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(54) Title: DISINFECTING SOLUTIONS EFFECTIVE AGAINST BACTERIAL ENDOSPORES

(57) Abstract: The present invention is directed to a biguanide-containing disinfecting solutions effective in inactivating bacteria endospores on surfaces, air-borne or in water. The methods of using the present invention are directed to disinfecting endospore laden surfaces, air and water with the subject biguanide-containing solutions.

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DISINFECTING SOLUTIONS EFFECTIVE AGAINST BACTERIAL ENDOSPORES

Background of the Invention:

The present invention is directed to biguanide-containing disinfecting solutions effective as rapid bacterial endosporicidals. More particularly, the present invention is directed to biguanide-containing rapid disinfecting solutions effective against bacterial endospores belonging to the Family of *Bacillaceae,* such as but not limited to those members of the Genus *Bacillus* and the Genus *Clostridium*, the two classified endospore forming bacteria. The present invention is likewise directed to methods for making and using the same.

Summary of the Invention:

The most resistant form of life is the bacterial endospore. This stage in the life of certain bacteria is a response to environmental signals that forecast problems for the survival of the vegetative (reproducing) cell. One such signal is a reduction in the water surrounding the cell. The vegetative cell undergoes a series of changes resulting in the formation of metabolically inert endospores, which are highly resistant to heat, chemicals and UV light. The vegetative cell changes include: decrease in internal water concentration; formation of several thick layers or coats that surround the cell's deoxyribonucleic

acid (DNA) and a portion of its cytoplasm; and the presence of large amounts of calcium and dipicolinic acid (DPA). The resultant endospore is a dormant form and does not reproduce. Endospores are difficult to kill except by strong chemicals, high heat, or gamma irradiation. When conditions signal a favorable environment, the endospores will germinate and the emergent vegetative cell can resume to replicate.

Although bacteria from about a half dozen genera can form endospores, the most common endospore-formers are members of the Genus *Bacillus* and the Genus *Clostridium*. Both are Gram positive rods, but *Bacillus* are aerobic or facultatively anaerobic whereas Clostridium are anaerobic. The endospore, which may survive for prolonged periods in the environment, may infect and germinate inside the body. For example, the etiological agent of tetanus, *Clostridium tetani*, will reside in the soil as an endospore, but if it enters the hospitable warmth, moisture and nutrients of a deep (anaerobic) wound, it will return to the actively reproducing vegetative stage.

The Genus *Bacillus* and the Genus *Clostridium* each contains organisms that are pathogenic. Such organisms may be infectious through one or more of the following mechanisms of exposure, depending upon the particular type of organism: inhalation, with infection through respiratory mucosa or lung tissues; ingestion; contact with the mucous membranes of the eyes, or nasal

tissues; or penetration of the skin through open cuts or abrasions. Chemical disinfection of endospore-forming bacteria and their endospores involves the use of strong chemical agents in the form of a liquid, gas or aerosol.

While many antibacterial and antimicrobial agents are known, few agents are effective in the destruction or inactivation of bacteria endospores. The principal disinfecting agents for destruction or inactivation of bacteria endospores are formaldehyde, glutaraldehyde (at pH 8.0-8.5), hydrogen peroxide and peracetic acid (Dietz and Bohm, 1980; Bohm, 1990). Hypochlorites are sporicidal but are rapidly neutralized by organic matter and, therefore, while good for disinfecting non-wooden surfaces, are unsuitable for disinfecting most environmental sites or materials. Hydrogen peroxide and peracetic acid are not appropriate disinfecting agents if blood is present.

Although effective endospore disinfecting agents, formaldehyde, glutaraldehyde, hydrogen peroxide, peracetic acid and chlorine compounds are toxic to humans and largely dangerous to handle. Formaldehyde (formalin) is poisonous and a suspect cancer hazard. The risk of cancer from formaldehyde depends on the level and duration of exposure. Formaldehyde (formalin) vapor is harmful if inhaled or absorbed through the skin. Formaldehyde is very corrosive and can not be made nonpoisonous. It is also flammable as a liquid

and as a vapor. The endospore disinfecting agent glutaraldehyde is likewise very corrosive and harmful if swallowed, inhaled, or absorbed through the skin. Hydrogen peroxide, while less dangerous to handle than formaldehyde and glutaraldehyde, may be harmful if swallowed and is known to cause eye irritation. The endospore disinfectant peracetic acid, like formaldehyde and glutaraldehyde is very corrosive and all contact should be avoided. Peracetic acid is corrosive to the eyes, the skin, the respiratory tract and upon ingestion. Inhalation of peracetic acid may cause lung oedema. It is also flammable and explosive. The endospore disinfectant chlorine dioxide is corrosive by inhalation and can cause serious skin burns. It is also flammable and explosive.

Accordingly, there is a need for improved bacterial endosporicidal agents that rapidly and effectively destroy bacterial endospores and maintain a low order of toxicity in humans.

Summary of the Invention:

In accordance with this invention, there is provided solutions effective for rapidly disinfecting bacterial endospore laden surfaces, air and/or water comprising bacterial endosporicidally effective amounts of a biguanide or water-soluble salts thereof. The subject biguanide-containing

solutions are useful in the rapid destruction of bacterial endospores, are non-toxic to humans for ease in handling, are simple to use and do not cause tissue irritation upon contact. The solutions of the present invention are particularly useful in instances where repeated hand washing and tissue contact is necessary, such as for example with medical care providers, since the subject solutions are non-irritating to tissue.

Accordingly, it is an object of the present invention to provide biguanide-containing solutions useful as bacterial endosporicidal agents.

Another object of the present invention is to provide a method for using biguanide-containing solutions useful as bacterial endosporicidal agents.

Another object of the present invention is to provide a method for making biguanide-containing solutions useful as bacterial endosporicidal agents.

Another object of the present invention to provide biguanide-containing solutions useful as bacterial endosporicidal agents that are non-toxic to humans.

Another object of the present invention to provide biguanide-containing solutions useful as bacterial endosporicidal agents that are easy to handle.

Another object of the present invention is to provide biguanide-containing solutions that do not cause tissue irritation upon contact therewith.

Still another object of the present invention is to provide biguanide-containing solutions with rapid bacterial endosporicidal activity.

These and other objectives and advantages of the present invention, some of which are specifically described and others that are not, will become apparent from the detailed description and claims that follow.

Detailed Description of the Invention:

The biguanide-containing solutions of the present invention are useful in killing bacterial endospores on surfaces, in the air or in water. The solutions of the present invention are particularly useful in instances where repeated hand washing and/or tissue contact is necessary, such as with medical care providers, since the same are non-irritating to tissue. "Non-irritating to tissue" means that the subject solutions do not cause adverse skin reactions such as redness, swelling and/or itching or burning sensations upon contact. Given the fact that bacterial endospores are the most resistant form of life, it is surprising that the subject biguanide-containing aqueous solutions are so highly

effective as rapid bacterial endosporicidals while being non-toxic to humans. The subject biguanide-containing solutions are also desirable as bacterial endosporicidals in that they have no harmful or offensive fumes.

The biguanide-containing solutions of the present invention comprise at least approximately 100 ppm, but more preferably at least approximately 100 ppm to less than approximately 200,000 ppm of one or more biguanides, and most preferably greater than approximately 1000 ppm to less than approximately 150,000 ppm of one or more biguanides. Suitable "biguanides" as referred to herein include biguanides, bis-biguanides and poly-biguanides. Such biguanides include for example but are not limited to chlorhexidine (1,1'-hexamethylene-bis[5-(p-chlorophenyl)biguanide]) or water soluble salts thereof, such as chlorhexidine gluconate, poly(hexamethylene biguanide) or water soluble salts thereof, such as poly(hexamethylene biguanide) hydrochloride, and 1,1'-hexamethylene-bis[5-(2-ethylhexyl)biguanide] (Alexidine) and chlorhexidine. Biguanides are described in U.S. Patent Number 5,990,174 incorporated herein in its entirety by reference. The preferred biguanide due to its ready commercial availability and superior endosporicidal effectiveness is poly(aminopropyl biguanide) (PAPB), also commonly referred to as poly(hexamethylene biguanide) (PHMB). The subject solutions preferably

likewise comprise deionized water and one or more buffers.

The pH of the subject solutions can range between 4.0 to 9.0 but will generally range between about 6.0 to about 8.0, and more preferably between 6.5 to 7.5. As mentioned above, one or more buffers may be employed to obtain the desired pH value. Tromethamine (tris(hydroxymethyl)aminomethane, (TRIS)) is a known buffer, which is suitable for use in the present invention. As an alternative, the subject solutions may include a supplemental buffering agent, meaning that the subject solutions may include a "mixed buffer" of tromethamine and one or more other buffer agents. Other suitable buffers include for example but are not limited to borate buffers such as but not limited to boric acid, potassium tetraborate, potassium metaborate, sodium borate and mixtures thereof, phosphate buffers such as but not limited to Na_2HPO_4, NaH_2PO_4, KH_2PO_4 and mixtures thereof, citrate buffers such as for example but not limited to sodium citrate, potassium citrate, citric acid and mixtures thereof, sodium bicarbonate, amino alcohol buffers and combinations thereof. Preferably, solutions of the present invention include TRIS and sodium borate in combination with sodium chloride or glycerin. One or more buffers are preferably added to solutions of the present invention in amounts ranging between approximately 0.01 to 2.0 weight percent by volume,

but more preferably between approximately 0.05 to 0.5 weight percent by volume.

Solutions of the present invention may be designed for a variety of osmolalities. Preferably, solutions in accordance with the present invention have an osmotic value of less than about 350 mOsm/kg, more preferably from about 175 to about 330 mOsm/kg, and most preferably from about 175 to about 320 mOsm/Kg. One or more osmolality adjusting agents may be employed in the subject solutions to obtain the desired final osmolality. Suitable osmolality adjusting agents include for example but are not limited to sodium and potassium chloride, monosaccharides such as dextrose, calcium and magnesium chloride, and low molecular weight polyols such as glycerin and propylene glycol. Typically, these agents are used individually in amounts ranging from about 0.01 to 5 weight percent and preferably, from about 0.1 to about 2 weight percent.

Preferably, the subject solutions likewise include one or more poly(ethylene oxide) (PEO) containing materials, which serve to assist in solubilization and hydration. Suitable PEO-containing materials include for example but are not limited to certain polyethyleneoxy-polypropyleneoxy block copolymers, also known as poloxamers. Such materials are commercially available under the trade name Pluronic™ and Tetronic™ (BASF Corporation, Parsippany, NJ), e.g., Tetronic 904, Tetronic 1307, Pluronic F127, Pluronic 105 and Pluronic 123. One or more PEO-containing materials are preferably added to

solutions of the present invention in amounts ranging between approximately 1.0 to 20 weight percent by volume, but more preferably between approximately 10 to 15 weight percent by volume and most preferably approximately 12 weight percent by volume. Suitable PEO-containing materials likewise include for example N-alkyl PEO monoethers and a material commercially available under the trade name Brij™ 700 (ICI Americas Corporation, Edison, NJ).

Preferably, the subject solutions likewise include one or more wetting agents, which serve to increase binding to surfaces by hydrogen-bonding interactions, hydrophobic interactions, and where appropriate, ionic interactions. Suitable wetting agents include for example but are not limited to cellulosic materials including cationic cellulosic polymers, hydroxypropyl methylcellulose, hydroxyethyl cellulose and methylcellulose, poly(vinyl alcohol), and poly(N-vinylpyrrolidone). A preferred class of wetting agent is the cationic cellulosic materials that have the ability to associate with anionic areas on surfaces. Such materials include polyquaternium-10 available under the trade name Polymer JR™-30 M (ICI Americas Corporation). Other wetting agents include quaternized guar gums such as guar hydroxypropyltrimonium chloride and hydroxypropyl guar hydroxypropyltrimonium chloride, and particularly products available under the trade name Jaguar™ (Rhodia, Cranberry, NJ). Such wetting agents may be used in a wide range of concentrations in the solutions of the present invention, generally within the range of approximately 0.01 to 10 weight percent by volume.

Optionally, the subject solutions may likewise include one or more additional surfactants to effect surface cleaning. Suitable surfactants include for example but are not limited to cationic, anionic, nonionic and amphoteric surfactants. Preferred surfactants are nonionic and amphoteric surfactants. The surfactants used in the present invention should be soluble in the aqueous solution and non-irritating to tissues.

Suitable non-ionic surfactants include for example polyethylene glycol esters of fatty acids, e.g., coconut, polysorbate, polyoxyethylene or polyoxypropylene ethers of higher alkanes (C_{12}-C_{18}), polysorbate 20 available under the trademark Tween® 20 (ICI Americas Corporation), polyoxyethylene (23) lauryl ether available under the trademark Brij® 35, (ICI Americas Corporation), polyoxyethyene (40) stearate available under the trademark Myrj® 52, (ICI Americas Corporation) and polyoxyethylene (25) propylene glycol stearate available under the trademark Atlas® G 2612 (ICI Americas Corporation).

Amphoteric surfactants suitable for use in solutions according to the present invention include materials of the type offered commercially under the trademark Miranol™ (Rhodia). Another useful class of amphoteric surfactants is exemplified by cocoamidopropyl betaine, commercially available from various sources.

Various other ionic as well as amphoteric and anionic surfactants suitable for use in the present invention are described in *McCutcheon's Detergents and Emulsifiers*, North American Edition, McCutcheon Division, MC Publishing Co., Glen Rock, NJ 07452 and the *CTFA International Cosmetic Ingredient Handbook*, Published by The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.

Preferably, one or more surfactants, when present in the subject solutions, are employed in a total amount from about 0.01 to about 17 weight percent, preferably 0.1 to 15 weight percent, and most preferably 0.1 to 12 weight percent.

One or more chelating agents can also be added to the subject solutions. Suitable chelating agents include for example but are not limited to hydroxyalkyl-phosphonates as disclosed in US Patent No. 5,858,937 (Richards et al.), and available under the tradename Dequest® (Monsanto Co., St. Louis, MO).

Test formulations of biguanide-containing solutions in accordance with the present invention, and in some instances non-biguanide-containing solutions, are set forth in Table 1 below.

Table 1

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.1	Soln.2	Soln.3	Soln.4	Soln.5	Soln.6	Soln.7	Soln.8
Water	QS to 100%	X	X	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X	X	X
Polymer JR	0.04%	X	X	X	X	X	X	X	X
Alexidine	10 ppm	X	X	X	X	X	X	X	X
PHMB	x ppm	5	5	5	1000	100	20	10	1
Tetronic 904	3%	X	X	X	X	X	X	X	X
Tetronic1307	3%	X	X	X	X	X	X	X	X
Pluronic F127	1%	X	X	X	X	X	X	X	X
Pluronic 105	1%	X	X	X	X	X	X	X	X
Pluronic123	1%	X	X	X	X	X	X	X	X
BRIJ	3%	X	X	X	X	X	X	X	X
Osmolality (mOsm/kg)		206	200	195	199	199	199	199	199
pH		7.00	7.00	7.01	7.03	7.03	7.03	7.03	7.03

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 - Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.9	Soln.10	Soln.11	Soln.12	Soln.13	Soln.14	Soln.15	Soln.16
Water	QS to 100%	X	X	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X	X	X
Polymer JR	x%	0.04	0.08	0.12	0.24	0.50	0.04	0.04	-
Alexidine	10 ppm	X	X	X	X	X	X	X	-
PHMB	x ppm	5	5	5	5	5	100	100	-
Tetronic 904	3%	X	X	X	X	X	X	-	X
Tetronic 1307	3%	X	X	X	X	X	X	-	X
Pluronic F127	1%	X	X	X	X	X	X	-	X
Pluronic 105	1%	X	X	X	X	X	X	-	X
Pluronic 123	1%	X	X	X	X	X	X	-	X
BRIJ	3%	X	X	X	X	X	X	-	X
Glycerin	1.2%	-	-	-	-	-	-	X	-
Osmolality (mOsm/kg)		212	212	212	212	212	201	199	208
pH		6.95	6.95	6.95	6.95	6.95	6.97	6.97	7.09

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 - Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.17	Soln.18	Soln.19	Soln.20	Soln.21	Soln.22
Water	QS to 100%	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X
Polymer JR	0.04%	X	X	X	X	X	X
Alexidine	10 ppm	X	X	X	X	X	X
PHMB	100 ppm	X	X	X	X	X	X
Tetronic 904	12%	X	-	-	-	-	-
Tetronic1307	12%	-	X	-	-	-	-
Pluronic F127	12%	-	-	X	-	-	-
Pluronic 105	12%	-	-	-	X	-	-
Pluronic 123	12%	-	-	-	-	X	-
BRIJ	12%	-	-	-	-	-	X
Osmolality (mOsm/kg)		234	231	195	206	213	215
pH		7.00	7.00	7.00	7.00	7.00	7.00

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 - Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.23	Soln.24	Soln.25	Soln.26	Soln.27	Soln.28	Soln.29
Water	QS to 100%	X	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X	X
Polymer JR	0.04%	X	-	-	X	X	-	X
Alexidine	10 ppm	-	X	-	X	-	X	X
PHMB	100 ppm	-	-	X	-	X	X	X
Tetronic 904	3%	X	X	X	X	X	X	X
Tetronic 1307	3%	X	X	X	X	X	X	X
Pluronic F127	1%	X	X	X	X	X	X	X
Pluronic 105	1%	X	X	X	X	X	X	X
Pluronic 123	1%	X	X	X	X	X	X	X
BRIJ	3%	X	X	X	X	X	X	X
Osmolality (mOsm/kg)		198	190	195	192	194	203	195
pH		7.00	7.00	7.00	6.9	7.00	7.00	7.00

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 – Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.30	Soln.31	Soln.32	Soln.33	Soln.34	Soln.35	Soln.36	Soln.37
Water	QS to 100%	X	X	X	X	X	X	X	X
Tris Base	x%	0.25	0.25	0.14	0.14	-	-	-	-
Na Borate	x%	-	-	0.22	0.22	0.018	0.018	-	-
NaCl	x%	0.51	-	0.50	-	0.50	-	0.45	-
Glycerine	x%	-	1.52	-	1.32	-	1.60	-	1.25
PHMB	100 ppm	X	X	X	X	X	X	X	X
Boric Acid	0.12%	-	-	-	-	X	X	-	-
Na Phos. (m)	0.08%	-	-	-	-	-	-	X	X
Na Phos. (di)	0.39%	-	-	-	-	-	-	X	X
Osmolality (mOsm/kg)		196	197	223	208	190	197	218	227
pH		6.9	6.98	7.08	6.98	6.99	7.02	7.00	7.04

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 - Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.38	Soln.39	Soln.40	Soln.41	Soln.42	Soln.43
Water	QS to 100%	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X
Polymer JR	0.04%	-	-	-	-	-	X
Alexidine	x ppm	5	10	20	50	100	100
PHMB	100 ppm	-	-	-	-	-	X
Tetronic 904	3%	X	X	X	X	X	X
Tetronic 1307	3%	X	X	X	X	X	X
Pluronic F127	1%	X	X	X	X	X	X
Pluronic 105	1%	X	X	X	X	X	X
Pluronic 123	1%	X	X	X	X	X	X
BRIJ	3%	X	X	X	X	X	X
Osmolality (mOsm/kg)		185	183	180	185	180	191
pH		6.92	6.96	7.17	7.03	6.95	7.00

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

Table 1 – Continued

Bacterial Endosporicidal Test Solutions

Components	Concentration	Soln.44	Soln.45	Soln.46	Soln.47	Soln.48	Soln.49	Soln50
Water	QS to 100%	X	X	X	X	X	X	X
Tris Base	0.12%	X	X	X	X	X	X	X
Na Borate	0.22%	X	X	X	X	X	X	X
Polymer JR	0.04%	X	X	X	X	X	X	X
Alexidine	10 ppm	X	X	X	X	X	X	X
PHMB	x ppm	100	100	100	100	100	100	1000
Tetronic 904	3%	X	X	X	X	X	X	X
Tetronic 1307	3%	X	X	X	X	X	X	X
Pluronic F127	1%	X	X	X	X	X	X	X
Pluronic 105	1%	X	X	X	X	X	X	X
Pluronic 123	1%	X	X	X	X	X	X	X
BRIJ	3%	X	X	X	X	X	X	X
Osmolality (mOsm/kg)		195	201	197	194	188	185	199
pH		4.00	5.00	6.00	7.00	8.00	9.00	7.03

X = the amount provided under "concentration" or that amount specified for the particular test solution

- = component not added to solution

The biguanide-containing bacterial endosporicidal solutions of the present invention are described in still greater detail in the examples that follow.

EXAMPLE 1 – Determination of Minimal Inhibitory Concentration of Selected Test Solutions Against 10^3 Endospores in Modified Trypticase Soy Broth on Cellulose Membrane:

The object of the present study was to determine the minimal inhibitory concentration of selected test solutions against 10^3 endospores in Modified Trypticase Soy Broth (MTSB) on cellulose membrane. To do this, 0.1 ml of a 10^4 suspension of *Bacillus stearothermophilus* endospores in 50 mM phosphate buffer with a pH of 7.0, was added to 20 ml of sterile phosphate buffer and the entire contents were then filtered through a 0.2 μm filter. Filters containing endospores (10^3 spores/membrane) were then placed on absorbent pads, which had been previously soaked with 1.8 ml of solution containing diluted test solution (2, 10, 50 and 100-fold dilutions) in MTSB. The pads/filters were then placed in individual petri dishes and incubated at 55° C. Recovery of spores was examined after both 24 hours and 48 hours.

Materials used in the subject study are listed below.

1. Test solutions 3-7, 14-29 and a 1:1 mixture of solutions 23 and 24
2. 50 mM phosphate buffer, pH 7.0

   Sodium phosphate monobasic -NaH_2PO_4 (Sigma # S-0751, Sigma Chemical Company, St. Louis, MO)

   Sodium phosphate dibasic – Na_2HPO_4 (Fisher # S-374, Fisher Scientific, Swanee, GA)
3. Modified TSB medium for spore recovery and growth of vegetative cells

   Bacto™ Tryptic Soy Broth (TSB), (DIFCO #211825, Lot #0341002, Becton, Dickinson, & Co., Sparks, MD)

   Sodium thiosulfate - $Na_2S_2O_3$-$5H_2O$ (Fisher #S-445)

   Tween™ 80 (J.T. Baker, #X257-07, Mallinckrodt, St. Louis, MO and Baker, Inc., Phillipsburg, NJ)
4. Certified spore suspension of *B. stearothermophilus*, 2 x 10^7 spores/ml (STS, Sterilization Technical Services, Rush, New York)
5. 15 ml polypropylene conical tube (Falcon, #352097, Becton, Dickinson, & Co. Sparks, MD)
6. Sterile petri dishes
7. 47 mm sterile cellulose pads

8. Sterile 0.2μ filters (Millipore, Billerica, MA)
9. 6-place filter manifold systems (Gelman, Pall Corporation, East Hills, NY)
10. 47 mm vacuum filter units (Pall, Pall Corporation, East Hills, NY)
11. A vacuum pump
12. Refrigerated incubator shaker (Innova 4230, New Brunswick Scientific, Edison, New Jersey)

In the subject study, dilutions of the test solutions (2-, 10-, 50- and 100-fold dilutions) were made from the original test solutions into MTSB. Test solution 4 was diluted 10-fold prior to making the above dilutions in MTSB. Duplicate 1.8 ml aliquots of the diluted test solutions in MTSB were added to 47 mm sterile cellulose pads contained in sterile petri dishes. Individual 0.1 ml aliquots of diluted *B. stearothermophilus* spore solution (1.0×10^4 spores/ml) were added to 20 ml buffer and the entire contents were filtered through a 0.2 micron membrane filter. Individual membrane filters were then transferred to petri dishes containing the prepared sterile cellulose pads. Membranes were slightly bent when placed on the wetted pads to ensure proper contact, and then rolled onto the pad to eliminate bubbles and voids. The petri dishes were

incubated at 55° C. Recovery was examined after 24 and 48 hours of incubation. A control with 1.8 ml MTSB only on the cellulose pad was also examined. The results of the subject study are set forth below in Table 2.

Table 2

Ranking of activity based upon recovery of *B. stearothermophilus* on cellulose membranes after incubation for 48 hours at 55° C

	Dilutions							
Solution	**2**		**10**		**50**		**100**	
14	-	-	-	-	-	-	+	+
27	-	-	-	-	-	-	+	+
15	-	-	-	-	1	1	+	+
25	-	-	-	-	1	1	+	+
20	-	-	-	-	1	1	+	+
18	-	-	-	-	2	1	+	+
4	-	-	-	-	4	2	+	+
22	-	-	-	-	3	11	+	+
19	-	-	-	-	6	3	+	+
5	-	-	-	-	5	5	+	+
21	-	-	-	-	10	10	+	+
17	-	-	-	-	8	15	+	+
28	-	-	-	-	22	5	+	+
29	-	-	-	-	52	58	+	+
6	-	-	47	49	+	+	+	+
7	-	-	136	+	+	+	+	+
3	-	-	+	+	+	+	+	+
24	-	-	+	+	+	+	+	+
23+24	-	-	+	+	+	+	+	+
26	-	-	+	+	+	+	+	+
16	+	+	+	+	nd	+	+	+
23	+	+	+	+	+	+	+	+
control				+	+			

+ = >150 cfu/membrane
- = no growth
nd = not determined

According to the above study, none of the test solutions was effective against 10^3 spores when diluted 100-fold with MTSB. A summary of the ranking of test solutions based upon recovery after 48 hours at 55° C is set forth below.

Group 1: Effective up to 50-fold dilution - **Solutions 14 and 27.**

Group 2: Moderately effective at 50-fold dilution, having slight break-through in growth with < 10 cfu recovered – **Solutions 15, 25, 20, 18, 4, 22, 19 and 5.**

Group 3: Partially effective at 50-fold dilution, having break-through in growth with >10 but <60 cfu recovered – **Solutions 21, 17, 28 and 29.**

Group 4: Partially effective at 10-fold dilution, having break-through in growth with >50 cfu recovered – **Solutions 6 and 7.**

Group 5: Effective at a 2-fold dilution – **Solutions 3, 24, 23+24 and 26.**

Group 6: Ineffective at a 2-fold dilution (no biguanide present) – **Solutions 23 and 16.**

EXAMPLE 2 – Determination Of Anti-Microbial Activity For Selected Solutions Against 10^4 Spores Of *B. globgii* (Membrane Recovery Test):

Solutions to be evaluated were diluted with Modified Trypticase Soy Broth (MTSB) and then 10^4 *B. globigii* spores were added to the diluted test solutions. Recovery of spores (as measured by outgrowth of vegetative cells) was determined after incubation at 30° C for 24 hours. Solutions 16 and 14 were used as controls. The ranking of solutions so tested, set forth below in Tables 4A and 4B, is based upon recovery after 24 hours at 30° C. When considering the results summarized in Tables 4A and 4B below, it should be noted that *B. globigii* grows much faster than *B. stearothermophilus*. Also, the criteria used for the ranking of solutions in Tables 4A and 4B as set forth below in Table 3 are the same criteria used to rank activity against *B. stearothermophilus* spores. Overall, the rankings tend to be better for Minimal Inhibitory Concentration (MIC) testing than the membrane testing.

Table 3

Criteria Used for Ranking Endosporicidal Test Solutions

Group 1: Effective at >100 fold dilution. 1++ (33 and 32) / 1+ (31) / 1 **(Solutions 45, 49, 44, 14 and 47)**

Group 2: Moderately effective at 50-fold dilution **(Solutions 48 and 46)**

Group 3: Partially effective at 50-fold dilution **(Solutions 35, 36, 37, 34, 42, and 30)**

Group 4: Partially effective at 10-fold dilution.

Group 5: Effective at a 2-fold dilution

Group 6: Ineffective at any dilution tested **(Solutions 41, 38, 40, 39 and 16)**

Table 4A

RANKING OF ANTI-MICROBIAL ACTIVITY AGAINST ENDOSPORES OF *B. globigii* : MEMBRANE RECOVERY TEST
(Recovery after 24 hours)

	Dilutions				
Solution	**2**	**10**	**50**	**100**	**Group rank**
31	-/-	-/-	1/-	1/-	1
33	-/-	-/-	-/-*	+/+	1
32	-/-	-/-	-/-*	+/+	1
30	-/-	-/-	-/-*	+/+	1
14 (- control)	-/-	-/-	-/-*	+/+	1
36	-/-	-/-	-/-*	+/+	1
35	-/-	1/5	-/-	+/+	1
46	-/-	11/14*	-/-	+/+	1
44	-/-	22/42	+/+	+/+	2
47	-/-	15/18	+/+	+/+	2
49	-/-	10/25	+/+	+/+	3
41	-/-	-/1	+/+	+/+	3
40	-/-	1/1	+/+	+/+	3
42	-/-	1/1	+/+	+/+	3
48	-/-	19/25	+/+	+/+	3
45	-/-	41/71	+/+	+/+	3
37	-/-	13/+	+/+	+/+	6
33	-/-	30/+	+/+	+/+	6
38	-/-	+/+	+/+	+/+	6
39	-/-	+/+	+/+	+/+	6
16 (+control)	+/+	+/+	+/+	+/+	6

* No growth at 24 hours, but growth noted at 48 hours.

Table 4B

RANKING OF ANTI-MICROBIAL ACTIVITY AGAINST ENDOSPORES OF *B. globigii*: Based upon 24h recovery MIC

	Dilutions					
Solution	**50**	**100**	**200**	**400**	**800**	**Group rank**
33	-/-	-/-	-/-	-/-	+/+	1++
32	-/-	-/-	-/-	-/-	+/+	1++
31	-/-	-/-	-/-	+/+	+/+	1+
45	-/-	-/-	+/+	+/+	+/+	1
49	-/-	-/-	+/+	+/+	+/+	1
41	-/-	-/-	+/+	+/+	+/+	1
14	-/-	-/-	+/+	+/+	+/+	1
47	-/-	-/-	+/+	+/+	+/+	1
48	-/-	-/+	+/+	+/+	+/+	2
46	-/-	+/-	-/-	+/+	+/+	2
35	-/-	+/+	+/+	+/+	+/+	3
36	-/-	+/+	+/+	+/+	+/+	3
37	-/-	+/+	+/+	+/+	+/+	3
34	-/-	+/+	+/+	+/+	+/+	3
42	-/-	+/+	+/+	+/+	+/+	3
30	-/-	+/+	+/+	+/+	+/+	3
41	+/+	+/+	+/+	+/+	+/+	6
38	+/+	+/+	+/+	+/+	+/+	6
40	+/+	+/+	+/+	+/+	+/+	6
39	+/+	+/+	+/+	+/+	+/+	6
16	+/+	+/+	+/+	+/+	+/+	6
+control	+/+	+/+	+/+	+/+	+/+	
- control	-/-	-/-	-/-	-/-	-/-	

No growth at 24 hours, but growth noted at 48 hours.

EXAMPLE 3 – Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore (5 minute exposure):

The objective of this study was to determine the efficacy of selected test solutions against 5 x 10^6 spores of *Bacillus globigii* on a glass surface. In this study, the ASTM (American Society for Testing and Methods) standard method for efficacy of sanitizers recommended for inanimate non-food contact surfaces, E-1153-94, was used but modified to assess the activity against endospores of *B. globigii* rather than the dried cells of *Klebsiella* as specified by the test. To do this, to the surface of previously cleaned and sterilized glass coupons (25 x 25 mm), 20 µl of a 2.5 x 10^8 spores/ml spore suspension of *Bacillus globigii* endospores was added and then spread over the surface of the coupon within 3 mm of the edge. The film was then allowed to air dry. Note: The ASTM test calls for drying at 35% relative humidity (RH). As the level of performance in this new test could not be anticipated, drying was accomplished under ambient conditions in a sterile hood. The test coupons were then exposed to 5.0 ml of a selected test solution at room temperature for 5 minutes. The same was then neutralized by the addition of 20 ml of sterile Dye-Engly neutralizing broth (D/E) followed by standardized mixing, i.e., jars containing the coupons were rotated in a single plane only, for 30 seconds at 300 rpm. The number of viable endospores remaining after treatment was enumerated by

standard plate count on TSA plate. Numbers of surviving, viable endospores were determined by the enumeration of colonies (cfu) of vegetative cells after 24 hours incubation at 30° C.

Materials used in the subject study are listed below.

1. Test solutions 4, 14, 41, 42, 43 and 61.
2. 50 mM phosphate buffer, pH 7.0

   Sodium phosphate monobasic -NaH_2PO_4 (Sigma # S-0751, Sigma Chemical Company, St. Louis, MO)

   Sodium phosphate dibasic – Na_2HPO_4 (Fisher # S-374, Fisher Swanee, GA)
3. Difco™ Tryptic Soy Agar (TSA), (DIFCO #236950, Lot #2190630, Becton, Dickinson, & Co., Sparks, MD)
4. Certified spore suspension of *B. globigii*, 2.5×10^8 spores/ml (STS, Sterilization Technical Services, Rush, NY)
5. Sterile petri dishes, 60 x 15 mm, polystyrene (Falcon, #3351007, Becton, Dickinson, & Co., Sparks, MD)
6. Glass slide cut to 25 x 25x 1 mm, polished and washed (VWR #48300-025)
7. Wide-mouth polypropylene autoclavable jars, 60 ml (Fisher #02891B, Swanee, GA)

8. Refrigerated incubator (Innova™ 4230, New Brunswick Scientific, Edison, New Jersey)
9. Mixer/titer plate shaker (Lab-Line Instruments, Inc., Melrose Park, IL)
10. Laminar hood, Class II, type A, biohazard cabinet (Envirco Corporation, Albuquerque, NM)
11. Dye-Engly Broth (D/E) Difco (Becton, Dickinson, & Co., Sparks, MD)

In the subject study, all manipulations were conducted within a sterile hood unless otherwise specified. To conduct the study, a single, cleaned, sterile, glass square (25 x 25 mm) was placed into a sterile 60 mm petri dish. To each coupon, 20 µl of a 2.5 x 10^8 spores/ml *B. globigii* spore suspension was added and then spread to within 3 mm of the edge of the coupon. The inoculated coupons were then air-dried overnight. The individual coupons were then transferred to sterile 60 ml jars. Test solutions were then prepared by i) making a 10-fold serial dilution of sample by transferring a 0.1 ml aliquot of neutralized test solution to 0.9 ml phosphate buffer, ii) transferring duplicate 0.1 ml aliquots from two appropriate dilution levels to TSA plates and spreading, and iii) transferring triplicate 0.01 ml aliquots from 4 dilution levels to TSA plates.

Control A solutions were then prepared by i) making a 10-fold serial dilution of sterile water by transferring 0.1 ml aliquot of neutralized sterile water to 0.9 ml phosphate buffer, ii) transferring duplicate 0.1 ml aliquots from two appropriate dilution levels to TSA plates and spreading, and iii) transferring triplicate 0.01 ml aliquots from 4 dilution levels to TSA plates. Control B solutions were then prepared by i) transferring 0.02 ml of a 2.5×10^8 spores/ml *B. globigii* spore suspension to a 25 ml solution containing 5 ml sterile water and 20 ml D/E neutralizing broth, ii) making a 10-fold serial dilution by transferring 0.1 ml aliquot to 0.9 ml phosphate buffer, iii) transferring duplicate 0.1 ml aliquots from two appropriate dilution levels to TSA plates and spreading, and iv) transferring triplicate 0.01 ml aliquots from 4 dilution levels to TSA plates. 5 ml of test solution, or the control, was added to each coupon so as to cover the inoculated coupon. The coupons were then allowed to stand for 5 minutes. This procedure was carried out in triplicate for each test solution. 20 ml of D/E neutralizing broth was added to each jar to neutralize the test solution. The jars were then rotated, in a single plane only, for 30 seconds at 300 revolutions per minute (rpm). The plates were incubated at 30 °C for 24 hours, at which time the colonies were examined and enumerated. The study results are set forth in Table 5 below.

Table 5

Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore After 5 Minutes

Solution	Survived spores per coupon (ss/c)	Log (ss/c)	Avg.	Std. Dev.	Log reduction in 5 minutes
4	0.00E + 00	0.00	0.00	0.00	6.09
	0.00E + 00	0.00			
	0.00E + 00	0.00			
14	1.88E + 03	3.27	3.95	0.60	2.14
	1.38E + 04	4.14			
	2.74E + 04	4.44			
31	1.25E + 05	5.10	5.15	0.26	0.94
	8.33E + 04	4.92			
	2.67E + 05	5.43			
32	4.60E + 04	4.66	4.86	0.20	1.23
	1.17E + 05	5.07			
	7.24E + 04	4.86			
33	1.92E + 05	5.28	5.44	0.16	0.65
	4.08E + 05	5.61			
	2.67E + 05	5.43			
43	8.00E + 03	3.90	4.69	0.70	1.40
	8.78E + 04	4.94			
	1.73E + 05	5.24			
Control A*	5.05E + 06	6.70	6.71	0.01	NA
	5.29E + 06	6.72			
Control B**	1.67E + 06	6.22	6.09	0.18	NA
	9.17E + 05	5.96			

* Control A spore suspension inoculated with 0.02 ml of 2.5e8 spores/ml was added to 0.9 ml phosphate buffer and was regarded as the first 10-fold dilution (dilution level -1).

** Control B non-treated glass inoculated with 0.02 ml of 2.5e8 spores/ml then treated as test samples except that 5.0 ml water, instead of test solution was used.

NA = Not Applicable.

EXAMPLE 4 – Efficacy of Selected Test Solutions and Disinfectants Against a Dried Film Containing *Bacillus globigii* Endospore (1 minute exposure):

An identical study to that of Example 3 was conducted with the exception that exposure of the endospores to the test solutions, disinfectants and controls was for 1 minute rather than for 5 minutes. The study results are set forth in Tables 6A and 6B below.

Table 6A

Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore After 1 Minute

Solution	Survived spores per coupon (ss/c)	Log (ss/c)	Avg.	Std. Dev.	Log reduction in 1 minute
4	7.00E + 04	4.85	4.42	1.17	2.15
	1.25E + 03	3.10			
	2.10E + 05	5.32			
14	2.84E + 05	5.45	5.61	0.21	0.96
	7.00E + 05	5.85			
	3.48E + 05	5.54			
31	1.75E + 05	5.24	5.44	0.17	1.13
	3.50E + 05	5.54			
	3.42E + 05	5.53			
32	3.09E + 05	5.49	5.56	0.06	1.01
	3.88E + 05	5.59			
	3.96E + 04	5.60			
33	8.33E + 05	5.92	5.57	0.31	1.00
	3.00E + 05	5.48			
	2.08E + 05	5.32			
43	2.28E + 05	5.36	5.05	0.35	1.53
	4.63E + 04	4.67			
	1.28E + 05	5.11			
Control A*	6.42E + 06	6.81	6.73	0.11	NA
	4.42E + 06	6.65			
Control B**	3.67E + 06	6.56	6.57	0.01	NA
	3.75E + 06	6.57			

* Control A spore suspension inoculated with 0.02 ml of 2.5e8 spores/ml was added to 0.9 ml phosphate buffer and was regarded as the first 10-fold dilution (dilution level -1).

** Control B non-treated glass inoculated with 0.02 ml of 2.5e8 spores/ml then treated as test samples except that 5.0 ml water, instead of test solution was used.

NA = Not Applicable.

Table 6B

Efficacy of Disinfectants Against a Dried Film Containing *Bacillus globigii* Endospore After 1 Minute

Disinfectant	Survived spores per coupon	Log (Survived spores per coupon)	Average	Std. Dev.	Log Reduction
1000ppm	8.38E+04	4.92	4.75	0.21	1.70
	6.63E+04	4.82			
	3.25E+04	4.51			
100ppm	8.38E+04	4.92	5.00	0.40	1.45
	2.71E+05	5.43			
	4.38E+04	4.64			
Ctrl-A	2.86E+06	6.46	6.52	0.09	N/A
	3.85E+06	6.59			
Ctrl-B	1.64E+06	6.21	6.45	0.33	N/A
	4.85E+06	6.69			

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1) Sporicidal efficacy of diluted samples of disinfectant at 1.0 minute exposure time was less than that achieved when Solution 4 was tested using a 1.0 minute exposure. This result supports the contention that the efficacy of the "active ingredient" is improved when the active ingredient is properly formulated.

2) It was noted during the recovery of endospores after the challenge that the neutralizing solution (D/E) was not fully effective in neutralizing the diluted disinfectant.

EXAMPLE 5 – Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore (30 seconds exposure):

An identical study to that of Example 3 was conducted with the exception that exposure of the endospores to the test solutions and controls was for 30 seconds rather than for 5 minutes. The study results are set forth in Table 7 below.

Table 7

Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore After 30 Seconds

Solution	Survived spores per coupon (ss/c)	Log (ss/c)	Avg.	Std. Dev.	Log reduction in 30 Seconds
4	3.43E + 05	5.53	5.35	0.28	1.09
	1.06E + 05	5.03			
	3.13E + 05	5.49			
14	4.93E + 05	5.69	5.80	0.15	0.64
	9.39E + 05	5.97			
	5.38E + 05	5.73			
31	6.69E + 05	5.83	5.91	0.19	0.53
	1.35E + 05	6.13			
	6.03E + 05	5.78			
32	6.70E + 05	5.83	5.78	0.39	0.66
	2.34E + 05	5.37			
	1.42E + 06	6.15			
33	8.18E + 05	5.91	5.54	0.32	0.90
	2.50E + 05	5.40			
	2.09E + 05	5.32			
43	3.04E + 05	5.48	5.54	0.09	0.90
	3.19E + 05	5.50			
	4.44E + 05	5.65			
Control A*	3.24E + 06	6.50	6.60	0.13	NA
	4.85E + 06	6.69			
Control B**	2.93E + 06	6.47	6.45	0.04	NA
	2.61E + 06	6.42			

* Control A spore suspension inoculated with 0.02 ml of 2.5e8 spores/ml was added to 0.9 ml phosphate buffer and was regarded as the first 10-fold dilution (dilution level -1).

** Control B non-treated glass inoculated with 0.02 ml of 2.5e8 spores/ml then treated as test samples except that 5.0 ml water, instead of test solution was used.

NA = Not Applicable.

EXAMPLE 6 – Efficacy of Selected Test Solution and Disinfectant Against a Dried Film Containing *Bacillus globigii* Endospore (30 seconds and 1 minute exposure):

An identical study to that of Example 3 was conducted with the exception that exposure of the endospores to the test solution, disinfectant (25 ppm = 0.0025% w/v) and controls was for 30 seconds and 1 minute rather than for 5 minutes. The study results are set forth in Table 8 below.

Table 8

Efficacy of Selected Test Solutions Against a Dried Film Containing *Bacillus globigii* Endospore After 30 Seconds and 1Minute

Solution	Survived spores per coupon (ss/c)	Log (ss/c)	Avg.	Std. Dev.	Log reduction 30sec	Log reduction 1min
PHMB 20w%	0	0	0	n/a	6.47	6.47
	0	0	0	n/a	6.47	6.47
	0	0	0	n/a	6.47	6.47
50	0	0	0	n/a	6.47	6.47
	0	0	0	n/a	6.47	6.47
	0	0	0	n/a	6.47	6.47
Control A*	2.88E + 06	6.46	6.54	0.11	n/a	
	4.10E + 06	6.61				
Control B**	4.00E + 06	6.60	6.47	0.18	n/a	
	2.21E + 06	6.34				

* Control A spore suspension inoculated with 0.02 ml of 2.5e8 spores/ml was added to 0.9 ml phosphate buffer and was regarded as the first 10-fold dilution (dilution level -1).

** Control B non-treated glass inoculated with 0.02 ml of 2.5e8 spores/ml then treated as test samples except that 5.0 ml water, instead of test solution was used.

NA = Not Applicable.

The present invention also contemplates that the bacterial endosporicidal solutions of the present invention may be employed as pharmaceutical agents to be advantageously employed in combating and/or treating infections. Thus, such pharmaceutical agents may be employed to reduce infection, kill microbes, inhibit microbial growth or otherwise abrogate the deleterious effects of microbial infection.

Particular examples of pharmaceutically acceptable forms of the subject solutions include for example but are not limited to oral, eye, topical or nasal spray or in any other form effective to deliver active components of the present invention to a site of microorganism infection. The route of administration is preferably designed to obtain direct contact of the active components of the present invention with the infecting microorganisms.

For topical applications, an acceptable carrier in any of a number of different forms may be used such as for example but not limited to a liquid, cream, foam, lotion or gel. Topical formulations of the subject solutions may additionally comprise organic solvents, emulsifiers, gelling agents, moisturizers, stabilizers, time release agents, dyes, perfumes, and like components commonly employed in formulations for topical administration.

In yet another embodiment, the subject solutions can be used in the personal health care industry in deodorants, soaps, acne/dermatophyte treatment agents and treatments for halitosis.

In other embodiments of the present invention, the subject solutions may be formulated as ready-to-use solutions or may be concentrated or formulated for later mixing with water or an appropriate carrier prior to use, as may be desired for a particular application. Such solutions and/or concentrates may advantageously be packaged and placed in kits with additional useful adjunct items for use against microbial infections, for decontamination and the like. In this case, the solution or concentrate container means may itself be an inhalant, eye dropper or other apparatus for application to decontaminate an infected area. Also included in such kits may be other adjunct items or instruments useful for assisting in the use of the subject solutions for decontamination, microbial infections or the like. Kits of the present invention typically also include a means for containing the contents of the kit in close confinement for commercial sale.

While the invention has been described herein in conjunction with specific examples thereof, this is illustrative only. Accordingly, many alternatives, modifications, and variations will be apparent to those skilled in the art in the light of the foregoing description and it is, therefore, intended to embrace all such alternatives, modifications, and variations as to fall within the spirit and scope of the appended claims.

We claim:

1. A disinfecting solution effective against bacterial endospores comprising:

a bacterial endosporicidally effective amount, non-irritating to tissue upon contact, of one or more biguanides in a solution.

2. A disinfecting solution effective against bacterial endospores comprising:

a bacterial endosporicidally effective amount less than 200,000 ppm of one or more biguanides in a solution.

3. A disinfecting solution effective against bacterial endospores comprising:

a bacterial endosporicidally effective amount of at least 100 ppm and less than 200,000 ppm of one or more biguanides in a solution.

4. A disinfecting solution effective against bacterial endospores comprising:

a bacterial endosporicidally effective amount greater than 1000 ppm and less than 150,000 ppm of one or more biguanides in an aqueous solution.

5. The disinfecting solution of claim 1, 2, 3 or 4 wherein said one or more biguanides are selected from the group consisting of poly(hexamethylene biguanide), alexidine, chlorhexadine, and salts thereof.

6. The disinfecting solution of claim 1, 2, 3 or 4 wherein said one or more biguanides are polymeric biguanides selected from the group consisting of salts of poly(hexamethylene biguanide).

7. The disinfecting solution of claim 1, 2, 3 or 4 wherein said one or more biguanides are bis-biguanides selected from the group consisting of alexidine or chlorhexidine, and salts thereof.

8. The disinfecting solution of claim 1, 2, 3 or 4 wherein said one or more biguanides is poly(hexamethylene biguanide).

9. The disinfecting solution of claim 1, 2, 3 or 4 wherein said one or more biguanides includes alexidine.

10. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more water-soluble polymers.

11. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more water-soluble polymers selected from the group consisting of hydroxypropylcellulose, hydroxypropylmethylcellulose,

methylcellulose, guar, cationic celluloses, cationic guars, neutral polysaccharides, cationic polysaccharides, poly(N-vinylpyrrolidone), cationic N-vinylpyrrolidone polymers, poly(vinyl alcohol) and cationic vinyl alcohol polymers.

12. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more water soluble surfactants based upon oligomers and polymers of ethylene oxide.

13. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes ethylene-oxide oligomers and polymers of poloxomers, reverse poloxomers, poloxamines, reverse poloxamines, and alkylethylene oxide-containing surfactants.

14. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more buffers.

15. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more buffers selected from the group consisting of tromethamine, borate buffers, phosphate buffers, citrate buffers, Tris (hydroxymethyl)aminomethane, sodium bicarbonate and amino alcohol buffers.

16. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution includes one or more buffers and glycerine.

17. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution has an osmolality of approximately 175 to 320 mOsm/kg.

18. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution has a pH of approximately 6.5 to 7.5.

19. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution contains greater than 1000 ppm of said one or more biguanides including one or more polymeric biguanides.

20. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution contains at least approximately 100 ppm alexidine.

21. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution contains greater than 1000 ppm of said one or more biguanides including one or more polymeric biguanides and alexidine.

22. The disinfecting solution of claim 1, 2, 3 or 4 wherein said solution contains approximately 50 ppm alexidine.

23. A method of using the disinfecting solution of claim 1, 2, 3 or 4 to inactivate bacteria endospores on surfaces comprising:

applying said solution to endospore laden surfaces.

24. A method of using the disinfecting solution of claim 1, 2, 3 or 4 to inactivate air-borne bacteria endospores comprising:

releasing an aerosol spray of said solution in endospore laden air.

25. A method of using the disinfecting solution of claim 1, 2, 3 or 4 to inactivate bacteria endospores in water comprising:

adding an effective amount of said solution to endospore laden water.

26. The method of claim 25 wherein an effective amount of said solution comprises at least 100 ppm but less than 200,000 ppm poly(hexamethylene biguanide) in endospore laden water.

27. A method of making the disinfecting solution of claim 1, 2, 3 or 4 comprising:

adding an endosporicidally effective amount of one or more biguanides to water.

28. A method of making the disinfecting solution of claim 1, 2, 3 or 4 comprising:

adding an endosporicidally effective amount of one or more polymeric biguanides and alexidine to water.

29. A method of making the disinfecting solution of claim 1, 2, 3 or 4 comprising:

adding an endosporicidally effective amount of one or more polymeric biguanides and said one or more bis-biguanides to water.

30. The method of claim 27 wherein said one or more biguanides are selected from the group consisting of alexidine or chlorhexidine, and salts thereof.

31. The method of claim 28 or 29 wherein said one or more polymeric biguanides are selected from the group consisting of salts of poly(hexamethylene biguanide).

32. The method of claim 29 wherein said one or more bis-biguanides are selected from the group consisting of alexidine or chlorhexidine, and salts thereof.

33. The method of claim 27 wherein said one or more biguanides is poly(hexamethylene biguanide).

34. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more water-soluble polymers.

35. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more water-soluble polymers selected from the group consistingof hydroxypropylcellulose, hydroxypropylmethylcellulose, methylcellulose, guar, cationic celluloses, cationic guars, neutral polysaccharides, cationic polysaccharides, poly(N-vinylpyrrolidone), cationic N-vinylpyrrolidone polymers, poly(vinyl alcohol) and cationic vinyl alcohol polymers.

36. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more water-soluble polymeric surfactants based upon oligomers and polymers of ethylene oxide.

37. The method of claim 23, 24, 25, 27, 28 or 29 include ethylene-oxide oligomers and polymers of poloxomers, reverse poloxomers, poloxamines, reverse poloxamines, and alkylethylene oxide-containing surfactants.

38. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more buffers.

39. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more buffers selected from the group consisting of tromethamine, borate buffers, phosphate buffers, citrate buffers, tris(hydroxymethyl)aminomethane, sodium bicarbonate and amino alcohol buffers.

40. The method of claims 23, 24, 25, 27, 28 or 29 wherein said solution includes one or more buffers and glycerine.

41. The method of claims 23, 24, 25, 27, 28 or 29 wherein said solution has an osmolality of approximately 175 to 320 mOsm/kg.

42. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution has a pH of approximately 6.5 to 7.5.

43. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution contains greater than 1000 ppm of said one or more biguanides.

44. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution contains at least approximately 100 ppm alexidine.

45. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution contains greater than 1000 ppm but less than 150,000 ppm of said one or more biguanides.

46. The method of claim 23, 24, 25, 27, 28 or 29 wherein said solution contains approximately 50 ppm alexidine.

47. A kit comprising:

a container containing said solution of claim 1, 2, 3 or 4; and

an instrument for use in applying said solution to an infected area.

48. A method of using the kit of claim 47 comprising:

opening said container containing said solution; and

using said instrument to apply said solution to an infected area.

49. A method of making the kit of claim 47 comprising:

closing a reopenable container filled with said solution; and

placing said reopenable container and said instrument in common packaging.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/008267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N47/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 308 611 A (THOMPSON CEDRIC B H) 3 May 1994 (1994-05-03) examples I,II,III column 7, line 15 column 10, lines 26-29 column 11, lines 8-22 claims 1,2,4-7,15,16 ----- -/--	1-5,7, 10-17, 23-25, 27,30, 34-41, 43,45, 47-49

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2004	29/09/2004

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