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The Effectiveness Comparison of Class VI Internal Chemical Indicator Strip with Rapid Readout Biological Indicator in Wet Heat Sterilization

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Abstract

Objective:
The objective of this study was to find out the effectiveness of the class VI internal chemical indicator strip in comparison with the effectiveness of the 3M attest 1292 rapid readout biological indicators.

Methods:
The laboratory experiment was done by proceeding stages of the research including formulation of the sodium chloride 0.9 % infusion, an application of both class VI internal chemical indicator strip and rapid readout biological indicator in the wet heat sterilization process and evaluation of the sodium chloride 0.9 % infusion which included sterility testing. The timing of sterilization used was 5, 7, 9, 10.5, 12, 13.5 and 15 min. The data were analyzed by means of descriptive statistics.

Results:
The class VI internal chemical indicator strip indicated color changes after 12 min of sterilization and the rapid readout biological indicator showed no color changes after 7 min of sterilization. The sterility test on the sodium chloride 0.9 % infusion had no microbial growth starting from sterilization time 10.5 min onwards.

Conclusion:
The class VI internal chemical indicator strip was more accurate as compared to the rapid readout biological indicator. The sterility level shown by the class VI internal chemical indicator strip was more effective compared to sterility level of rapid readout biological indicator.

Keywords: class VI internal chemical indicator strip, rapid readout biological indicator, wet heat sterilization, sodium chloride 0.9 % infusion

INTRODUCTION

Wet heat sterilization which utilizes the autoclave is an effective and fast method of sterilization. The purpose is to ensure sterilized products, material, and medical equipment, and not only for producing sterilized goods. All goods which have been sterilized must have a sterility assurance.[1-3]

Sterility testing can be done based on the usual combination of mechanical, chemical and biological indicators as sterilization parameters.[2] Upon use of the equipment, we initially have to validate and state that the equipment is utilizable. This can be achieved using the rapid readout biological indicator and 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 packaging to ensure that the sterilization agent penetrates and truly reaches the instruments. An external chemical indicator is used when the internal chemical indicators could not be observed from outside the package. These indicators change color after being exposed to a certain temperature. Resulting from 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.[4] 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 process cycle.[4,5]

The biological indicator is a component in a through quality assurance program for sterilization in hospitals and health facilities as well as aseptic equipment installation qualification, process cycle development, quality assurance of sterilization programs and decalcification of sterilization equipment.[7] Until this moment, this indicator is the basic reference to determine if a sterilized condition is achieved. Taking into account the fact that the purpose of sterilization is to kill microorganisms. Therefore, there is no other clearer way of demonstrating the killing of microorganisms than the biological indicator.[6]

Rutala *et al.* have conducted research by comparing four types of biological indicator and five types of chemical indicators for wet heat 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 living after 5 min of sterilization, 0 %, 0 %, 0 % and 8 % percentage of spores living after 10 min of sterilization and all 0 %

percentage of spores living after 15 min of sterilization. After 3 h of incubation, the attest 1292 rapid readout biological indicator shows 100%, 72% and 0% of fluorescence after 5, 10 and 15 min of sterilization severally. The chemical indicators comply, propper, chemdi, sterigage and thermalog s severally showed the 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.[8]

Based on the above facts, a comparison of the effectiveness of the use of the class VI internal chemical indicator strip and the rapid readout biological indicator in wet heat sterilization was carried out to obtain a good and reliable sterilization process. So we can determine which indicators are the most effective and efficient to use in giving assurance of sterility.

MATERIALS AND METHODS

Chemicals and reagents

Alcohol 70% (Brataco®), phenol (Brataco®), aquadest (Brataco®), aqua pro injection (Ikhapharmindo®), trypticase soy agar (E-Merck®), trypticase soy broth (E-Merck®), fluid thioglycollate medium (E-Merck®), sodium chloride (E-Merck®), active carbon (Bratachem®), *bacillus subtilis* bacteria (micro lab), *candida albicans* fungus (micro lab).

Instruments/equipment/apparatus

Autoclave (All American™), spirit lamp (Pyrex®), Petri dish (Pyrex®), incubator (Memmert™), test tube rack (standard), test tubes (Pyrex®), digital balance (Ohaus™), gke steri-record® class VI internal chemical indicator strip, 3M attest 1292™ rapid readout biological indicator, laminar air flow cabinet (Esco™).

Preparation of apparatus and materials

The apparatus and materials were sterilized first for sterility assurance and not to affect the results of the experiment. The apparatus used in this research were the autoclave, spirit lamp, Petri dish, incubator, test tube rack, test tubes, digital balance, and glassware. The whole research procedure conducted in the laminar air flow 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 laminar air flow room was cleaned with phenol solution.[9,10]

Sterilization of apparatus

Glassware was washed thoroughly first, dried and wrapped in scrap paper and sterilized using an autoclave at 121 °C for 15 min. The sterilized glassware was then placed into an oven at 100 °C for 10 min.[9,11]

Preparation of the growth mediums

Preparation of the growth mediums composes of: [11-13]

1. Trypticase soy agar
   Trypticase soy agar 10 g was dissolved in 250 ml of aquadest, then heated until fully dissolved. The solution was sterilized in the autoclave at 121 °C for 15 min.
2. Trypticase soy broth
   Trypticase soy broth 7.5 g was dissolved in 250 ml of aquadest, then heated until fully dissolved. The solution was sterilized in the autoclave at 121 °C for 15 min.
3. Fluid thioglycollate medium
   Fluid thioglycollate medium 7.5 g was dissolved in 250 ml of aquadest, then heated until fully dissolved. The solution was sterilized in the autoclave at 121 °C for 15 min.

Microbial contamination testing of laminar air flow room

The microbial contamination testing of the laminar air flow room was carried out as follows:[12]

1. Swab method
   A sterile transport swab was dipped into pyrogen-free aqua pro injection aseptically. It was then carefully swabbed in certain areas. The transport swab was squeezed into a petri dish filled with sterilized trypticase soy agar. The petri dish was covered, labeled and incubated at 37 °C for 18-24 h.
2. Settling plate method
   A sterile petri dish containing trypticase soy agar was placed into the laminar air flow cabinet which has been sterilized with alcohol 70 %. The lid of the petri dish was removed, and the petri dish was left exposed for 15 min. After that, it was covered, labeled and incubated at 37 °C for 18-24 h.

Preparation of sodium chloride 0.9 % infusion

A. Formulation
   In this study, the product tested was sodium chloride 0.9 % infusion. It was prepared in the laboratories following the formula given in the Indonesian pharmacopeia. According to the formula, each 100 ml consisted of sodium chloride 0.9 %, active carbon 0.1 % and aqua pro injection until 100 ml.[13]
B. Preparation of sodium chloride 0.9 % infusion
   Sodium chloride 0.945 g was dissolved in a certain volume of aqua pro injection, and the volume topped up until 105 ml. Then, 0.1 g of active carbon was added and the solution was heated to 60-70 °C for 15 min. The pH of the solution was checked and controlled until a stable pH value of 7.0 was obtained. After that, the hot solution was filtered using a filter paper. The first filtrate was discarded, and the second filtrate was collected. The collected filtrate was then transferred into a sterile infuse bottle until it reached the state volume of 100 ml. After the filling up completely done, the infuse bottle was shut tightly.[14,15]

Fertility testing of growth medium

Fluid thioglycollate medium was inoculated with *bacillus subtilis* at 30-35 °C, while trypticase soy broth was inoculated with candida albicans at 20-25 °C. Both media were allowed to incubate for seven days at certain temperatures.[12,13,16,17]

Application of class VI internal chemical indicator in wet heat sterilization

The methods used in application of the chemical indicator in wet heat sterilization were:[4,18-21]

1. The first step was to ensure that the autoclave used was in proper working conditions throughout the study.

The autoclave was conditioned to a 15 min cycle at each trial.

2. A comparative study was conducted based on the time variations of 5, 7, 9, 10.5, 12, 13.5, and 15 min (based on d-value calculations).
3. After that, the chemical indicator strip was stuck to the sodium chloride 0.9 % infusion and packaged properly, and the sterilization process was carried out at 121 °C.
4. After the sterilization process was completed, the color change of the chemical indicator strip was observed.

Application of a rapid readout biological indicator in wet heat sterilization[22-24]

1. The first step was to ensure that the autoclave used was in proper working condition throughout the study. The autoclave was conditioned to a 15 min cycle at each trial.
2. A comparative study was conducted, based on the time variations of 5, 7, 9, 10.5, 12, 13.5, and 15 min (based on d-value calculations).
3. After that, the rapid readout biological indicator placed together with the sodium chloride 0.9 % infusion and packaged properly, and the sterilization process was carried out at 121 °C.
4. After the sterilization process was completed, the rapid readout biological indicator was placed into the incubator for 24 h and the color change was observed.

Sterility testing of sodium chloride 0.9 % infusion

Test tubes, fluid thioglycollate medium, and trypticase soy broth growth media were placed in the laminar air flow cabinet aseptically. After that, 15 ml of fluid thioglycollate medium and trypticase soy broth growth media were separately filled into the test tubes accordingly. One ml of the sample was then filled into each test tube and the mouth of the test tubes was covered aseptically. Samples in trypticase soy broth growth medium were incubated at temperatures within 20-25 °C whereas samples in fluid thioglycollate medium at temperatures within 30-35 °C for no longer than 14 d. Positive and negative controls were used for both growth media. Inoculations of *candida albicans* in trypticase soy broth and *bacillus subtilis* in fluid thioglycollate mediums were done as positive controls. For negative control, sterile growth media of fluid thioglycollate medium and trypticase soy broth were poured into separated test tubes.[2,13]

RESULTS AND DISCUSSION

Microbial contamination test of laminar air flow space

The microbial contamination test was usually conducted using two common methods, which were the swab method and settling plate method. The aim of the microbial contamination test was to ensure that the laminar air flow was free from all forms of living organism that could cause contamination. The results of this test showed that the laminar air flow space fulfilled the sterility condition (Table 1).

Table 1: Microbial contamination test of laminar air flow space

Method	Petri dish	Result
Swab method	1	-
	2	-
Settling plate method	1	-
	2	-
	control (-)	-

Description: (+) = microbial growth; (-) = no microbial growth

The laminar air flow space was an area for production of sterile preparations and categorized as class 1 or white area and has to meet the total number of microbes allowed requirement. According to the good manufacturing practice guidelines, the growth limit of microbes in class 1 area is less than 1 cfu and it turns out that based on the results obtained in table 1 shows that the growth of bacteria was 0 cfu.[9,10] In order to obtain a sterile room/space and meet the number of microbes and particle's requirement, the inner and outer parts of the laminar air flow cabinet had disinfected first with alcohol 70 % and phenol to clean the floors, walls, and ceiling of the room. The mechanism of action of alcohol was by penetrating the bacterial cell wall with the help of water and cause denaturation of the cell wall protein, causing cell lysis. Alcohol 70 % was used for the disinfection of laminar air flow cabinets because it did not corrosive towards metal and evaporates easily thus minimizing the time of contact with the surface of the laminar air flow cabinet. Phenol works by coagulating proteins, which led to the leakage of the bacterial cell membrane. Phenol is commonly used for the disinfection of floors, walls and tabletops and so on. Phenol is unsuitable for cleaning the inner surface of the laminar air flow cabinet due to its longer contact time with the surface compared to that of alcohol and its more corrosive property.[5,12,16]

Growth medium fertility test

The fertility test of the growth mediums was done to ensure that the growth medium was in good condition so that bacteria can grow. The media used in the sterility test were thioglycolate fluid medium (FTM) and tryptone soy broth (TSB). The medium fertility test was performed by inoculating b*acillus subtilis* bacteria into FTM and c*andida albicans* fungus into TSB. Then, FTM and TSB were incubated within 30-35 °C and 20-25 °C, respectively. The results were indicated in Table 2.

Table 2: Fertility test of FTM and TSB growth media

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

Description: (+) = microbial growth; (-) = no microbial growth

The data in Table 2 showed that there was the growth of *bacillus subtilis* in FTM and *candida albicans* in TSB media. These mean that both of the growth media were good for microbial growth and could be used for sterility test.[13]

Application of class VI internal chemical indicator strip in wet heat sterilization

The results of sterility test on the class VI internal chemical indicator strip were observed according to the color change on the indicator after the wet heat 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 strips are shown in Table 3.

Table 3: Color change of class VI internal chemical indicator strip with time variations at 121 °C

Sterilization 121 °C	Indicator Color Change			Sterility
	Time	Temperature	Wet heat	
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 : (+) = color change; (-) = no color change

Table 3 shows that at 5 to 10.5 min sterilization, color change occurred only in the temperature indicator. This means that sterilization time of 5 to 10.5 min did not reach a sterile state. The sterile condition could be achieved at 12 to 15 min sterilization.

The class VI internal chemical indicator strip was a chemical indicator designed to react to all critical variables in a wet heat sterilization cycle which are temperature, time and wet heat. In theory, this indicator can indicate a complete cycle in the presence/absence of a specific time and temperature parameters throughout sterilization. There are two-color changes that can occur in class VI internal chemical indicator strip, which are brownish, red and black, as they appear on the surface of the indicator. The color change from red to brownish red indicates that temperature is the only variable achieved, whereas time of exposure and wet heat are still lacking. It shows that the sterilization requirement does not meet. If the color change from red to black occurs in the three parameters of wet heat sterilization, it shows that the sterililization meets the requirements.[5,20,21]

Application of rapid readout biological indicator in wet heat sterilization

The results of sterilization test on rapid readout biological indicator were shown through the color changes of the fluorescence media, a mixture of bacterial spore *Geobacillus stearothermophilus* in the rapid readout indicator which was incubated for 24 h. *G. stearothermophilus* bacterial spore was used because it withstood heat in wet heat sterilization using an autoclave. It can be assumed that the bacterium were killed through a sterilization process. The rapid readout biological indicator was sterilized using wet heat sterilization at 121 °C with variations time of 5, 7, 9, 10,5, 12, 13.5, and 15 min. The results can be seen in Table 4.

Table 4: Color changes of rapid readout biological indicator with time variation at 121 °C

Sterilization 121 °C	Indicator color changes
5 min	+
7 min	-
9 min	-
10.5 min	-
12 min	-
13.5 min	-
15 min	-

Description: (+) = microbial growth; (-) = no microbial growth

The results above indicated that a color change of the media in the ampoule from purple to yellow was observed in the 5 min sterilization. It was assumed that the infuse preparation sterilized in the 5 min did not achieve a sterile state. However, in the sterilization time of 7 to 15 min, no color change was observed in the media, where the color remained purple. This indicated that the infuse preparations sterilized at 7, 9, 10.5, 12, 13.5 and 15 min achieved a sterile state. Theoretically, the color changes in the ampoule of the rapid readout biological indicator are caused by the activity of alpha-glucosidase enzyme and changes in media pH. Alpha-glucosidase enzyme reacts by hydrolysis of 4-methylumbellipheril-alfa-D-glucopyranosidase (alfa-MUG), which is a florigenic substrate present in the indicator's growth media if it is exposed to an effective temperature and sterilization time. The hydrolysis reaction produces fluorescence 4-metilumbeliferon (4-MU) and alfa-D-glucose.[22-24]

Variation of sterilization timing for both class VI internal chemical indicator strip and rapid readout biological indicators was determined using F_0 method for calculation of d-value.[4]

Sterility test of sodium chloride 0.9 % infusion

Sterility test was carried out on all sodium chloride 0.9 % infuse preparations sterilized using wet heat sterilization at 121 °C with time variations of 5, 7, 9, 10.5, 12, 13.5, and 15 min. Sterility testing of sodium chloride 0.9 % infusion was proved the results of usage of both the indicators and to compare the effectiveness of both indicators. The results can be seen in Table 5.

Table 5: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 5 min time sterilization in TSB media

Observation day	Control	Sodium chloride 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 5 shows that at 3 d onwards, microbial growth occurred. This means that the 5 min of sterilization of sodium chloride 0.9 % infuse was not effective.

The results of the sterility test of sodium chloride 0.9 % infuse at 7 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 6.

Table 6: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 7 min time sterilization in TSB media

Observation day	Control	Sodium chloride 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 6 shows that at 6 d onwards, microbial growth occurred. This means that the 7 min of sterilization of sodium chloride 0.9 % infuse was not effective.

The results of sterility test of sodium chloride 0.9 % infusion at 9 min in TSB media incubated at 20-25 °C for 14 d can be seen in Table 7.

Table 7: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 9 min time sterilization in TSB media

Observation day	Control	Sodium chloride 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 7 shows that at 13 d onwards, microbial growth occurred. This means that the 9 min of sterilization of sodium chloride 0.9 % infuse still was not effective.

The results of sterility test of sodium chloride 0.9 % infusion 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.

Table 8: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 10.5, 12, 13.5, and 15 min time sterilization in TSB media

Observation day	Control	Sodium chloride 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 shows that no microbial growth occurred. This means that the 10.5 min onwards of sterilization of sodium chloride 0.9 % infuse were effective.

Next, the experiment carried on by sterility test of sodium chloride 0.9 % infuse preparation in FTM media. The sterilization times used of the infuse were 5, 7, 9, 10.5, 12, 13.5, and 15 min. The results of sterility test of the sodium chloride 0.9 % infusion at 5 min in FTM media incubated at 30-35 °C for 14 d can be seen in Table 9.

Table 9: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 5 min time sterilization in FTM media

Observation day	Control	Sodium chloride 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

Based on the results obtained from Table 9, there was no microbial growth from the 1 until the 4 d (for preparation 2) and 5 d (for preparation 1 and 3). The growth can only be seen starting from 5 (for preparation 2) and 6 (for preparation 1 and 3) until 14 d. It showed that 5 min of sterilization was not effective for sterilization of sodium chloride 0.9 % infusion.

The results of sterilization test of sodium chloride 0.9 % infusion at 7 min in FTM media incubated at 30-35 °C for 14 d can be seen in Table 10.

Table 10: Sterility test of sodium chloride 0.9 % infuse reparations which sterilized at 7 min time sterilization in FTM media

Observation day	Control	Sodium chloride 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

Based on the results obtained from Table 10, there was no microbial growth from the 1 until the 6 d. The growth can only be seen starting from the 7 d onwards. It showed that 7 min of sterilization was not effective for sterilization of sodium chloride 0.9 % infusion.

The results of sterility test of sodium chloride 0.9 % infusion 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.

Table 11: Sterility test of sodium chloride 0.9 % infuse preparations which sterilized at 9, 10.5, 12, 13.5, and 15 min time sterilization in FTM media

Observation day	Control	Sodium chloride 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

Based on the results obtained from Table 11, there was no microbial growth from the 1 until the 14 d. They showed that sterilization of sodium chloride 0.9 % infusion at 9, 10.5, 12, 13.5, and 15 min was effective.

The comparison of the effectiveness of class VI internal chemical indicator strip and rapid readout biological indicator was done by referring to the results of sterility testing of sodium chloride 0.9 % infusion. Based on the observation using both the indicators with sterility test of sodium chloride 0.9 % infusion 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 showed by the class VI internal chemical indicator strip was more accurate compared to that of the rapid readout biological indicator. The data showed that the application of class VI internal chemical indicator strip showed intended color changes from red to black from the 12 min onwards. Meanwhile, the results of the sterility test on sodium chloride 0.9 % infuse preparation showed that a sterile preparation was obtained after sterilization at 10.5 to 15 min. The results of the rapid readout biological indicator showed no color changes from purple to yellow on the 7 min, which theoretically proves that sodium chloride 0.9 % infuse preparation is sterile. Based on this, it can be concluded that sterility level shown by class VI internal chemical indicator strip was more effective compared to sterility level of rapid readout biological indicator.

Theoretically, the use 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 use of chemical indicators is more as a marker for exposure of sterilization on the sterilized object.[14,24]

A few reasons may cause the sterility level of the class VI internal chemical indicator strip to be more effective compared to that of the rapid readout biological indicator. One of the reasons was due to the incubator used in this study. The incubator used was a common one which may not suit the incubation of the rapid readout biological indicator. Besides that, incubation timing of the rapid readout biological indicator was 24 h while incubation timing of sodium chloride infuses sample, which was inserted in TSB and FTM media was 14 d. Rapid readout biological indicator could not be incubated for a longer time because the media in an ampoule will dry up causing difficulties in the observation. It was because the biological indicator was designed specifically to give results within 3 h of incubation using a specific instrument which was the rapid auto-reader 290 wet heat. Based on this, it was assumed that the rapid readout biological indicator was more suitable for monitoring instrument sterilization while the class VI internal chemical indicator strip was suitable for monitoring sterilization of both instrument and preparations.

CONCLUSION

The class VI internal chemical indicator strip was more accurate compared to that of the rapid readout biological indicator. The sterility level shown by class VI internal chemical indicator strip was more effective compared to sterility level of rapid readout biological indicator.

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