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Biological indicator design and efficacy evaluation parameters

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Abstract

In its most general definition, biological indicators are systems used in many different areas to control the effectiveness of the sterilization processes. Indicator of achieving the target of the sterilization process; it is to determine the performance of this process with specific indicators after the material and/or material exposed to the sterilization process are completely cleaned off the live microorganisms. With its most common usage, biological indicators are designed using bacterial spores that have been determined to be the most resistant to the sterilization method and are used when evaluating the effectiveness of the sterilization processes. It is expected that the SAL (Sterility Assurance Level) value should be 10^6 after the complete sterilization process. The design of the Biological Indicators is achieved by the absorbing bacterial spores on filter paper and placing them in a plastic tube. It consists of a glass ampoule with a color indicator to observe the growth of bacteria in the tube and a specially formulated culture medium for the growth of the relevant bacteria. In traditionally used biological indicators, the result can be reached in long periods such as 24 hours (steam), and 48 hours (ethylene oxide and moist heat), depending on the type of the indicator used. However, based on the recent studies, biological indicators that give in a very short time have been developed to overcome the time constraints. In this manuscript we are presenting a review on the biological indicators and novel approaches in this field.

1. Introduction

Sterilization, with its most well-known definition, terminates the activities of the microorganisms in the environment and enables the safe use of non-disposable medical materials. At the end of the processes that must be carried out following the international standards defined for sterilization, instructions for the production of the medical materials can be created. It is expected that the sterilization process of all medical materials/devices that are used repeatedly will be completed successfully within the scope of the relevant standards and the effectiveness of the result will be checked and reported. In particular, the records of the sterilization process and its results should be kept by institutions and organizations and archived for presentation to the competent authorities of the relevant countries (such as the Ministry of Health for Turkey) [1].

When sterilization methods are examined, it is known that there are different approaches such as steam, H_2O_2, EO, dry heat, and Gamma, and different types of biological indicators are needed to measure the effectiveness of each method. Sterilization processes provide reusability for both hospital and medical equipment/devices but create additional costs. For this reason, the most effective sterilization type with the most accurate method should be selected as soon as possible and the process should be validated. Users can operate safe sterilization processes by fulfilling the requirements of the legal regulations and international sanctions. It is vital to make sure that every step of the sterilization practices is done correctly and that the process is working correctly. For this reason, it is

necessary to apply physical, chemical, and biological tests to control each step of the preferred sterilization method and to create records. The most reliable method is to take the microbiological samples from the materials whose sterilization process has been completed and to carry out growth controls in the media. While this method is reliable, it is not practical and feasible for every sterilization cycle. Considering the ease of use, feasibility, and reliability, indicators and indicators with different properties have been used for the sterilization process control purposes [2].

Figure 1. Biological Indicator Steam Sterilization - 3M website

The working principle of the biological indicators can be defined as the removal of the microorganisms from the media surface by providing precise information about the efficiency of the sterilization process. The usage areas of sterilization units show a great variety. Different industrial groups aim to reach the SAL 10^6 value as a result by choosing different sterilization methods. For this reason, the sterilization type and the related biological indicator type vary according to the material and sterilization method that is planned to be utilized [1].

The compatibility of the sterilized medical device with the sterilization agent is determined by the chemical indicators, and the steps that affect the effectiveness of the sterilization process are determined by the biological indicators [3].

Spores containing *Geobacillus* and *Bacillus* are frequently used because the bacteria species generally used in the design of the biological indicator have high resistance and represent difficult conditions. *Escherichia coli*, which is in the vegetative form, is also widely used in the experimental studies [4]. In biological indicators, the area required for the bacterial spores and growth are combined in a glass lantern and monitored. After sterilization is completed, the part containing spores is transferred to the medium and transferred to the incubator operating in the temperature range required for the reproduction. The environment that feeds the system of the indicator must be validated against the sterilization agent. If the vital activities of the bacterial spores cease at the end of this process, the sterilization process is completed. Again, the process should be evaluated in terms of the residue of the sterilization agent and the damage caused by the sterilization systems that cause toxic effects on the vital activities [5].

2. Material and Method

While designing the Biological Indicators, the bacterial spores selected for the relevant sterilization method are increased, a pH meter is used to measure the number of acid metabolites that increase in the environment.

Biological Indicators are produced by three different methods;

1. Paper Strips

Paper strips inoculated in the envelope - Incubation period min 7 days, Evaluation; Growth medium turbidity [5]

2. Self-Contained Closed Systems-Generally Response within 1-48 hours

With practical use, all components (spore strip, growth medium, etc.) are ready-made systems.

3. Enzyme-Based Biological Indicators - Rapid response within 1-4 hours [6]

The standards to be complied with while preparing the design, production, validation, and usage instructions of Biological Indicators can be listed as follows [7];

- ISO 14161: 2009 Biological Indicators
- ISO 11140-1: 2014 Chemical Indicators
- ISO 13485:2016 Medical device standard and the requirements for a quality management system (QMS)
- ISO 11138-1: 2017 Biological Indicators

Additional product and application-oriented standards can be added to the list. In particular, all standards defined for the selected sterilization method should be examined and their requirements should be met [8-11]. Evaluation results of the Biological Indicators can be determined by three different methods listed below [7];

1. Turbidimetric
2. Fluorimetric
3. Spectrophotometric

The fact that the response times of the indicators that are expected to yield results by choosing one of these methods are not short. Regular samples are taken in each cycle following the sampling plan from the material or medical device, the sterilization process of which has been completed, extends the process even to longer period of times. Evaluation of the results and approval process is directly proportional to the response rate of the biological indicator [8]. For this reason, if sterilization takes place in a hospital environment, the material awaiting approval cannot be used until the result is reached for safety reasons, causing disruptions in the work. This situation provides us with an explanatory example of the importance of the biological indicator yield times. A large number of studies are carried out to shorten the time to get the results of the Biological Indicators and they are sold by prominent companies in the sector. The new generation indicator studies, which significantly shorten the working time for all the users, have begun to be accepted, which will start eliminating the indicators that respond over a long period of time with the traditional methods.

It is known that 3M™ Attest™ Rapid Readout Steam indicators, one of the products approved by 3M company, give precise and clear results after 3 hours. It is known that the incubation period (RIT) is shortened as a result of the studies carried out by the company [12].

When the properties of this biological indicator are examined, it is observed that the highest-strength Geobacillus stearothermophilus spores are used, incubated for three hours for fluorescence reading, and the correct result is achieved when the process is completed [12].

Figure 2. 3M™ Attest™ Rapid Readout Steam- 3M website [12]

3. Results

Sterilization Experiment studies with *G.stearothermophilus, B.atrophaeus, B.subtilis* bacteria:

The change in bacterial population with exposure time was observed. A positive result indicates that the bacteria continue their vital activities. The results are summarized in Table 1.

Table 1. Sterilization Experiment on *G.stearothermophilus, B.atrophaeus, B.subtilis* [13]

Type of biological indicator	Exposure time(min)			
	30	60	90	120
G.stearothermophilus ATCC 7953(1.76*10^5 CFU)		*	*	
B.atrophaeus ATCC 9372 (8.48*10^5 CFU)	*	*		
B.subtilis ATCC 6633 (1.58*10^6 CFU)	*	*		

4. Discussion

Biological indicators and sterilization efficiencies have been tried to be evaluated by many different studies. While trying to shorten the time to get results with different design studies, it is also aimed to get the right result. Glucose and starch were used as nutrient sources in the study in Table 2 conducted with *G. stearothermophilus and E. coli* bacteria. Measurements were made at intervals of two hours periodically after sowing. Table 2 shows the results of the study and Figure 2 shows the graph of the reproduction [1].

Table 2. Hourly growth rate of bacteria in media varieties (log10 kob/mL) [1]

Control Time	Fattening place *+ E.coli*	Fattening place 1 *+G.stearothermophilus*	Fattening place 2 *+E.coli*	Fattening place2 *+G.stearothermophilus*
2	2,869232	3,908485	2,491362	3,982271233
4	3,748188	5,78533	3,380211	4,653212514
6	5,819544	6,041393	5,875061	6,32219295
8	6,838849	8,662757832	6,892095	7,838849091

The growth curve of the 4 mediums is given in Figure 1, it is defined as the growth level unit (log10 cfu/mL).

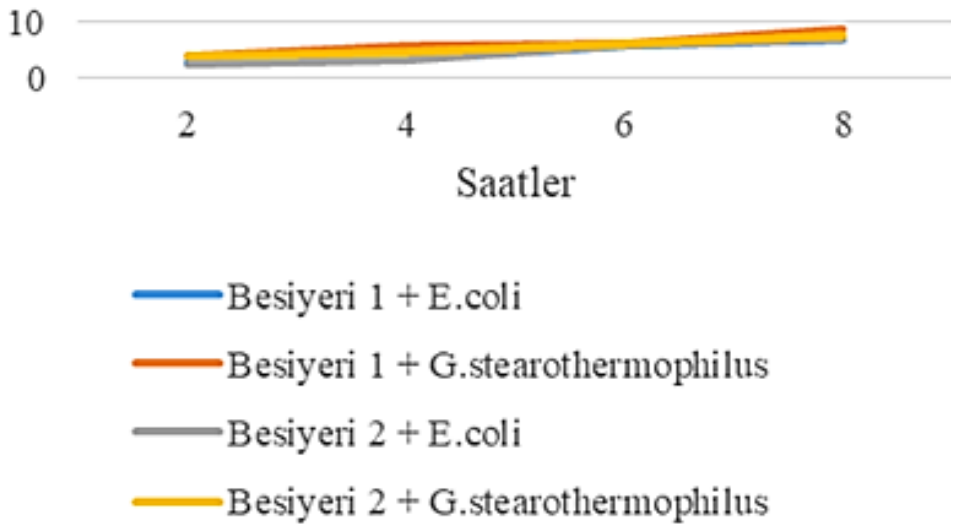

Figure 2. Reproduction Curve (log10 kob/mL) [1]

Of the two different types of color indicators (PR and BCP), which are planned to be used in the design of the biological indicator, the faster one is preferred. When Table 3 is examined, it is observed that the response time of the PR ren indicator is shorter.

When the results of Table 4 and Table 5 were examined, it was observed that there was a growth after the 4th hour according to the optical reading results of *G. stearothermophilus bacteria*. Within the scope of this evaluation, a positive result is observed for this bacterial species after the 4th hour.

Tablo 3. Summary of detection times according to bacteria type and energy source [1]

Bacteria	Indicators	Energy Source	2.hours	4.hours	6.hours	8.hours	24.hours
E.Coli	PR	Strach	-	+	+	+	+
		Glucose	-	+	+	+	+
	BCP	Strach	-	+	+	+	+
		Glucose	-	+	+	+	+
G.stearothermophilus	PR	Strach	-	+	+	+	+
		Glucose	-	+	+	+	+
	BCP	Strach	-	-	-	-	+
		Glucose	-	-	-	-	+

PR: Fenol Red BCP: Brom Krezol Purple

Tablo 4. Detection times according to bacteria type and energy source summary of results -Glucose [1]

Hour	Bacterium's name	Energy Source	Optical hardware readings results			
			R	G	B	Evaluation
0	*G.stearothermophilis*	Glucose	38	31	32	Negative
2			38	31	33	Negative
4			30	26	35	Positive
6			29	29	38	Positive
8			29	31	40	Positive
24			29	31	40	Positive

R: Red G: Green B: Blue

Tablo 5. Summary of detection times according to bacteria type and energy source [1]

Hour	Bacterium's name	Energy Source	Optical hardware readings results			
			R	G	B	Evaluation
0	*G.stearothermophilis*	Strach	38	30	33	Negative
2			38	31	33	Negative
4			32	27	38	Positive
6			29	41	41	Positive
8			29	31	40	Positive
24			30	24	32	Positive

R: Red G: Green B: Blue

5. Conclusion

Traditional biological indicators take 24 hours to give the final results. This leads to increased operational costs and the field is aiming to overcome this hurdle. However, recent studies have shown us that new generation biological indicators produced using high-strength *E. coli* and *G. stearothermophilus* bacteria. These can be used for sterilization safety control by giving accurate results in as little as 4 hours. With the use of biological indicators that we can get a quick response in a short time, significant reductions in operating costs can be observed and effective sterilization safety can be achieved. It is possible to gain serious benefits by expanding their use and production.

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Author contributions

Ebru Öner Usta: Conceptualization, Methodology, Writing-Original draft preparation, **Furkan Ayaz:** Data curation, Writing-Reviewing and Editing.

Conflicts of interest

The authors declare no conflicts of interest.

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