Jan. 16, 1973 K. KERELUK 3,711,378

MICROBIOLOGICAL-SCALED STERILITY CONTROLS

Filed Sept. 15, 1969

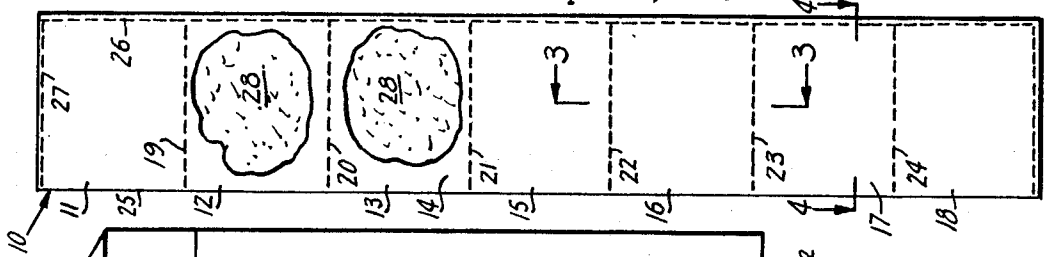



FIG. 2




FIG. 3

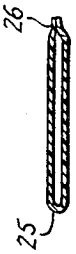



FIG. 4

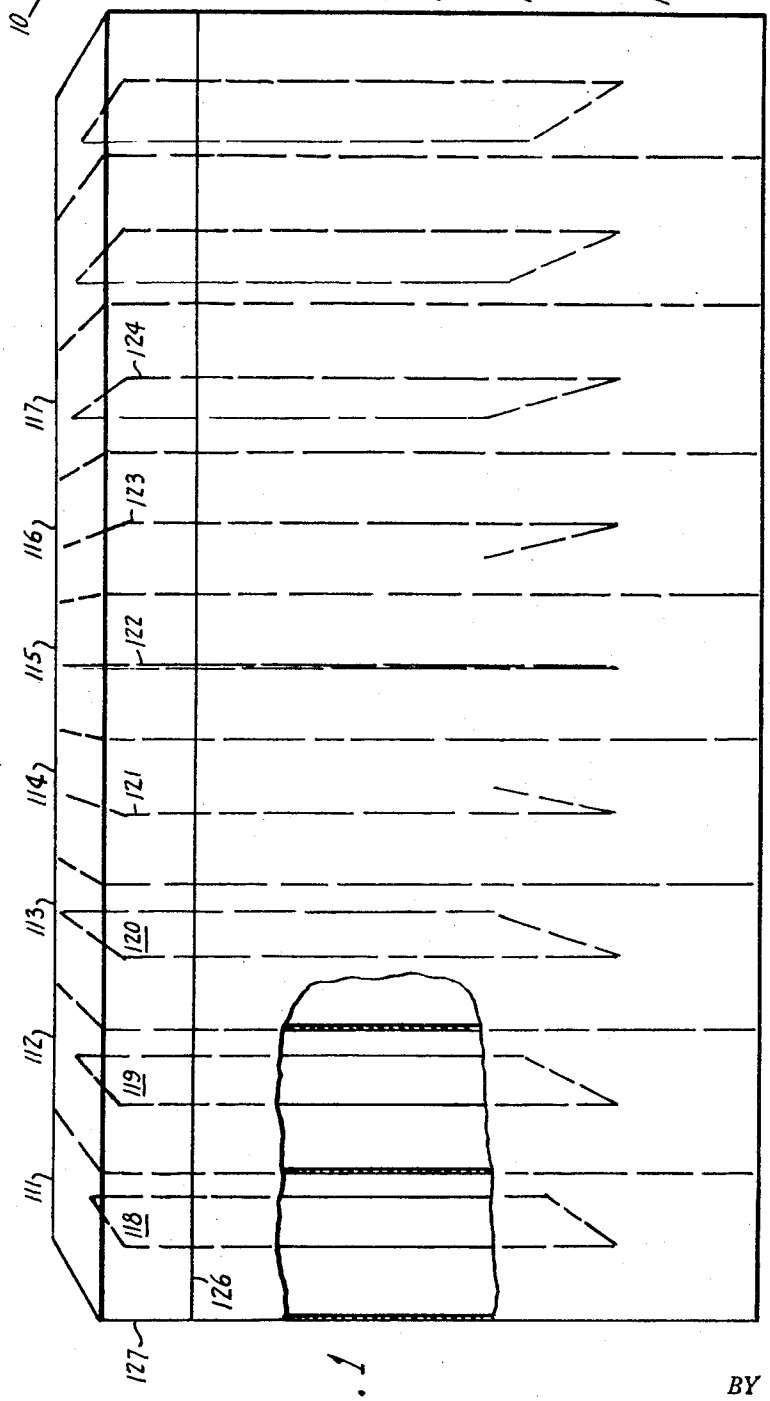



FIG. 1

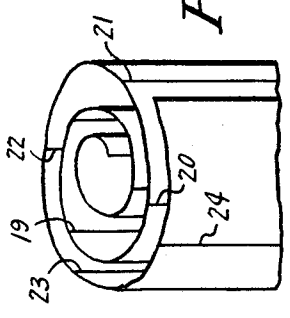



FIG. 6

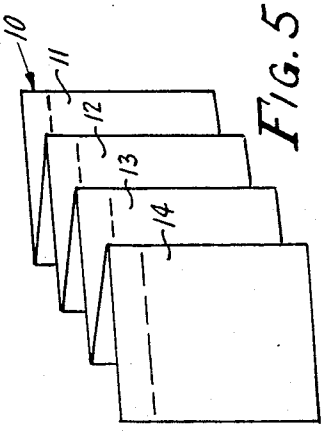



FIG. 5

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# MICROBIOLOGICAL-SCALED STERILITY CONTROLS

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U.S. Cl. 195—103.5 R 14 Claims


## ABSTRACT OF THE DISCLOSURE

A method and apparatus for determining the effectiveness of a sterilizing cycle by preparing a plurality for example seven spore impregnated strips of filter paper sealed in glassine envelopes and exposing the strips to the sterilizing cycle under study. Each of the seven strips has a different spore population, e.g. $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, and $10^9$. The sterility control is either exposed directly to the active elements of the sterilizing process or the control is placed in the load of materials to be sterilized. The strips are then removed and incubated and studied. The effectiveness of the cycle can be concluded from the spore strip containing the highest population on which the entire population is killed.


## STATEMENT OF THE INVENTION

This invention relates to sterility controls, and more particularly, to control devices for determining the effectiveness of a sterilizing cycle.

## OBJECTS OF THE INVENTION

It is an object of the invention to provide an improved sterility control.

Another object of the invention is to provide an improved control for determining the effectiveness of a sterilizing cycle.

Another object of the invention is to provide an improved structure of a sterility control.

Another object of the invention is to provide a sterility control, which is simple in construction, economical to manufacture, and simple and effifficient to use.

Another object of the invention is to provide an improved method of controlling a sterilizing process in a sterilizer.

## GENERAL STATEMENT OF THE INVENTION

It is well known in the field of sterilization that the sterility of an article or commodity is predicated in its exposure to fairly precise conditions which cannot be varied to any great extent without sacrificing sterility. In many cases, even slight variations in exposure conditions may result in so-called "hits" or "misses" or situations where sterility may be achieved on one occasion, fail on the next and succeed on the third attempt even though the test conditions were supposedly the same each time.

Because of these situations, a method is hereby proposed which enables an operator or manufacturer of sterile disposables to evaluate the effectiveness and/or efficiency of the sterilizing process. With this method, it is possible to determine (1) whether the conditions of the process are capable of achieving sterility, (2) whether the conditions of the process will achieve sterility each time the process is conducted, and (3) whether some safety factor, such as increased exposure time or increased temperature, should be considered in order to attain sterility and/or reproducibility of results.

## DESCRIPTION OF THE PROCESS

The proposed method is based on the use of specially prepared bacterial spore-impregnated strips of filter paper, sealed in glassine envelopes and exposed to the conditions of the sterilization process under study, and the proper interpretation of the test results.



The bacterial spore-impregnated strips, employed in this method, are prepared in a manner similar to currently used spore strips with one exception. A total of seven spore strips are prepared as a set with each strip containing a different concentration or population of bacterial spores, i.e., the first strip will contain a population of $1\times10^2$ bacterial spores, the second strip will contain a population of $1\times10^3$ spores, and so on until the final or seventh strip will contain a population of $1\times10^8$ bacterial spores.

The organism used in this method comprises a bacterial sporeforming specie known to be resistant to the sterilizing process being used or tested. For example, the bacterial specie used to test the efficiency of a steam sterilizing process is usually *Bacillus stearothermophilus,* while *Bacillus subtilis* var. *niger* is more commonly employed to test the efficiency of such sterilizing processes as dry heat or ethylene oxide.

In practice, the microbiological-scaled sterility controls are exposed either directly to the sterilization process or are placed in the load of materials to be sterilized. The sterilization process is conducted in the usual manner following which the sterility controls are removed from the sterilizing apparatus or the load, aseptically removed from their respective glassine envelopes and cultured in tubes of recommended bacteriological culture media. The tubes are then incubated at the optimum temperature of the test organism (usually stipulated on the glassine envelope) for a period of seven days. Results are recorded in terms of growth or no growth.

On the basis of the results, the effectiveness and/or efficiency of the sterilizing process under the conditions employed can be determined. To illustrate, in the event that the sterility control strips covering spore populations ranging from $10^2$ to $10^6$ are sterilized but those strips containing $10^7$ through $10^8$ spores are not sterilized, it can be concluded that the sterilizing conditions used are capable of sterilizing materials containing microbial contamination levels up through $1\times10^6$ organisms. If successive tests produce the same result, it can be concluded that the conditions of the sterilizing process are reproducible and will provide consecutive sterilizing cycles. However, if the above results are not achieved in each successive cycle, it can be concluded that the sterilizing conditions are not capable of producing reproducible results and will require some modification. If all seven of the sterility control strips are repeatedly sterilized, it can be concluded that the sterilizing conditions are capable of sterilizing materials or articles which are *unusually heavily contaminated.* However, if results show that only the lower ranged sterility controls ($10^2$ or $10^3$) are sterilized, it can be concluded that the sterilizing process is of low order and is not capable of sterilizing even moderately contaminated articles, i.e., those having contamination levels in the magnitude of $1\times10^4$ to $1\times10^6$ organisms.

## EXAMPLE

(1) Sterile carriers (Spordex strips and ceramic tiles) were inoculated with varied spore populations ($10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, $10^9$).

(2) They were exposed to 500 mg./l. of Eto @ 50% R.H. and 130° F. at varied time intervals. The shortest period of exposure for the inoculated carriers was five minutes. The exposure period was increased to 30 minutes by five-minute increments.

(3) At each exposure period for each of the seven populations 10 inoculated carriers were exposed (5 spordex strips, 5 ceramic tiles) to the test conditions. After exposure each carrier was transferred individually to a tube

of Trypticase Soy Broth(TSB). The tubes were incubated at 37° C. for one week. Following incubation each tube of TSB with a carrier was observed for the presence or absence of growth.

## STATEMENT OF PRIOR ART

Test strips have been used for various purposes to indicate various intensities of process, for example, Pat. 3,245,882 discloses a test strip for testing the phenylalanine in blood.

FIG. 1 is an isometric view of one embodiment of the invention.

FIG. 2 is a top view of another embodiment of the invention.

FIG. 3 is a cross-sectional view taken on line 3—3 of FIG. 2.

FIG. 4 is a cross-sectional view taken on line 4—4 of FIG. 2.

FIG. 5 is a view showing the embodiment of FIG. 2 folded.

FIG. 6 is a view showing the embodiment of FIG. 2 formed into a roll.

Now with more particular reference to the embodiment of FIG. 2 through 6, the glassine envelopes are indicated at **10**. They are divided into containers **11**, **12**, **13**, **14**, **15**, **16**, **17**, and **18** by the scored lines **19**, **20**, **21**, **22**, **23**, and **24**. The material which makes up the envelope may be made of a sheet which is folded back on itself at **25** and sealed along lines **26** and **27** by a suitable process. A spore carrier **28** will be enclosed in each compartment of the envelope. The spore carrier **28** will be specially prepared bacterial spore impregnated strips of filter paper, sealed in the glassine envelopes, and they will be exposed to the condition of sterilizing by placing the envelope **10** either rolled up, as shown in FIG. 6, or folded, as shown in FIG. 5, or lying flat as shown in FIG. 2.

In the embodiment of the invention shown in FIG. 1 a container **110** is shown, which is made of relatively rigid material and which has compartments **111**, **112**, **113**, **114**, **115**, **116**, **117**. The compartments will each contain at least one bacterial spore impregnated strip **118**, **119**, **120**, **121**, **122**, **123**, and **124**. The strips will be made according to a well known process, such as for example shown in Pat. No. 2,931,757, and will be impregnated with different numbers of spores, for example, spore strips **118** through **124** will be impregnated with $1\times10^2$ bacterial spores, $1\times10^3$ bacterial spores, $1\times10^4$ bacterial spores, on through $1\times10^8$ bacterial spores, respectively. A scored liner **126** formed in the walls of the compartments, by which the upper part **127** of the container may be removed. The spore strip may then be removed and incubated. The container **110** may be molded from a plastic material for example vinyl or polyethylene.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

**1.** A method of determining the effectiveness of a sterilizing cycle comprising providing a set of at least seven spore carrying devices, each having a different spore population,

the number of spores in each said population being equal to a real positive integer greater than zero raised to a real positive exponential integer, each said exponential power being different from the others,

exposing said set of devices to the sterilizing cycle,

incubating said devices and observing the devices having the highest population that is completely killed.

**2.** The method recited in claim **1** wherein said devices are inoculated with at least $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, and $10^8$ spores respectively,

said spores are inoculated with spores taken from the group of *Bacillus stearothermophilus* and *Bacillus subtilis* var. *niger*.



**3.** The process recited in claim **2** wherein said spore devices are incubated after exposure.

**4.** A sterility control comprising a plurality of containers adapted to be disposed in a sterilizing chamber,

a spore carrier in each said container,

each said spore carrier being impregnated with a different population of spores,

the number of spores in each said population being equal to a real positive integer greater than zero raised to a real positive exponential integer, each said exponential power being different from the others, whereby the effectiveness of the cycle can be determined by observing said spore carrier having the largest number of said spores wherein all said spores are killed.

**5.** The control recited in claim **4** wherein said containers are connected together in edge to edge relation.

**6.** The control recited in claim **5** wherein said containers are in the form of glassine envelopes.

**7.** The control recited in claim **6** wherein said envelopes are adapted to be disposed in said sterilizer in the form of a roll.

**8.** The control recited in claim **7** wherein at least seven of said devices are provided in said containers.

**9.** A container having at least seven separate compartments,

said compartments being isolated from each other,

a spore strip in each said compartment,

each said strip being impregnated with a different population of spores from the other strips,

the number of spores in each said population being equal to a real positive integer greater than zero raised to a real positive exponential integer, each said exponential power being different from the others,

said container with said spore strips being adapted to be placed in a sterilizing chamber during sterilizing cycle and said strips subsequently incubated and the strips having all spores thereon killed whereby the effectiveness of said cycle can be determined by observing the said strip having the largest number of said spores wherein all said spores are killed.

**10.** The control recited in claim **9** wherein said compartments are generally rectangular in cross section.

**11.** The control recited in claim **9** wherein said containers are in the form of glassine envelopes, and a removable cover on each envelope,

said envelope being scored along a line adjacent on edge whereby the cover can be removed.

**12.** The control recited in claim **9** wherein said container is scored adjacent one edge whereby said cover may be removed and said container is made in the form of glassine envelopes attached together.

**13.** The control recited in claim **9** wherein said container is made of a relatively rigid material.

**14.** The control recited in claim **13** wherein said container is made from a material taken from the group of vinyl and polyethylene.


**References Cited**

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U.S. Cl. X.R.

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