Preventing mildew growth on cotton swabs and other products containing cotton

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ABSTRACT

The action of microorganisms is generally dependent on factors such as availability of organic or inorganic nutrients, sunlight, and moisture. In this study, a combination of factors occurring during manufacturing and storage were found to cause mold growth on cotton swabs. These factors included improper or inadequate sanitization, contaminated Methocel, inadequate amounts of preservative (0.1% or less) in the infested Methocel, high moisture levels (30% or more) in cotton swabs, plastic sticks/put ups, and warehousing cotton swabs under hot and humid conditions. Sealed plastic bags used for storage, essentially unventilated units, were found to provide an ideal moisture chamber for mildew development. Implementation of certain control options solved the above problems, ensuring mildew-free manufacturing of cotton swabs.

Application:

While this research was performed on cotton swabs, the recommendations for preventing mildew may also be applied to other products such as incontinent pads, tampons, hydroentangled nonwovens, and other products containing cotton.

MICROBIAL DAMAGE TO cotton, fiber, fabric, and cloth; paper and books; photographic supplies; paper pulp in paper mills; wood pulp; and bread in storage is well known, and losses run into hundreds of millions of dollars annually. Recently, an Indonesian company complained to a California-based cotton producer that a lot it had received was fungus-infested. Of the 33,690 bales weighing 225 kg each, 21,000, worth US$ 18 million, were tainted with fungus (1).

The presence of mildew is often accompanied by a musty odor, discoloration, and staining of cloth. Discoloration is dependent on the fungi present and may vary from white to yellow, pink, green, brown, red, and black.

Finished cotton goods, such as tarpaulins, sandbags, awnings, towels, clothes, and rugs, are particularly susceptible to microbial deterioration when stored under warm and humid conditions. Often loom-state (Greige) cotton goods are damaged during shipment overseas. The rate of decay is accelerated in areas of high temperatures, dampness, and rain *(2)*. There is extensive literature on the subject *(2-5)*. However, a literature search did not reveal any microbial attack on cotton swabs.

In the summer of 1994, mold/mildew on double-tipped cotton swabs was observed at a leading healthcare products manufacturing company. It had stored 1750 cases of 48 plastic bags containing 1000 swabs per bag in Florida for two months. Mildew development, as indicated by greenish and blackish discoloration, occurred in nearly 50% of the cases. An investigation into the possible sources of the contamination was performed, followed by corrective actions.

The mildew produced a major rejection with an approximate loss of $95,000 to the company. The company has 35 swab making machines, and under a regular production schedule, it would produce 15 million swabs per five-day week. This troubleshooting report discusses causes and prevention of such biological attack on nonsterile cotton swabs during storage.

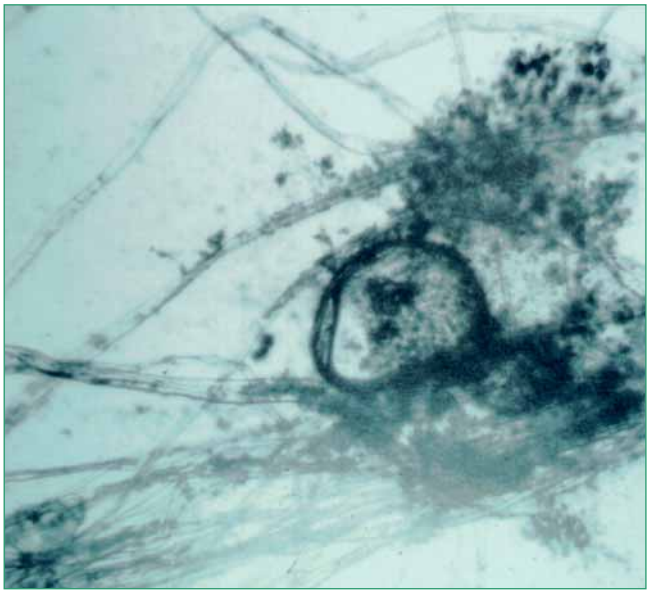
SWAB MANUFACTURING OPERATIONS

The following operations are performed in the manufacturing process:

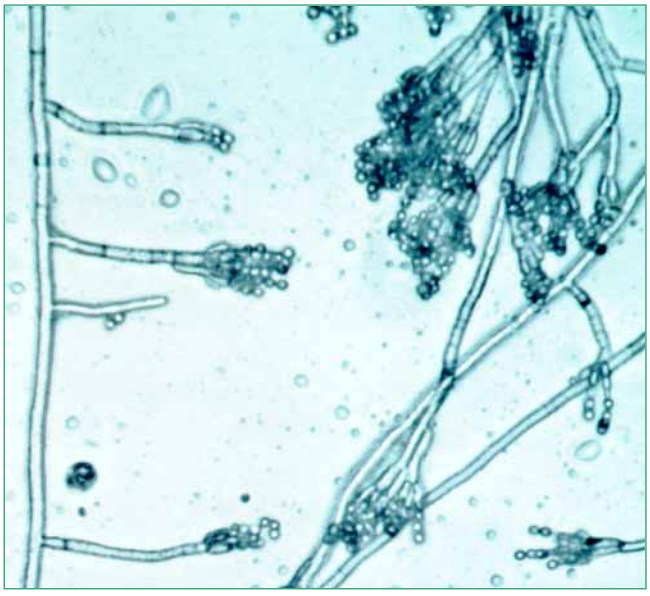
- Loading the sticks
- Stick transfer system
- Tip gluing
- Cotton feeding
- Formation/modeling of the cotton buds
- Drying of cotton buds
- Packaging turntable

Standardized sticks (length 2.875 in. ±0.0625 in., 2.2 mm diameter) of paper, wood, or plastic are supplied by the stick hopper. Short cotton fibers of 8-20 mm are preferred. Bleached short fibers, comber noil, gin motes, and mill waste are the fibers used in making swabs. Carded round slivers (diameter 10-12 mm) having 1.5 gm per linear meter are fed to the machine. The finished cotton buds have a diameter of 4.8 mm. Glue anchors the buds to the stick ends. Formation of the cotton buds is carried out by spinning the cotton on the stick tips and shaping the buds.

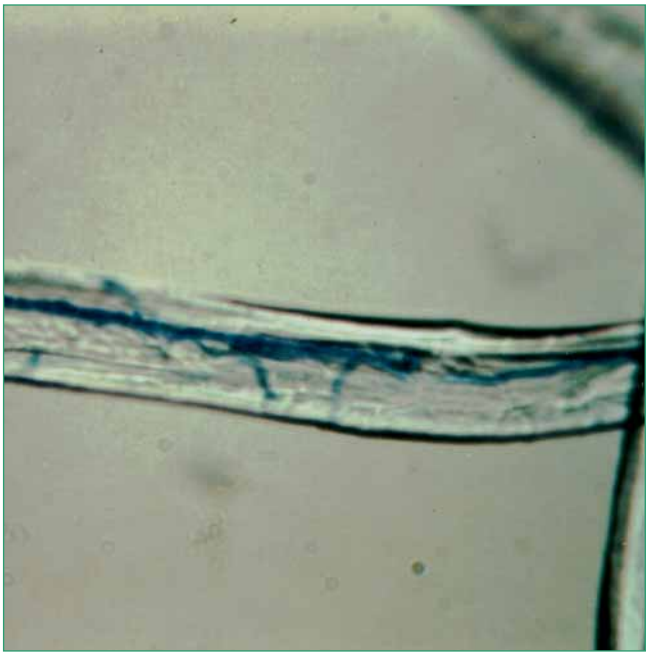
While the buds are formed, a 1% solution of Methocel *(6)* slurry (the sizing solution) containing 0.1% Dowicil *(6)* is injected and absorbed by the buds as the sticks run through. The normal amount is about one drop of solution on each side for about 15 tips. Moisture and the sizing solution help in producing aesthetically pleasing buds, free from a fuzzy appearance. Generally, the higher the moisture, the greater the production speed of making swabs.



1. Microbial damage in cotton



2. Branched filaments (hypae)



3. Optical micrographs of stained filaments (hypae)

The next operation is drying the cotton buds. The purpose of drying is to reduce the moisture of cotton buds before the sticks are taken over for packaging. The temperature of the hot plates as well as of the hot air section is adjusted to suit the production requirements.

EXPERIMENTAL

Observations on discolored swabs

Only a few swabs (20-90) were contaminated out of a thousand in a bag, and that discoloration encompassed one-third to one-fourth of the contaminated cotton tip, indicative of limited growth. However, even this small level of contamination is not acceptable. A few of the plastic bags were foggy, with tiny water droplets condensed in the interior of the bag, and the swabs were found to be wet. (These swabs were probably produced out of specifications and escaped the Quality Assurance inspection).

Identifying the discoloration

The discolored swabs were examined by the microbiologist of an independent laboratory. Microscopic examination and pH determination verified the presence of fungal hypae and spores on the discolored cotton. The environment of the growth area was found to be acidic (pH approximately 5). An acidic environment is known to be conducive to the growth of fungi rather than bacteria.

Mold growth was microscopic, not macroscopic — that is, not visible to the naked eye. Fungi photographs of the discolored cotton were visually compared to photographs of known molds and filamentous yeasts. Green spots exhibited mature saprophytes and hypae of fungi; hypae represented the vegetative stage of fungal growth. Matured fungal colonies were observed as black spots.

Staining of cotton for the presence of fungi was carried out with a solution of Aniline Blue, C. I. No. 42755 (Acid Blue) in lactophenol *(7)*. Fungal materials appear deep blue on colorless cotton fibers. **Figures 1-3** illustrate some of the fungal materials observed.

Microscopic observations of mildew growth were further confirmed by fungal growth on SMA (Sabouraud Maltose Agar) plates. Colonies, too numerous to count, developed on green and black discolored cotton by both the standard streak method and sample pour plate method *(8)*.

No attempt was undertaken to assess the damage due to biodeterioration. It has been our impression that the biodeterioration damage was

probably in the initial stages.

Although a Scanning Electron Microscope (SEM) was not used in the present investigation, it can be used to study mildew-infested cotton fabrics. SEM identifies and pinpoints mildew growth by using three-dimensional micrographs that show various stages of growth on different portions of the fabric *(5)*.

Testing water for contamination

The bioload of water collected from brass nozzles (in humidification) was tested to be lower than that of the potable water. (The bioload of water was routinely tested every three to six months.)

Water used in the plant is from the city supplies. It is heavily chlorinated. (Occasionally one can detect an odor of sodium hypochlorite.) The incoming water goes first through a series of cascading filters that have the capability of retaining microbes, and then through impingement of UV light before using it in humidification of carding and converting operations. Thus water has not been the culprit. Furthermore, the entire water supply line and the humidification system are sanitized once a year.

Evaluating moisture levels for mold growth

A planned experiment was carried out in which nine study groups of swabs were made with varying levels of both moisture and Dowicil 200, as described in **Table I**. The goal was to determine moisture levels that would encourage mold/discoloration under controlled conditions of incubation. Each test group consisted of 10 plastic bags with 1000 swabs per bag. Swabs were made with moisture levels of 8%, 14%, and greater than 20%. These samples were incubated at 30-35°C for 41 days. Visual inspection was performed for any discoloration or mold and logged daily.

After 15 days, very slight mold growth was observable in nine out of 10 study groups. Black or green discoloration appeared in small areas on the cotton tips. The only test group that remained free of even slight contamination was No. 7 (0.2% Dowicil, 8% moisture).

At day 41, final observation showed that in group No. 6 (0.1% Dowicil, >20% moisture), six out of 10 bags had contamination similar to the swabs that were returned from Florida.

On rechecking moisture levels, it was found that swabs from group No. 6 were substantially wetter than groups 3 and 9, the other two groups with moisture levels greater than 20%. Group No. 6 was the wettest (35-40%). Apparently the high level of moisture in the swabs stimulated mold growth. These results suggest that moisture should be closely monitored and kept below 14% to prevent mildew development.

Although 0.1% Dowicil was present in group No. 6, it did not prevent mold growth. Therefore fungal plate counts were performed, revealing that the Methocel slurry was contaminated before the addition of Dowicil.

The count for Methocel alone was 234,000 colony forming units (CFU)/ml; for Methocel with 0.1% Dowicil, it was 23,000 CFU/ml. High contamination in Methocel deactivated Dowicil.

The lessons learned are these:

- Sanitization of the Methocel tank, transfer tank, and swab making machines should be done before making a fresh batch of Methocel
- Any unused Methocel slurry should be discarded.

Start with clean microbe-free tanks, make fresh batches of Methocel (free from microbes), and then add preservative. The preservative will prevent contamination. This finding was confirmed by a "challenge test" performed by Dow Chemical, as described in the appendix. The preservative Dowicil was effective in that test in a concentration of 0.1%.

Standardizing the

MANUFACTURING

- Segregating contaminated cases. Cases without visible contamination were put in a drying room and repacked.
- Studying the possible sources of the microbes: water; environment; and Methocel tanks, transfer tanks, and the swab equipment.
- Checking the moisture levels of the swabs at the time of production and restandardizing size-drops per minute on the swab.
- Experimenting to produce contaminated swabs (without inoculation) by manufacturing swabs with higher moisture levels and incubating them in the lab.
- Developing a test protocol for optimizing preservative level in Methocel.
- Increasing quality assurance inspection.
- Implementing the weekly sanitization procedure.

INDEPENDENT LAB

- Detection of fungi on discolored swabs.

VENDOR S LAB

- Studying the challenge test per the test protocol and determining the preservative (Dowicil 200) level.

II. Efforts to determine causes of and remedial steps to eliminate mold growth

Group	Dowicil Level (%)	Moisture Approx. %
1	0.0	8
2	0.0	14
3	0.0	>20
4	0.1	8
5	0.1	14
6	0.1	>20
7	0.2	8
8	0.2	14
9	0.2	>20

I. Swabs with varying levels of Dowicil and moisture

Bacteria		Fungi	
Rating	No. of colonies	Rating	Growth
1	0	-	
2	1-4		
3	5-10		
4	11-25	+/-	Slight
5	26-50		
6	51-100		
7	101-200	+	Light
8	201-300		Moderate
9	Too many to count		
10	Confluent growth	++	Heavy

Organisms used in multiple challenge test

ATCC bacterial pool

Bacilus subtilis — ATCC 8473
Enterobacter aerogenes — ATCC 13048
Escherichia Coli — ATCC 11229
Klebsiella pneumoniae — ATCC 8308
Proteus Vulgaris — ATCC 881
Pseudonomonas aeruginosa — ATCC 10145
Pseudonomonas aeruginosa — ATCC 15442
Salmonella choleraesuis — ATCC 10708
Staphylococcus aureus — ATCC 6538

Mold

Aspergillus niger — ATCC 16404

Yeast

Saccaharomyces cerevisiae — ATCC 4105

IV. Growth rating chart

- Tightly controlled moisture levels on the swab.
- Used a drying room for high level moisture swabs.
- Increased quality assurance audits.
- Established good weekly sanitization practices for Methocel preparation; swab making machines; and transfer tanks, hoses, and 10 gal pails, thereby controlling the bioloads.
- Increased concentration of preservative (Dowicil) to 0.2% from 0.1%.
- Discontinued use of the plastic putups.

III. Implemented changes

sanitization procedure

Chlorox bleach has 5.2% active sodium hypochlorite. Sterilant is prepared by using 2.5 oz of bleach solution per 10 gal of water.

All the size preparation equipment, including the mixing paddle, stainless steel pails, tanks, plastic transfer containers, water pumps, formers, and plastic/Teflon drain tubings are washed thoroughly in hot water and rinsed before treating with the above sterilant. Sterilant must remain in contact with the equipment for at least 1 min before draining it out, rinsing, and allowing the equipment to dry. Simultaneous sanitization of the Methocel/Dowicil floor area and the swab making equipment (for the areas in contact with Methocel/ Dowicil solution) is equally important. Weekly sanitization was instituted. It takes 10-15 min to sanitize a swab machine. It takes 90-100 min to clean, sanitize, and prepare Methocel/ Dowicil slurry.

Reference *(9)* provides definitions for the terms decontamination, disinfection, and sterilization.

Evaluating effectiveness of the preservative by a multiple challenge test

In collaboration with Dow Chemical Company, a test protocol was prepared to determine optimum concentration of the preservative (Dowicil 200 and/or Dowicide A) to be used along with the Methocel slurry. Dowicil 200 and Dowicide A are two preservatives often used by the cosmetics industry. It was felt that Dowicil and Dowicide would make a synergistic combination to overcome attack from a high level of microbials. The following freshly prepared samples were sent to Dow Chemicals for evaluation by a 10-cycle Challenge test:

A. Methocel slurry
B. Methocel slurry + 0.1% Dowicil 200
C. Methocel slurry + 0.2% Dowicil 200
D. Methocel slurry + 0.3% Dowicil 200
E. Methocel slurry + 0.1% Dowicil 200 + 0.05% Dowicide A
F. Methocel slurry + 0.2% Dowicil 200 + 0.05% Dowicide A

In this test protocol, each test sample was inoculated with American Type Culture Collection (ATCC) bacterial pool, subsequently incubated, and examined for any mold/discoloration. The detailed test procedure and test report are described in the appendix.

The test report indicated that 0.1% Dowicil (the lowest level used in this set of experiments) was effective in preserving the Methocel slurry even after multiple inoculations.

Table II summarizes the efforts made by manufacturing, an independent lab, and a vendor's lab to resolve the problem.

While this research was performed on cotton swabs, the recommendations for preventing mildew may also be applied to other products, such as incontinent pads, tampons, hydroentangled nonwovens, and other products containing cotton.

REVIEW OF FACTORS IN MILDEW GROWTH

Behavior of cotton with respect to the deterioration due to mildew involves a somewhat complex set of interacting factors. It is considered worthwhile to review them briefly.

Bleached cotton

Cotton, as obtained from fields, is inherently susceptible to attack by many microorganisms. These microorganisms are invariably present in the soil, water, air, and surface of the fiber. They become active whenever favorable situations for their growth occur. Practical methods for the prevention of mildew growth lie in understanding the growth habits of the microorganisms.

Description: Slurry of Methocel® preserved with Dowicil® 200 preservative

Sample	ATCC Bacterial Pool Challenge Number											A. niger Challenge Number				S. cerevisiae Challenge Number		
	IS*	1	2	3	4	5	6	7	8	9	10	IS*	1	2	3	1	2	3
Sample A Unpreserved slurry	9	9	9	9	9	9	9	9	10	10	10	-	-	+	±	++	++	++
Sample B 0.1% Dowicil 200 preservative	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
Sample C 0.2% Dowicil 200 preservative	1	1	1	1	4	1	1	1	1	1	1	-	-	-	-	-	-	-
Sample D 0.3% Dowicil 200 preservative	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
Sample E 0.1% Dowicil 200 0.05% Dowicide A	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
Sample F 0.2% Dowicil 200 0.05% Dowicide A	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-

V. Multiple challenge test results

Bleached cotton used in the manufacture of swabs, often U.S. Pharmacopoeia grade, is almost 100% pure cellulose, free from nitrogenous and other growth-stimulating materials. After hydrogen peroxide treatment at 100°C, the cotton becomes sterile. However, bacteria or microorganisms can be introduced during the subsequent stages of storage, transportation, carding, and/or converting operations.

Methocel sizing

A 1% solution of Methocel has been used in the manufacture of swabs as a binder for making an aesthetically pleasing cotton tip. Although an extremely small quantity of this sizing is present on the cotton tip, it can be a significant factor in the cause of mold, particularly in the presence of moisture and warm temperature and the absence of sunlight. Food such as nitrogen, sulfur, and organic/inorganic substances, in addition to a source of organic carbon, stimulate the growth.

Methocel/Dowicil solution

The Methocel cellulose ether product is a carbohydrate polymer that dissolves in water. It is important to disperse Methocel thoroughly in hot water (90°C) for at least 20 min before dissolving it with cold tap water (32-41°C) under good agitation. Good dispersion prevents lumping caused by formation of gels. Although Methocel is considered to be resistant to microorganisms, resistance to microorganisms is greatly increased by adding 0.1% Dowicil 200, a preservative. Dowicil 200 solution is added to Methocel solution and agitated for 30 min before use in swab manufacturing. Dowicil 200 is highly soluble in water, while remaining virtually insoluble in oils or organic solvents. As a result, it stays in the aqueous component, where microorganisms can live. Extensive toxicologic studies conducted on Dowicil 200 show a quite favorable toxicologic profile *(10)*. Therefore, it is safe for use on cotton swabs.

Moisture

All microorganisms require moisture for their continued growth. Many of them will withstand desiccation for a while and will quickly resume growth when moisture again becomes available. Plastic sealed bags are essentially unventilated storage units. They have been found to provide an ideal moisture chamber (under hot temperatures) for mildew growth. Most cotton-deteriorating microbials exhibit growth in the temperature range of 15-35°C. On average, a temperature of 30°C would be most suited for growth. An incubating temperature of 30°C is therefore accepted by scientists after inoculating with a known fungus.

Too high a temperature kills bacteria and fungi. Thus, sterilization of cotton is done by autoclaving it at a steam pressure of 15 psi for 30 min followed by vacuuming for 10 min.

Sunlight

Direct sunlight, and UV rays in particular, exert an inhibitory action upon

most microorganisms. UV rays are excellent for killing microbes.

Oxygen

Generally, the presence of oxygen tremendously increases the rate of growth.

Thus it is easy to see that action of microorganisms on cotton is greatly dependent on the environmental factors such as availability of moisture, oxygen, nitrogen, organic and inorganic food material, and absence of sunlight.

Storage environment

Four most important deteriorating situations have been:

- Shaded aboveground exposures
- Soil-contact exposures
- Sunny aboveground exposures
- Aquatic exposures

The present situation involved the storage of swabs in hot Florida aboveground indoor exposure — a shaded aboveground exposure, as described above. This environment is less drastic in its action on cotton than in soil-contact exposures. In shaded indoor storage, photochemical effects of sunlight on microbes are essentially absent. Mildew, in hot temperatures and high humidity (moisture provided by wetness of the swabs), manifests itself in the form of discoloration.

CONCLUSIONS

Sanitization of the Methocel preparation room, Methocel preparation tank, transfer tank, and swab making machines should be done simultaneously so that everything is free from any microbial contamination. Sanitization is carried out with a weak solution of sodium hypochlorite bleach solution. (Methocel slurry seems to get infested first. Introducing preservative in an infested slurry deactivates the capability of the preservative). If 0.1% Dowicil is added to freshly made Methocel, it gives excellent protection against mold and mildew development.

The healthcare products manufacturing company uses 0.2% Dowicil at present. We have recommended that if they exercise the other control options, they can reduce the preservative to the 0.1% level. (Dowicil costs $25/lb; therefore, significant savings will result.)

Apparently the swabs under investigation were manufactured with higher than normal moisture. The situation could have been aggravated by the use of sealed plastic bags and storing the swabs in Florida summer temperatures. Wetness in the buds provided the necessary moisture that, coupled with inadequately preserved Methocel, resulted in discoloration of the swabs.

Moisture level in the swabs should be low (preferably around 14% and definitely not above 20%). Swabs with higher moisture should be dried before storing and shipping.

The plant described in this article has discontinued the practice of using sealed plastic bags. Plastic bags with ventilation (punctured with 3 to 4 holes) could be used without causing mildew growth. A list of changes implemented is given in **Table III**. Since implementing these changes, mildew has not recurred in two and half years. **TJ**

KEYWORDS

Biological tests, cotton, fungi, microorganism control, moisture content, preservatives, problem solving, protectants, sanitation, storage, test methods, trouble shooting.

Parikh is a materials and research engineer and Calamari is a research leader, Textile Engineering Research, with the U.S. Department of Agriculture, Agricultural Research Service, Mid South Area Southern Regional Research Center, New Orleans, LA. Tao is a postdoctoral research associate with the USDA. Briggs is president, Wyant Health Care, Wyant Health Care (formerly Hosposable Products), Sommerville, NJ.

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6. Methocel (methyl cellulose ether) and Dowicil 200 (a preservative for antimicrobial protection) are from Dow Chemical USA. The mention of company or commercial products does not imply recommendation or endorsement by the U. S. Department of Agriculture to the exclusion of others that may also be suitable.
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9. Biological cleanliness depends to a large extent on end use of a medical or hospital grade product. Decontamination usually denotes total or substantial removal of infectious or disease- causing microorganisms from materials to a level considered safe from health or medical perspective. Disinfection is done by use of disinfectant. Disinfectant is an agent that would free anything from infection. Sterilization is a process of freeing from living microorganisms. The sterilized material is "germ-free."
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APPENDIX

Multiple challenge test method for the evaluation of preservative effectiveness.

1. 50g samples of the formulation to be tested are weighed into 2 oz screwed-capped glass bottles.
2. Cotton swabs (control) are used to prepare Initial Streaks (IS) on the appropriate medium. Tryptic Soy Agar (Difco) is used for bacterial and Malt Agar (Difco) with 0.3% yeast Extract (Difco) is used for fungi. Plates are incubated at 30-32°C for 48 h and rated for growth. (See growth rating chart.)
3. Each sample is inoculated with 0.1 ml of a mixture of 24 h cultures of ATCC Bacterial Pool, grown individually in pure culture on a shaker at 30-32°C and mixed in equal amounts just before use. The final concentration is approximately 2 million colony forming units (CFU)/g of formulation. Additional samples may be tested against typical yeast and/or mold. Cells for fungal inoculation are harvested from pure-culture slants with saline. When used at 0.1 ml/50 g, this inoculum 5 10 yields a final concentration of approximately 100,000 CFU/g of formulation.
4. Inoculated samples are incubated at 30-32°C for 24 h (48 h for fungi) and then are streaked into agar plates, as above. Plates are incubated at 30-32°C for 48 h and rated for growth.
5. Steps 3 and 4 are repeated for the desired number of challenge cycles. Any sample which exhibits a rating of "4" ("+" for fungi) or higher will not be reinoculated unless the rating drops below "4" (or "+") in a subsequent incubation-enumeration period.

Table IV lists the classifications of growth rates. **Table V** provides the results of the multiple challenge texts.

Organisms used in multiple challenge test

ATCC bacterial pool

Bacilus subtilis — ATCC 8473
Enterobacter aerogenes — ATCC 13048
Escherichia Coli — ATCC 11229
Klebsiella pneumoniae — ATCC 8308
Proteus Vulgaris — ATCC 881
Pseudonomonas aeruginosa — ATCC 10145
Pseudonomonas aeruginosa — ATCC 15442
Salmonella choleraesuis — ATCC 10708
Staphylococcus aureus — ATCC 6538

Mold

Aspergillus niger — ATCC 16404

Yeast

Saccaharomyces cerevisiae — ATCC 4105