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Research Article

Effectiveness of the Class VI Internal Chemical Indicator Strip on Steam Sterilization of Sodium Chloride Infusion

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ABSTRACT

Steam sterilization which utilizes the autoclave is an effective and fast method of sterilization. The purpose of sterilization is to provide sterile products, material and medical equipment. All goods which have been sterilized must have a sterility assurance. Sterility testing can be done based on the usage combination of mechanical, chemical and biological indicators as sterilization parameters. The purpose of this research was to find out the effectiveness of the Class VI Internal Chemical indicator strip on steam sterilization of sodium chloride infusion. This research is a laboratory experiment with procedural stages of this research includes formulation of the NaCl 0.9 % infuses, the application of class VI Internal Chemical indicator strip in the steam sterilization process and evaluation of the NaCl 0.9 % infuse which includes sterility testing. The timings of sterilization used were 5, 7, 9, 10.5, 12, 13.5 and 15 min. The results show that class VI internal chemical indicator strip shows intended color change after 12 min of sterilization. The results of the sterility test on the sodium chloride 0.9 % infuse has no microbial growth starting from sterilization time 10.5 min onwards. The class VI internal chemical indicator strip is effective with 12 min of steam sterilization at 121 °C.

Keywords: Class VI Internal Chemical Indicator Strip, Steam Sterilization, Sodium chloride 0.9 % infusion

INTRODUCTION

Steam sterilization which utilizes the autoclave is an effective and fast method of sterilization. The purpose of sterilization is to ensure sterile products, material and medical equipment, and not only for producing sterile goods. All goods which have been sterilized must have a sterility assurance[1-3]. Sterility testing can be done based on the usage combination of mechanical, chemical and biological indicators as sterilization parameters[2]. Upon usage of the equipment, we initially have to validate and state that the equipment is utilizable. This can be done by the usage of chemical strip indicator[2,4]. There are two types of chemical indicators, which are the external chemical indicator and internal chemical indicator. The chemical indicator uses a sensitive chemical compound to assess physical conditions such as temperature throughout the sterilization process. The internal chemical indicator should be placed in each sterilization pack to ensure that the sterilization agent penetrates the packaging and truly reaches the instruments within the package. An external chemical indicator is used when the internal chemical indicators could not be observed from outside the package. These indicators change colour after being exposed to a certain temperature. Due to this, the chemical indicator can verify that temperature is achieved and the sterilization process is successful[4-6]. The advantages of the chemical indicator are that it can give immediate information whether an object has undergone sterilization and whether the parameters or conditions required for sterilization is achieved[3]. Besides that, the chemical indicator can indicate specific information on each packaging. The disadvantage of the chemical indicator is that it cannot assure a sterile condition, but can only indicate that if an object has undergone sterility conditions in a sterilization process cycle[4,5]. William A. Rutala, 1996, has conducted a research by comparing four types of biological indicator and five types of chemical indicators for steam sterilization at 121 °C to monitor the effectiveness of sterilization. The results show that after 48 h of incubation, the conventional biological indicators Attest 1262, Proof Plus, Assert and Biosign each showing 100 %, 95 %, 88 % and 93 % percentage of spores alive after 5 min of sterilization, 0 %, 0 %, 0 % and 8 % percentage of spores alive after 10 min of sterilization and all 0 % percentage of spores alive after 15 min of sterilization. After 3 h of incubation, the Attest 1292 Rapid Readout biological indicator shows 100 %, 72 % and 0 % of fluorosence after 5, 10 and 15 min of sterilization respectively. The chemical indicators Comply, Propper, Chemdi, Sterigage and Thermalog S respectively showed sterilization failure rate of 100 % each after 5 min of sterilization, 0 %, 0 %, 0 %, 92 % and 100 % after 10 min of sterilization and 0 %, 0 %, 0 %, 3 % and 27 % after 15 min of sterilization[7]. Based on the above matters, the effectiveness of the usage of the class VI internal chemical indicator strip in steam sterilization will be conducted to obtain a good and reliable sterilization process.

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MATERIALS AND METHODS

Preparation of apparatus and materials

The apparatus and materials that are to be used are sterilized first for sterility assurance and to not affect the results of the experiment. The apparatus used in this research is the autoclave, spirit lamp, Petri dish, incubator, test tube rack, test tubes, digital balance, and glassware that is usually used in the Sterile Product Technology and Formulation Laboratorium. The whole research procedure is to be conducted in the Laminar Air Flow (LAF) cabinet which has been sterilized with alcohol 70 % and exposed to UV rays for 2 h before starting any work and the floor of the LAF room is cleanes with phenol solution[8,9].

Sterilization of apparatus

The glassware that is to be used is washed thoroughly first, dried and wrapped in scrap paper and sterilized using an autoclave at 121 °C for 15 min. The sterilized glassware is then placed into an oven at 100 °C for 10 min[8].

Preparation of growth medium

Preperation of growth medium consist of[10,11]:

Trypticase Soy Agar (TSA)

10 g of TSA is weighed and then dissolved with 250 ml of aquadest, then heated till fully dissolved. The solution is sterilized in the autoclave at 121 °C for 15 min.

Trypticase Soy Broth (TSB)

7.5 g of TSB is weighed and then dissolved with 250 ml of aquadest, then heated till fully dissolved. The solution is sterilized in the autoclave at 121 °C for 15 min.

Fluid Thioglycolate Medium (FTM)

7.5 g of FTM is weighed and then dissolved with 250 ml of aquadest, then heated till fully dissolved. The solution is sterilized in the autoclave at 121 °C for 15 min.

Microbial contamination testing of Laminar Air Flow (LAF) room

The methods used for the microbial contamination testing of the LAF room are[10]:

Swabb method

A sterile transport swab is dipped into pyrogen free Aqua pro injectionum asecptically. It is then carefully swabbed in certain areas. The transport swab is then squeezed into a Petri dish filled with sterilized Trypticase Soy Agar (TSA). The Petri dish is then covered, labelled and incubated at 37 °C for 18-24 h.

Settling plate method

A sterile Petri dish containing Trypticase Soy Agar (TSA) is placed into the Laminar Air Flow (LAF) cabinet which has been sterilized with alcohol 70 %. The lid of the Petri dish is then removed and the Petri dish is left exposed for 15 min. After that, it is covered, labelled and incubated at 37 °C for 18-24 h.

Preparation of NaCl 0.9 % infuse

Formulation: In this study, the product which is going to be tested is NaCl 0.9 % infuse. The infuse is prepared in the laboratorium following the formula given in The Indonesian Pharmacopeia. According to the formula, each 100 ml consists of natrium chloride 0.9 %, active carbon 0.1 % and aqua pro injectionum till 100 ml.[11]

Preparation of NaCl 0.9 % infuse: Firstly, 0.945 g of NaCl is weighed and dissolved in a certain volume of aqua pro injectionum and the volume topped up till 105 ml. Then, 0.1 g of active carbon is added and he solution is heated till 60-70 °C for 15 min. The pH of the solution is constantly checked and monitored until a stable pH value is obtained which is 7.0. After that, the solution is filtered while still hot using a filter paper whereby the first filtrate is discarded and the second filtrate is collected. The collected filtrate is then transferred into a sterile infuse bottle till it reaches the stated volume in the Drug Formulation Manual which is 100 ml. The infuse bottle is shut tightly after filling up is done[12].

Fertility testing of growth medium

FTM growth medium is inoculated with *Bacillus subtilis* bacteria while TSB growth medium is inoculated with *Candida albicans* fungus. Both mediums are allowed to incubate for seven days at certain temperatures. FTM is incubated between 30-35 °C while TSB is incubated at 20-25 °C[10,11,13].

Application of class VI internal chemical indicator in steam sterilization

The methods in the application of the chemical indicator in steam sterilization are[4,14,15,16,17]:

The first step is to ensure that the autoclave to be used is in proper working condition throughout the study. The autoclave is conditioned to a 15 min cycle at each trial. A comparative study is conducted, based on the determined timing variation which are 5; 7; 9; 10.5; 12; 13.5 and 15 min (based on D-value calculations). After that, the chemical indicator strip is stuck to the NaCl 0.9 % infuse and packaged properly, and the sterilization process is carried out at 121°C with the determined variation times which are 5; 7; 9; 10.5; 12; 13.5 and 15 min. After the sterilization process with the varied times is completed, the colour change of the chemical indicator strip is observed.

Sterility testing of NaCl 0.9 % infuse

Test tubes, FTM and TSB growth mediums are placed inside the LAF cabinet aseptically. Then, 15 ml of FTM and TSB growth mediums are separately filled intothe test tubes accordingly. 1 ml of sample is then filled into each test tube and then the mouth of the test tubes is covered aseptically. Samples in TSB growth medium are incubated at temperatures within 20-25 °C whereas samples in FTM growth medium are incubated at temperatures within 30-35 °C for no longer than 14 d. Both positive and negative controls are done for both growth mediums. Inoculations of *Candida albicans* in TSB and *Bacillus subtilis* in FTM are done as postice controls. For negative control, sterile growth media of FTM and TSB are poured into separate test tubes[2,11].

RESULTS AND DISCUSSION

Results of microbial contamination test of Laminar Air Flow (LAF) space

The microbial contamination test is usually conducted using two common methods, which are the Swabb method and Settling Plate method. The aim of the microbial contamination test is to ensure tha the LAF is free from all forms of life that could cause contamination. The results of this test shows that the LAF space fulfils the sterility conditions where there is no bacterial growth in the Petri dishes containing growth medium, as shown in Table 1

Table 1: Results of Microbial Contamination Test of LAF Space

Method	Petri Dish	Result
Swabb Method	1	-
	2	-
Settling Plate Method	1	-
	2	-
	Control	-

Description: (+) = Microbial growth (-) = No microbial growth

Table 2: Result of Fertility Test of FTM and TSB Growth Medium

Culture	Microbe	Observation day						
		1	2	3	4	5	6	7
FTM	Bacillus subtilis	+	+	+	+	+	+	+
TSB	Candida albicans	+	+	+	+	+	+	+

Description: (+) = Microbial growth (-) = No microbial growth

below. The LAF space is an area for production of sterile preparations and is categorized as Class 1 or white area and has to meet the total number of microbes allowed requirement. According to the GMP guidelines, the growth limit of microbes in Class 1 area is less than 1cfu and it turns out that based on the results obtained in Table 1 shows that the growth of bacteria was 0 cfu[8,9]. In order to obtain a sterile room/ space and meet the number of microbes and particles requirement, the inner and outer parts of the LAF cabinet has to be disinfected first with alcohol 70 % and phenol to clean the floors, walls and ceiling of the room. The mechanism of action of alcohol is by penetrating the bacterial cell wall with the help of water and cause denaturation of the cell wall protein, causing cell lysis. Alcohol 70 % is used for the disinfection of LAF cabinets because it is no corrosive towards metal and evaporates easily thus minimizing the time of contact with the surface of the LAF cabinet. Phenol works by coagulating proteins which leads to the leakage of the bacterial cell membrane. Phenol is commonly used for the disinfection of floors, walls and table tops and so on. Phenol is not suitable for cleaning the inner surface of the LAF cabinet due to its longer contact time with the surface compared to that of alcohol and its more corrosive property[5,10,13].

Results of growth medium fertility test

The fertility test of growth medium is done to ensure that the growth medium is in good condition so that bacteria can grow. The media used in the sterility test is Thioglycolate Fluid Medium (FTM) and Tryptone Soy Broth (TSB). The medium fertility test is conducted by inoculating *Bacillus subtilis* bacteria into FTM and *Candida albicans* fungus into TSB. Then, FTM is incubated within 30-35 °C and TSB is incubated within 20-25 °C. The results can be seen in Table 2.

The data in Table 2 shows that there is growth of *Bacillus subtilis* in FTM medium and *Candida albicans* in TSB medium. This proves and guarantees that both the growth mediums are able to grow microbes and can be used in the sample sterility test[11].

Table 3: Colour Change of Class VI Internal Chemical Indicator Strip with Time Variations at 121 °C

Sterilization 121 °C	Indicator Colour Change			Sterility
	Time	Temperature	Steam	
5 min	-	+	-	Not Sterile
7 min	-	+	-	Not Sterile
9 min	-	+	-	Not Sterile
10.5 min	-	+	-	Not Sterile
12 min	+	+	+	Sterile
13.5 min	+	+	+	Sterile
15 min	+	+	+	Sterile

Description: (+) = Colour change (-) = No colour change

Results of application of class VI internal chemical indicator strip in steam sterilization

The results of sterility test on the class VI internal chemical indicator strip can be observed by the colour change on the indicator after the steam sterilization process at 121 °C with variation timings of 5; 7; 9; 10.5; 12; 13.5 and 15 min. The results of the class VI internal chemical indicator strip are shown in Table 3. Based on the results obtained, it can be said that from the 5 min to the 10.5 min, there is colour change and only temperature is the variable achieved whereas time and temperature are the variables which are not achieved. From this, it is assumed that that the infuse preparations sterilized at 5; 7; 9 and 10.5 min have not reached a sterile state. From the 12 min to the 15 min, all three variables which are time, temperature and steam are achieved. From this, it is assumed that the infuse preparations sterilized at 12; 13.5 and 15 min are in a sterile state. The class VI internal chemical indicator strip is a chemical indicator designed to react to all critical variables in steam sterilization cycle which are temperature, time and steam. In theory, this indicator can indicate a complete cycle with the presence/absence of a specific time and temperature parameters throughout sterilization. There are two colour changes that can occur in the class VI internal chemical indicator strip which are brownish red and black, as it appears on the surface of the indicator. The colour change from red to brownish red indicates that temperature is the only variable that is achieved whereas time of exposure and steam is still lacking. This shows that the sterilization requirement is not met. The colour change from red to black indicates that the three parameters of steam sterilization which are temperature, time and steam are achieved and this shows that the sterility requirement is met[5,16,17]. Variation of sterilization timing for class VI internal chemical indicator strip was determined using F0 method with calculation of D- value[4].

Results of sterility test of Nacl 0.9 % infuse

Sterility test was carried out on all the Nacl 0.9 % infuse preparation sterilized using steam sterilization at 121 °C with time variations of 5; 7; 9; 10,5; 12; 13,5 and 15 min. Sterility testing of Nacl 0.9 % infuse is to prove the results

Table 4: Formulation NaCl 0.9 % infuse

Ingredients	Amount
NaCl	0.9 gram
Activated carbon	0.1 gram
Aquabidestilata	100 mL

Table 5: Sterility Test Results of 5 min in TSB Media

Observation Day	Control	NaCl 0.9 % Infuse Prepartion		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	+	-	+
4	-	+	+	+
5	-	+	+	+
6	-	+	+	+
7	-	+	+	+
8	-	+	+	+
9	-	+	+	+
10	-	+	+	+
11	-	+	+	+
12	-	+	+	+
13	-	+	+	+
14	-	+	+	+

Description: (+) = Microbial growth (-) = No microbial growth

Table 6: Sterility Test Results of 7 min in TSB Media

Observation Day	Control	NaCl 0.9 % Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	+	+
7	-	+	+	+
8	-	+	+	+
9	-	+	+	+
10	-	+	+	+
11	-	+	+	+
12	-	+	+	+
13	-	+	+	+
14	-	+	+	+

Description: (+) = Microbial growth (-) = No microbial growth

of the effectiveness of the indicator. The results of sterility test of Nacl 0.9 % infuse at 5 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 5. Based on the results obtained from Table 5, there is no microbial growth on the 1 and 2 d. The growth can only be seen starting from the 3 d onwards. This result indicates that 5 min of sterilization of the Nacl 0.9 % infuse is not effective. The results of the sterility test of Nacl 0.9 % infuse at 7 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 6. Based on the results obtained from Table 6, there is no microbial growth from the 1 till the 5 d. The growth

Table 7: Sterility Test Results of 9 min in TSB Media

Observation Day	Control	NaCl 0.9 % Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	+	-	-
14	-	+	-	+

Description: (+) = Microbial growth (-) = No microbial growth

Table 8: Sterility Test Results of 10,5; 12; 13,5 and 15 min in TSB Media

Observation Day	Control	NaCl 0.9 % Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-

Description: (+) = Microbial growth (-) = No microbial growth

can only be seen on 6 d onwards. These results show that sterilization time of 7 min is not effective as well for Nacl 0.9 % infuse. The results of sterility test of Nacl 0.9 % infuse at 9 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 7. Based on the results obtained from Table 7, there is no microbial growth from the 1 till the 12 d. The growth can only be seen starting from the 13 and 14 d. These results show that 9 min of sterilization is still not effective for the sterilization Nacl 0.9 % infuse. The results of sterility test of Nacl 0.9 % infuse at 10,5; 12; 13,5 and 15 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 8. Based on the results obtained from Table 8, there is no microbial growth from the 1 till the 14 d. These results shows that 10,5; 12; 13,5 and 15 min are effective for sterilization of Nacl 0.9 % infuse.

Table 9: Sterility Test Results of 5 min in FTM Media

Observation Day	Control	NaCl 0.9 %Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	+	-
6	-	+	+	+
7	-	+	+	+
8	-	+	+	+
9	-	+	+	+
10	-	+	+	+
11	-	+	+	+
12	-	+	+	+
13	-	+	+	+
14	-	+	+	+

Description: (+) = Microbial growth (-) = No microbial growth

Table 10: Sterility Test Results of 7 min in FTM Media

Observation Day	Control	NaCl 0.9 % Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	+	+
8	-	+	+	+
9	-	+	+	+
10	-	+	+	+
11	-	+	+	+
12	-	+	+	+
13	-	+	+	+
14	-	+	+	+

Description: (+) = Microbial growth (-) = No microbial growth

Next, the experiment is carried on by sterility test of Nacl 0.9 % infuse preparation in FTM media. The sterilization times used for the infuse are 5; 7; 9; 10.5; 12; 13.5 and 15 min. The results of sterility test of the Nacl 0.9 % infuses at 5 min in FTM media incubated in 30-35 °C for 14 d can be seen in Table 9. Based on the results obtained from Table 9, there is no microbial growth from the 1 till the 4 d. The growth can only be seen starting from 5 till 14 d. This result shows that 5 min of sterilization is not effective for sterilization of Nacl 0.9 % infuse. The results of sterilization test of Nacl 0.9 % infuse at 7 min in FTM media incubated in 30-35 °C for 14 d can be seen in Table 10. Based on the results obtained from Table 10, there is no microbial growth from the 1 till the 6 d. The growth can only be seen starting from the 7 d onwards. This result shows that 7 min of sterilization is not effective for sterilization of Nacl 0.9 % infuse. The results of sterility

Table 11: Sterility Test Results of 9; 10,5; 12; 13,5 and 15 min in FTM Media

Observation Day	Control	NaCl 0.9 % Infuse Preparation		
		1	2	3
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-

Description: (+) = Microbial growth (-) = No microbial growth

test of Nacl 0.9 % infuse at 9; 10,5; 12; 13,5 and 15 min in FTM media incubated at 30-35 °C for 14 d can be seen in Table 11. Based on the results obtained from Table 11, there is no microbial growth from the 1 till the 14 d. This result shows that sterilization of Nacl 0.9 % infuse at 9; 10,5; 12; 13,5 and 15 min is effective. The effectiveness of class VI internal chemical indicator strip is done by referring to the results of sterility test of the Nacl 0.9 % infuse. Based on the observation using the indicator with sterility test of Nacl 0.9 % infuse at 5; 7; 9; 10,5; 12; 13.5 and 15 min at 121 °C in TSB and FTM media, it can be concluded that results shown by the class VI internal chemical indicator strip is accurate indicator. The data shows that the application of class VI internal chemical indicator strip showed intended colour changes from red to black from the 12 min onwards. Meanwhile the results of the sterility test on the Nacl 0.9 % infuse preparation showed that a sterile preparation was obtained after sterilization at 10.5 to 15 min. Based on this, it can be concluded that sterility level shown by class VI internal chemical indicator strip is effective. Theoretically, the usage of the biological indicator is an effective method of monitoring sterilization compared to the chemical indicator. In fact, sterilization monitoring method was accepted as the most effective method. The usage of chemical indicators is more as a marker for the exposure of sterilization on the sterilized object[12,18].

CONCLUSION

The usage of class VI internal chemical indicator strip on steam sterilization is effective in 12 min meanwhile the usage of rapid readout biological indicator is effective in 7 min with temperature of 121 °C.

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