durham tubes incubated at 37°C and 44°C

**Table 4:** Number of bacterial colonies found in Petri dishes

Dilution	Sample A		Sample B		Sample C	
	C1	C2	C1	C2	C1	C2
10 <sup>-1</sup>	582	549	721	656	spreader	spreader
10 <sup>-2</sup>	476	432	spreader	521	578	600
10 <sup>-3</sup>	394	321	300	114	512	478
10 <sup>-4</sup>	267	96	27	21	425	376
10 <sup>-5</sup>	26	10	18	6	288	96

the number of bacteria present in the sample. This method is only to calculate bacteria that are able to ferment lactose by producing gas. For samples of high estimated density of bacteria, varieties were used: 3 × 10 ml, 3 × 1 ml, and 3 × 0.1 ml.<sup>[8,10]</sup>

#### Test forecast

Prepare 9 test tubes:

- Three test tubes each containing 5 ml of double strength LB medium (up).

- Six test tubes each containing 10 ml of single-strength (and) LB medium.
- Into tube  $a_1$  to  $a_3$ , was inoculated each 10 ml of water sample.
- Into tube  $b_1$  to  $b_3$ , was inoculated each 1 ml of water sample.
- Into tube  $b_4$  to  $b_6$ , was inoculated 0.1 ml of water sample, tube content was sucked and released repeatedly.
- Cover each test tube using clean cotton.
- Incubate on temperature  $37^\circ$  for 48 h.
- Observe each tube to see whether there is a gas, the presence of a gas indicates a positive estimate test. Proceed to Test of Affirmation for the tubes that produce gas.

#### Test of affirmation

- Take 1–2 lactose contents from each positive test tube tested, transferred into a Brilliant Green Lactose Bile Broth (BGLBB) reaction tube.
- A series of BGLBB tubes which have been planted, buried at  $37^\circ\text{C}$  to ensure the presence of coliform bacteria. Another series was screamed at  $44^\circ\text{C}$  to confirm the presence of coli fecal bacteria.
- The reading is done after 48 h by looking at the number of BGLBB tubes that showing positive gas.
- Record the number of tubes in the BGLBB confirmation test which indicates both positive and negative gas. The number obtained is matched with the MPN table per 100 ml sample, and the MPN index will be obtained.

#### Total Plate Count Test (TPC)

Quantitative methods are used to determine the number of microbes present in a sample, commonly known as the TPC. In this TPC method, the medium which was used is the Endo agar in a Petri dish and incubated at a temperature of  $30^\circ\text{C}$  for 72 h with the position reversed with the end result of colonies that can be observed. The total number of ice cubes allowed by Indonesian National Standard (SNI) number 7388: 2009 is  $1 \times 10^4$  koloni/g.

- Provide a sample of each 100 g of ice cubes and placed in sterile backerglass aseptically.
- Prepare 15 test tubes containing 9 ml of sterile Aquadest used for dilution and labeled for each sample.
- Prepare 30 Petri dish and labeled for dispersion.
- Take 1 g of sample, then input to first dilution tube.
- Pipe 1 ml sample from first dilution move to second dilution, proceed to third dilution, and so on.
- From each dilution of 1 ml input to the Petri dish according to each label, then poured 15 ml Endo into the Petri dish, and petri dish immediately shake.
- After the media solidifies, immediately incubate the Petri dish at  $30^\circ\text{C}$  for 72 h with the position upside down.
- Count colonies on the cup using a colony counter.

## RESULTS AND DISCUSSION

### Results

#### MPN

The results of the non-fecal MPN coli and coli fecal household ice cubes used by the drink seller in Aur market using double tube method are as follows:

- The research result of the estimated test of MPN
- Test of affirmation

#### TPC

- In Sample A, the number of colonies:  $181 \times 10^4 \times \text{coliform/g}$  sample.
- In Sample B, the number of colonies:  $20.710^4 \times \text{coliform/g}$  sample.
- In Sample C, the number of colonies:  $192010^4 \times \text{coliform/g}$  sample.

## DISCUSSION

The samples in this study are ice cubes from three different places that sold by beverage sellers on Aur Market, Bukittinggi City. Water which has met the health requirements is sometimes still polluted, either at the time of collection or transport, until the time of storage of water already cooked.<sup>[13,14]</sup> In this research, we test MPN and TPC. In MPN test, a double tube method consisting of an approximate test and an affirmation test was used. This method is a common method used to determine the MPN of coliform and coli fecal bacteria.

Coli bacteria are a group that often contaminates water, and its existence is not expected.<sup>[15]</sup> The presence of this bacteria group on water signifies that the water has been contaminated.<sup>[7]</sup> Coli bacteria are divided into two types, namely, fecal coli, such as *Escherichia* derived from human feces, and nonfecal coli, such as *Aerobacter* and *Klebsiella* that are not derived from human feces but derived from other sources.<sup>[13]</sup>

Based on its growth temperature, bacteria are classified as follows:

- Thermophile bacteria, which require high temperatures to grow well and optimum temperature above  $50^\circ\text{C}$ ,
- Mesophyll bacteria, which have an optimum temperature between  $20$  and  $45^\circ\text{C}$ ,
- Psychrophilic bacteria, which grow at low temperatures between  $5$  and  $10^\circ\text{C}$ .<sup>[16]</sup>

For bacteria coli growth, it required suitable media. The medium used is lactose broth (LB) liquid media which is usually used for the approximate test. LB media have different strengths that are double and single. Nine test tubes were divided into three groups consisting of three test tubes each. The first three

test tubes were filled with 5 ml of LB dual strength media. While the other six test tubes are filled with 10 ml of single strength LB media, which put into Durham tube then put them in reverse position. Durham tube serves to provide air cavity and see the presence or absence of gas bubbles.

Before doing research, all the tools are sterilized. Sterilization is the removal of all life forms, either pathogenic, non-pathogenic, vegetative, or non-vegetative forms of an object or material.<sup>[17]</sup> However, most of the equipment and media which commonly used in microbiological work will be damaged if it was used in burning. Fortunately, there are other methods available which are effective.<sup>[18]</sup> Heat sterilization with pressure or steam sterilization is sterilization performed using autoclave and using saturated water vapor at 1 atm at 121 for 15 min. So that, the release of latent energy of steam was occurred that resulted in the death of microorganisms irreversibly due to denaturation or coagulation of cell proteins.<sup>[19]</sup>

The reaction tube containing the media was sterilized before the samples were added in the autoclave at 121 for 15 min. After sterile, each medium is filled with  $3 \times 10$  ml,  $3 \times 1$  ml, and  $3 \times 0.1$  ml samples. The addition of the sample is done inside the aseptic cabinet, and this aims to reduce the contamination from the outside. Then, the entire test tube was covered with gauze and incubated at 37 for 48 h.

If the sample is polluted, then gas will arise on the tube Durham, and the incidence of gas occurs due to LB fermentation by coli bacteria. If the sample is not polluted, then there is no visible gas on the tube Durham. Hence, this indicates that the sample is not contaminated by non-faecal coli bacteria and the fecal coli can be seen in Table 2.<sup>[20]</sup> The samples with positive results on Forecast Test then being tested again on Affirmation Test. This test is performed by inoculating bacterial cultures from positive forecast tests into the BGLBB medium. Inoculation is done using a sprayed ose needle inside the aseptic cabinet. The reaction tube containing the BGLBB media and the sample culture was covered with cotton and then incubated into the incubator at 37 and water bath incubator at 44 for 48 h. The results are positive if there are air bubbles in the tube Durham can be seen in Table 3. Non-fecal coli group will show a positive result on incubation temperature 37° whereas bacteria class coli fecal at temperature 44°.

TPC is a method used to calculate the number of bacterial colonies. The common medium used to calculate the number of *coliform* bacteria is the Endo agar medium. In the total plate number method, dilution is used starting from till and done by Duplo. Then incubated at a temperature of 30 for 72 h in reverse position aims to keep the water vapor contained in the petri dish does not fall over the medium, so it does not affect bacterial growth. The calculation is done after 48 hours using colony counter.

Table 4 shows the results of the TPC study of household, ice cubes used by drink sellers in the Aur City market of

Bukittinggi on media incubated at temperatures of 30 for 72 h, and there were pale pink spots. This indicates the growth of bacteria in the agar endo media. Then, do the calculation of colony by using colony counter. The number of bacteria that grow can be calculated ranging from 30 to 300 colonies. If the number of colonies grows too high > 300, it will be difficult to calculate so the probability of calculation error is very large. The number of colonies >300 is categorized as too numerous to count sample or spreader so that the sample cannot be included in the calculation data.

The results obtained from this study Samples A, B, and C do not meet the requirements determined by the National Standard Agency that is  $1 \times \text{colony/g}$  can be seen in Table 1. This can be caused in the process of making ice cubes and the water is not perfectly cooked, so that coliform bacteria in the water does not die.<sup>[21]</sup> Contaminated ice packs may contaminate the ice when the wrapping is opened or when ice is removed from the plastic wrap. Therefore, people are expected to be more careful in choosing a drink because ice cubes are a food product that is ready to eat and does not require heating process first.<sup>[22]</sup>

## CONCLUSION

From research determination of *Coliform* bacterial contamination on ice stone household in Aur market of Bukittinggi, it can be concluded:

1. Ice cubes used beverage sellers in the Aur market of Bukittinggi contaminated with non-fecal coli bacteria and fecal coli.
2. Samples A, B, and C are contaminated with non-fecal coli bacteria and coli fecal.
  - a. In Sample A obtained = 1100 *Coliform*/100 ml sample.
  - b. In Sample B obtained = 1100 *Coliform*/100 ml sample.
  - c. In Sample C obtained = >2400 *Coliform*/100 ml sample.
3. TPC on household ice cubes in Aur market of Bukittinggi is as follows:
  - a. In Sample A, the number of colonies was obtained: 181/g samples.
  - b. In Sample B, the number of colonies was obtained: 20.7 g samples.
  - c. In Sample C, obtained the number of colonies: 1920 g sample.
  - d. Household ice cubes in the Aur market of Bukittinggi are not feasible for consumption when compared to the maximum bacterial contamination limits set by the Indonesian National Standardization Agency.

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