Roles of Microwave Oven in Preparation of Microbiological Growth Media

Christian Prijana,[1] Yanti Mulyana,[2] Basuki Hidayat[3]
[1]Faculty of Medicine Universitas Padjadjaran, [2]Department of Microbiology and Parasitology Faculty of Medicine Universitas Padjadjaran, [3]Department of Nuclear Medicine Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung

Abstract

Background: Sterilization of a growth medium before being utilized is a very important step in a microbiology laboratory. The common method for this purpose is by using the autoclave. However, autoclaving takes more time. To overcome this limitation, we tried to use the microwave oven. The aim of this study was to evaluate the ability of microwave oven in preparing the growth media.

Methods: This was a laboratory experimental study conducted at Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran, from October to November 2014. The growth media used were: MacConkey agar, in petri dishes, inoculated with Escherichia coli; Sabouraud agar, in petri dishes, inoculated with Candida albicans; Kligler iron agar (KIA), in reaction tubes, inoculated with Escherichia coli and Salmonella Typhi; Simmons citrate agar, in reaction tubes, inoculated with Klebsiella pneumoniae; Mueller-Hinton (M-H) broth, in reaction tubes, inoculated with Escherichia coli; and Motility Indole Urea (MIU) semisolid agar, in reaction tubes, inoculated with Proteus sp.The media would be heated by microwave for 1, 2, and 3 minutes.

Results: From the total 54 dishes/tubes of various microwave-sterilized media, contaminations were only seen at 5 dishes/tubes. Most of the media, except the one-minute-heated Mueller-Hinton broth, were sterilized more than half dishes/tubes. The identification function of all media in this study was performed well.

Conclusions: The utilization of microwave oven as an alternative sterilizing apparatus for microbiological growth media is very potential, particularly for two and three minutes duration of heating. **[AMJ.2016;3(1):1–5]**

Keywords: growth media , microbiology, microwave, sterilization

Introduction

Sterilize the growth medium before used is very important in the microbiology. The process of growth medium sterilization is commonly performed by using an apparatus named autoclave.[1] There are two kinds of growth media sterilization process in between the microbiology laboratory; they are the sterilization of about-to-used-media, as well as the sterilization of the utilized media in order to decontaminate the media from the infectious contaminants. The disadvantage of autoclave sterilization process is taking a long period of time. During the autoclaving process, the temperature is 121°C and the pressure is 2 atm, for 15 minutes. Sterilized objects are placed in an apparatus after heated around 1 hour. After the sterilization process, it still needs several hours to wait until the pressure inside the apparatus drops again up to 1 atm to permit opening the apparatus.[2]

The alternative sterilizing apparatus which will be tested in this study is the microwave oven. Microwave oven operates by transmitting a very short invisible wave, named microwave. That will be immediately absorbed evenly by water, carbohydrates, and lipids. The microwave will excitate the atoms of the molecules which absorb wave and produce heat.[3] A 600–750 W microwave oven which

Correspondence: Christian Prijana, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +628551100188, Email: christianprijana@keromail.com

is used at 100% power level will generate approximately 218–260°C of temperature. The heat produced by the microwave will at last denaturize the protein.[4] The advantage of using microwave oven is the shorter time needed to produce heat. The objective of this study was to evaluate the ability of microwave oven in sterilizing growth media in different durations as well as the identification function of the microwave-sterilized growth media.

Methods

This was a laboratory experimental study. The study was conducted at Microbiology Laboratory, Faculty of Medicine, Universitas Padjadjaran, from October to November 2014. This study was approved by Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia . The media would be heated by microwave for 1, 2, and 3 minutes; each of which consisted of two dishes/tubes, except Kligler Iron Agar with three tubes, and then incubated for 1x24 hours in the temperature of 37°C. Afterwards, one of the dishes/tubes would be kept for 7x24 hours in the refrigerator in with temperature between 2–8°C to subsequently observe if there were contaminant growth on the media. Rest of the media would be inoculated with certain microbes to subsequently observe the identification function of the microwave-sterilized growth media, compared to the autoclaved media as control.

The role of microwave oven was called potential in preparing a medium in certain duration if it could sterilize more than half of the dishes/tubes as well as preserving the identification function of media. The growth media used in this study were: MacConkey agar, in petri dishes, inoculated with Escherichia coli; Sabouraud agar, in petri dishes, inoculated with Candida albicans; Kligler iron agar (KIA), in reaction tubes, inoculated with Escherichia coli and Salmonella Typhi; Simmons citrate agar, in reaction tubes, inoculated with Klebsiella pneumoniae; Mueller-Hinton (M-H) broth, in reaction tubes, inoculated with Escherichia coli; and Motility Indole Urea (MIU) semisolid agar, in reaction tubes, inoculated with Proteus sp.

Results

Most media were still sterile after being kept in the incubator for 24 hours and then in the refrigerator for seven days (Table 1). Contaminations were just seen in 5 out of 54 dishes/tubes, four of which were seen on Mueller-Hinton Broth and one of which was seen on KIA. All contaminations were found after 24 hours incubation, no other contaminations seen during the 7 days period in the refrigerator.

In the first experiment , one tube of one-minute-heated Kligler iron agar was contaminated. In the second experiment , one tube of one-minute-heated Mueller-Hinton broth was contaminated. Meanwhile, in the third experiment, four tubes of Mueller-Hinton broth, one tube of one-minute-heated media, one tube of two-minute-heated media, and two tubes of three-minute-heated media were contaminated.

The two minute duration of microwave heating succeed to sterilize all the media more than half of the dishes/tubes. The three

Table 1 Result of Recapitulation after Sterilizing Growth Media Using Microwave Oven and Keeping in the Refrigerator for 7 Days

Type of Medium	One Minute Sterilization		Two Minute Sterilization		Three Minute Sterilization		Total	
	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated	Sterile	Contaminated
MacConkey	3	0	3	0	3	0	9	0
Sabouraud	3	0	3	0	3	0	9	0
KIA	2	1	3	0	3	0	8	1
Citrate Simmons	3	0	3	0	3	0	9	0
MH Broth	1	2	2	1	2	1	5	4
MIU	3	0	3	0	3	0	9	0
Total	15	3	17	1	17	1	49	5

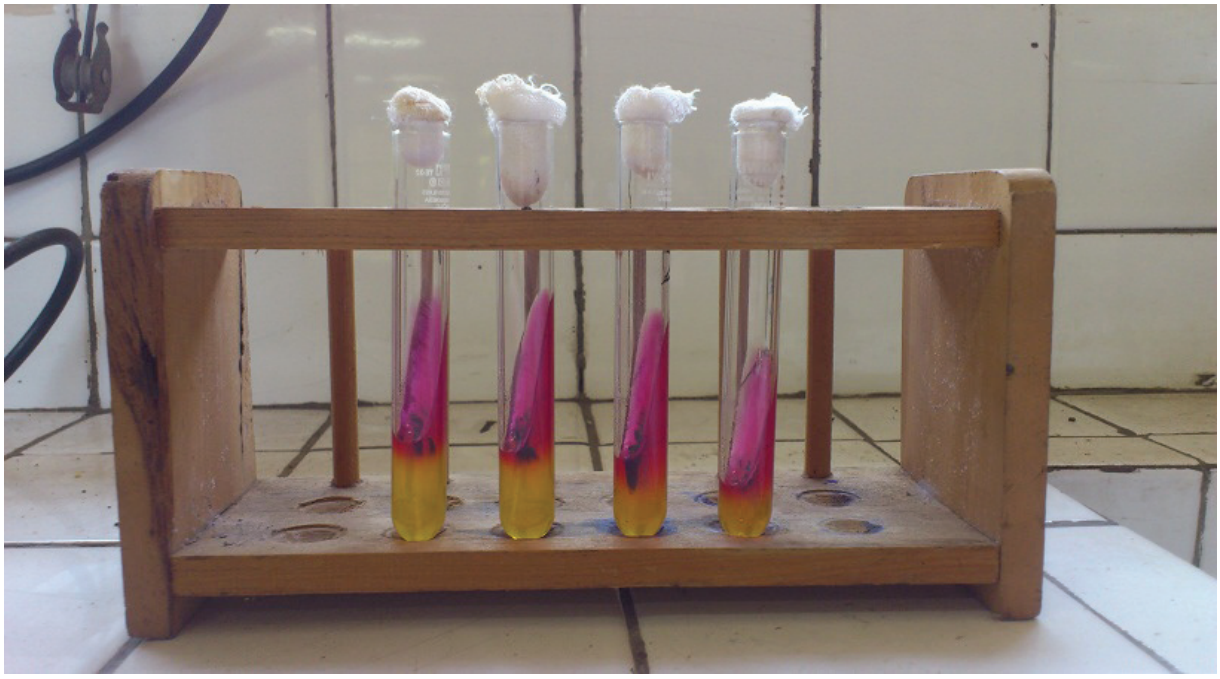

Figure 1 Inoculation Results of S. Typhi to Kligler Iron Agar (KIA)
From left to right: one minute microwave-heated medium; two minutes microwave-heated medium; three minutes microwave-heated medium; and autoclaved medium as control.

minute duration of microwave heating succeed to sterilize all the media more than half of the dishes/tubes as well. The one minute duration of microwave heating failed to sterilize more than half of the Mueller-Hinton broth tubes; however, it succeed to sterilize the other five media more than half of the dishes/tubes.

Since both tubes of the three-minute-heated Mueller-Hinton broth got contaminated after 24 hours of incubation, inoculation of E. coli for the three-minute-heated Mueller-Hinton broth could not be performed. One of the tubes was kept in the refrigerator for seven days.

Inoculation of microbes to the microwave-heated media generated similar changes with the autoclaved-media. Apart from that, there was another phenomenon seen in this study. The microwave-sterilized media tended to have brighter or more transparent color than the autoclaved media.

Discussion

Table 1 showed that microwave oven succeed to sterilize most of the media. From the total 54 dishes/tubes of various microwave-sterilized media, contaminations were only seen at 5 dishes/tubes. The results of this study is in accordance with the study conducted by Bhattacharjee, et al. 5 which stated that the microwave is able to sterilize the microbiological growth media quickly.
All contaminations in this study were found in tubes, no contamination was found on the petri dish. This result might be due to difficulties of burner's heat in reaching the whole depth of a tube during the aseptic technique. The heat also probably failed to sterilize the tube because it was absorbed by hand while holding the tube.

Inoculation performed to microwave-sterilized media generated similar changes to the autoclaved growth media. It indicated that the identification function of the media in this study was still performed well. E. coli fermented lactose that changed the color of MacConkey agar into red. C. albicans produced cream-like colonies on Sabouraud agar. KIA turned into yellow at both the butt and slant as the result of glucose and lactose fermentation, meanwhile S. Typhi fermented only glucose but not lactose, so that it changed the color of the butt only. S. Typhi also generated black color near the site of inoculation due to the production of H2S. Simmons citrate agar turned into blue due to the utilization of citrate by K. pneumoniae. E. coli colony generated turbidity

at the Mueller-Hinton broth. The motility of Proteus produced swarming around the site of inoculation, the utilization of urea decreased the pH and changed the color of media into pink.[6,7]

Even though the temperature inside the microwave oven can destruct the identification component of a medium, the identification function of the media in this study was still performed well. This was probably due to the ability of microwave oven to excite the molecules contained in the media.[8] The media immediately converts the microwave into heat energy that increases the temperature inside the microwave oven in such a short time, so that it is not long enough to destruct the identification component of growth media. Based on study conducted by Kothari, et al.[9] microwave-sterilized liquid growth media is more fertile than the autoclaved media. In that study, various media were heated by microwave oven for 10 minutes. Various bacteria and yeast inoculate to the microwave-sterilized media generated higher cellular density and growth velocity than the bacteria, and yeast inoculates to the autoclaved.[9]

There were differences in color between the microwave-sterilized media and the autoclaved media. Similar results were also stated by Geczi et al.[10] The study showed that liquid food treated with microwave have different color with untreated control and traditional-heated samples. They arecaused by the Maillard reaction which occured during autoclaving.[11] Maillard reactions are group of various complicated non-enzymatic reactions between free amino groups of protein, usually the ε-amino groups of protein, and carbonyl groups of reducing sugars.[12] This reaction results in the darkening of the autoclaved media's color due to the production of melanoidines, the final products of the reaction.[13]

This study has some limitations. The temperature in the microwave oven, as well as in the refrigerator, could not be measured precisely but could only be estimated around 218–260oC for microwave oven and 2–8°C for the refrigerator. The longest duration of microwave heating in this study is only three minutes. Even though there are still more sterile than contaminated Mueller-Hinton broth, the results of sterilizing the Mueller-Hinton broth generated some recommendations for further studies. There should be further studies in microwave-sterilizing the liquid media using more tubes and variations of heating duration.

From this study, it can be concluded that the utilization of microwave oven, particularly with two and three minute duration of microwave heating, as an alternative sterilizing apparatus for microbiological growth media is very potential. The most potential heating durations are two and three minutes since they are able to sterilize more than half dishes/tubes of all media in this study.

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