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MIL-STD-969
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MILITARY STANDARD

BIOLOGICAL INDICATORS

FOR STERILIZATION PROCESSES

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DEPARTMENT OF DEFENSE
WASHINGTON, DC 20301

Biological Indicators for Sterilization Processes

MIL-STD-969

1. This Military Standard is approved for use by all Departments and Agencies of the Department of Defense.

2. Recommended corrections, additions, or deletions should be addressed to: Defense Personnel Support Center, ATTN: Directorate of Medical Materiel, DPSC-ATT, Philadelphia, PA 19101.

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TABLES

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1. SCOPE

1.1 Scope. This standard covers biological indicators (BI) consisting of carriers containing bacterial spores suitable for use as biological monitors in determining the efficacy of sterilization processes.

1.2 Classification. The BI system described by the manufacturer's literature shall be one of the following types and classes and shall have the following storage requirements. The system shall be constructed to assure that the sterilizing agent of the sterilizing process is the agent accounting for the bacterial spore lethality.

Types.

I. Indicators containing bacterial spores of a single species, at a known level of population and resistance.
II. Indicators containing bacterial spores of two species, with each species at a known level of population and resistance.
III. Indicator sets containing bacterial spores of a single species, at various (graduated) levels of population and known resistances within the set.
IV. Indicators containing the bacterial spores of a single species, at a known level of population and resistance, and containing the culture medium within the individual unit but not in direct contact with the bacterial spores until time of incubation.
V. Indicators containing the bacterial spores of two species with each species at a known level of population and resistance, and containing the culture medium within the individual unit but not in direct contact with the bacterial spores until time of incubation.
VI. Indicator sets containing the bacterial spores of a single species, at various (graduated) levels of population and known resistances within the set, and containing the culture medium within the individual units, but not in direct contact with the bacterial spores until time of incubation.
VII. Indicators containing the bacterial spores of a single species, in a culture medium at a known level of population and resistance.

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Classes.

1. Contains bacterial spores of Bacillus stearothermophilus.
2. Contains bacterial spores of Bacillus subtilis var niger (or globigii).
3. Contains bacterial spores of Bacillus pumilus.
4. Contains bacterial spores other than class 1, 2, or 3 and must be specified.
5. Contains bacterial spores of classes 1 and 2.

Storage requirements.

A. Indicators requiring storage from 2^{o} to $10^{o}C$ (35.6^{o} - $50^{o}F$).
B. Indicators requiring storage below $0^{o}C$ ($32^{o}F$).
C. Indicators requiring storage from 0^{o} to $50^{o}C$ (32^{o} - $122^{o}F$).
D. Indicators requiring storage from 15^{o} to $30^{o}C$ (59^{o} - $86^{o}F$).
E. Indicators requiring storage conditions other than those listed above, as specified by the manufacturer.

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2. REFERENCED DOCUMENTS

2.1 Issues of documents. The following documents of the issue in effect on date of invitation for bid or request for proposal form a part of this standard to the extent specified herein.

STANDARDS

MILITARY

MIL-STD-105	- Sampling Procedures and Tables for Inspection by Attributes.
MIL-STD-966	- Monitoring Sterilization Systems for Medical Devices (Industrial).

(Copies of specifications, standards, and publications required by contractors in connection with specific procurement functions should be obtained from the procuring activity or as directed by the contracting officer.)

2.2 Other publications. The following document forms a part of this standard to the extent specified herein. Unless otherwise indicated, the issue in effect on date of invitation for bid or request for proposal shall apply.

THE WILLIAMS AND WILKINS COMPANY, A DIVISION OF WAVERLY PRESS, INC.

Bergey's Manual of Determinative Bacteriology.

(Application for copies should be addressed to the Williams and Wilkins Co., A Division of Waverly Press Inc., 428 E. Preston Street, Baltimore, MD 21202.)

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3. DEFINITIONS

3.1 Biological indicator (BI). A known quantity of micro-organisms (of species defined in 1.2) in or on a carrier whose units have measurable resistance to a specified sterilization procedure and which can be used to demonstrate by microbiological methods that sterilizing conditions were achieved.

3.2 Finished BI. Finished BI refers to the indicator in the normal form that is supplied by the manufacturer to the customer or user.

3.3 Bacterial spores. Bacterial spores, as used in this standard, refers to dried spores on a carrier material, unless otherwise noted (such as Type VII).

3.4 Bacterial species identification. Bacterial species identification shall be in accordance with Bergey's Manual of Determinative Bacteriology.

3.5 Known level of population. Known level of population refers to a "known" population at time of manufacture, testing and release.

3.6 Survival of BI. Survival refers to growth of the BI in a growth medium after exposure to the conditions given in table I and after incubation in accordance with the manufacturer's recommendation. The viability of the BI shall meet or exceed the minimum indicated by the manufacturer.

3.7 Kill of BI. Kill refers to no growth of the BI in a growth medium after exposure to the conditions given in table I and after incubation in accordance with the manufacturer's instructions.

3.8 Up time. Time required from introduction of the sterilizing agent into the exposure chamber and until the lower limit of conditions stated in table I is reached. Cycle exposure timing shall start when the minimum specified test parameters have been reached.

3.9 Down time. The total time required to remove the sterilizing agent from the exposure chamber and to lower the conditions of the chamber to a safe level for the removal of the material.

3.10 Decimal-reduction value (D-value). The number of survivors is a direct relation of the resistance of the organism to the sterilization process being used. One method for this determination is the D-value. A D-value is the time required at a given set of conditions (i.e., for steam, the parameters are temperature, pressure and time; for ethylene oxide, the parameters are temperature, relative humidity, gas concentration and time) to reduce the initial microbial population by 1 logarithm or 90 percent. The D-value must be determined from a straight line graph plot on semi-log graph paper of the log number of survivors versus time of exposure to a set

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of defined sterilization conditions, or from the mathematical statement $D=t/(\log N_o - \log N)$, where N_o is the initial homogenous microbial population, N is the surviving microbial population at any time (t), and D is the D-value for any specific set of conditions. The line shall be based on substantial statistical data.

3.11 Inspection. Inspection, as used in this standard, is defined as both examination (such as visual and auditory investigation without the use of special laboratory appliances or procedures) and technical testing of the item.

3.12 Unit of product. The unit of product for test purposes shall be individual BIs from each manufacturer's designated lot or batch.

3.13 Unit package. The package or box which contains a quantity of individual or immediate containers.

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4. GENERAL REQUIREMENTS

4.1 Design. The BI shall be designed to permit intimate contact between the biological organisms and the sterilizing agent being monitored.

4.2 Package or product markings.

4.2.1 Immediate container. Each immediate container of BIs, as described in the product specification, must be marked with:

a. Manufacturer's name or registered trademark.

b. Name of organism(s) contained in BI.

4.2.2 Unit package. Each unit package of BIs must contain the following information on the package or as an insert within each package:

a. Type, class, and storage requirement.

b. Name or registered trademark of manufacturer.

c. Manufacturer's lot or batch number.

d. Expiration date.

e. Number of BIs within the package.

f. Dose - response characteristics:

(1) Resistance performance test (minimum D-value).

(2) Performance characteristics (survival/kill).

(3) Spore population (average range or minimum to be defined and stated by manufacturer).

g. Instructions for use.

h. Complete instructions for incubation, including incubation time, temperature, and culture medium, if not part of the basic system.

i. Recommendations for disposal after expiration date, and after use and culturing.

j. Cautions that BIs not be stored in close proximity to any sterilizing agent, such as ethylene oxide, steam, radiation, or at high temperatures.

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4.3 Workmanship. Workmanship and material shall be first class throughout. The product shall be free from all defects which detract from its appearance or impair its serviceability.

4.4 Responsibility for inspection. Unless otherwise specified in the contract, the contractor is responsible for the performance of all inspection requirements as specified herein. Except as otherwise specified in the contract, the contractor may use his own or any other facilities suitable for the performance of the inspection requirements specified herein, unless disapproved by the Government. The Government reserves the right to perform any of the inspections set forth in the standard where such inspections are deemed necessary to assure supplies and services conform to prescribed requirements.

4.5 Records. Records of materials, examinations and tests performed by or for the contractor shall be maintained by the contractor and made available to the Government, upon the Government's request, at any time, or from time to time, during the performance of the contract and, as to any expiration-dated items, for the expiration dating period specified by the contractor for such item, or such longer period as may be required by regulation of any federal agency; or as to non-dated items, for not less than three years after delivery of the item to the Government.

4.6 Decimal-reduction value (D-value). D-value determinations shall be conducted on each batch or lot of finished BIs at time of manufacture.

4.7 Expiration dating period. Not less than fifteen months of the expiration dating period shall remain at time of delivery to the Government (storage conditions specified by manufacturer).

4.8 Retained samples. The manufacturer shall retain samples in storage for each lot or batch and conduct scheduled periodic test evaluations of the samples during the potency period. The results of this periodic evaluation shall be available for review by the procuring agency.

4.9 Shipping package. The BIs shall be shipped in substantial commercial containers of the type, size, and kind commonly used for the purpose, so constructed as to insure acceptance and safe delivery by common or other carriers.

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5. DETAILED REQUIREMENTS

5.1 Performance characteristics testing. Survival/kill resistance shall be conducted as indicated in table I for each batch or lot of BIs for each type of sterilization agent for which the BI is recommended. Use of an alternate testing method, after extensive parallel testing against one of the below methods, may be authorized by the procuring agency after review of test results. Tests are to be conducted on BIs packaged in their normal finished product form.

TABLE I. BI resistance performance.

Sterilizing agent	Conditions of Exposure				Maximum Up time	Maximum Down time
	Temperature	RH%	Survival 1/	Kill 2/		
Steam	121° ± 0.5°C (250° ± 1°F)	100	5	15	10 sec	10 sec
Ethylene oxide 3/						
600 ± 60 mg/liter	54° ± 1°C (129° ± 2°F)	60 ± 20	15	120	1 min	1 min
1200 ± 120 mg/liter	54° ± 1°C (129° ± 2°F)	60 ± 20	5	30	1 min	1 min
1200 ± 120 mg/liter	25° ± 1°C (77° ± 2°F)	60 ± 20	20	120	1 min	1 min

1/ Minimum time in minutes.

2/ Maximum time in minutes.

3/ One of the following concentrations and conditions shall be used.

5.1.1 Sampling for tests. Sampling for tests shall be conducted in accordance with MIL-STD-105. The inspection level shall be S-4. The AQL shall be 0.65 for kill and the acceptance number for survival shall be zero. The unit of product for test purposes shall be the individual BI.

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5.1.2 Conditions other than table I. For BIs for use under conditions not covered in table I, the manufacturer shall include the survival/kill performance for that particular usage in the test results. (The sampling plan in 5.1.1 shall apply.) Examples of other BI usage are:

a. Steam (at 132°C).

b. Dry Heat.

c. Radiation.

d. Sets of BIs with graduated levels of spore population.

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