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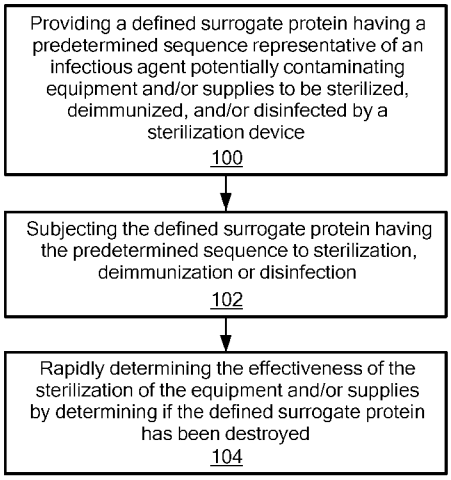


FIG. 1

(57) Abstract: A method for rapidly determining effective sterilization, deimmunization, and/or disinfection of equipment and/or supplies by a device. The method includes providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating the equipment and/or the supplies to be sterilized, deimmunized, and/or disinfected by the device. The defined surrogate protein having the predetermined sequence is subjected to sterilization, deimmunization, or disinfection. The effectiveness of the sterilization deimmunization, or disinfection is rapidly determined by determining if the defined surrogate protein has been destroyed.

WO 2017/172879 A1

METHOD FOR RAPIDLY DETERMINING EFFECTIVE STERILIZATION, DEMMUNIZATION, AND/OR DISINFECTION

RELATED APPLICATIONS

This application claims benefit of and priority to U.S. Patent Application Serial No15/472,499 filed March 29, 2017 under §§119, 120, 363, 365, and 37 C.F.R. §1.55 and §1.78, and that application and this application also claim benefit of and priority to U.S. Provisional Application Serial No. 62/314,617 filed March 29, 2016, under 35 U.S.C. §§119, 120, 363, 365, and 37 C.F.R. §1.55 and §1.78, and each of U.S. Patent Application Serial No. 15/472,499 and U.S. Provisional Application No. 62/324,617 are incorporated herein by this reference.

FIELD OF THE INVENTION

This invention relates to a method for rapidly determining effective sterilization, deimmunization, and/or disinfection.

BACKGROUND OF THE INVENTION

A wide range of infectious agents, including infectious proteins, spore forming bacteria, vegetative bacteria, fungus and viruses have major impacts in medical settings. The process to remove infectious organisms or render them non-infectious from medical equipment makes use of a wide range of sterilization devices or equipment and disinfection devices and processes. The CDC lists examples of infectious agents and microorganisms by resistance to standard disinfection and sterilization processes. *See* Table 1 below from CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008.

Some infectious agents, such as the HIV virus, may be easy to remove from medical equipment. Many infectious agents, including vegetative bacteria, are moderately difficult to eliminate. Other infectious agents, such as prions, can only be destroyed by extremely harsh conditions that damage and/or destroy modern medical equipment. Failure to eliminate infectious agents from medical equipment before use can put patients at extreme risk of injury and death.

Agent Category	Example Organisms or Diseases
Prions	Creutfeldt-Jakob Disease
Bacterial spores	Bacillius atrophaeus
Coccidica	Cryptosporidium
Mycobacteria	M. tuberculosis, M. terrae
Nonlipid or small viruses	polio, coxsackie
Fungi	Aspergillus, Candida
Vegetative bacteria	S. aureus, P. aeruginosa
Lipid of medium-sized viruses	HIV, herpes, hepatitis B

Table 1. Decreasing order of resistance of infectious agents and microorganisms to disinfection and sterilization.

Some conventional methods to determine if sterilization equipment functions effectively may rely on FDA approved Biologic Indicator process (BI strips) in a multi-step process. This widely accepted conventional process starts with filter papers infused with a defined number of bacterial spores (BI strips). The BI strips are subjected to a standard cycle by the sterilization equipment or device, e.g., an ethylene oxide (EtO) sterilization, radiation, or steam sterilization equipment which is being qualified. After the sterilization process is completed, the treated strips are then placed in a defined bacterial media for growth, frequently for days to weeks. If no growth is seen after the defined period, the sterilization process by the medical equipment being certified is declared a success. Together this combination of

supplies and techniques is the approved process to qualify sterilization equipment in positive or negative process. If there is growth, the sterilization equipment fails and if there is no growth the sterilization equipment passes.

The conventional biologic indicator tests may use one of three different species of bacteria. The standard species used to test the effectiveness of ethylene oxide (EtO) sterilization is *B. atrophaeus*. To test the effectiveness of gamma radiation sterilization, the bacteria species used is *B. pumilis*. To test the effectiveness of steam sterilization the bacteria species used is *G. stearothermophilus*. However, the three species that are used to qualify sterilization capacity of equipment are not bacteria that commonly cause disease in humans. Instead, the species are surrogate species, strains of soil bacteria that form high persistent spores. They are used instead of medically relevant infectious agents, because, *inter alia*, the spores of the bacteria are extremely difficult to damage such that they can no longer replicate, and if for some reason a health care worker or patient accidently comes in contact with the spores through use or on improperly cleaned equipment, there is very little chance that the human will become ill. As spores from the surrogate species are scientifically known to be more difficult to destroy than medically relevant species, such as *Polio* or *S. aureus*, e.g., Methicillin-resistant *Staphyloccus aureaus* (MRSA), when the sterilization equipment is qualified to destroy all spores on a BI test strip, the FDA accepts that the equipment is also able to destroy all organisms that rank lower for resistance to sterilization.

The conventional methods discussed above used to qualify effective sterilization, deimmunization, or disinfection may only measure the ability of the surrogate organisms to grow after sterilization treatment. However, such

conventional methods do not indicate how the surrogate organisms are damaged and/or destroyed resulting in the absence of growth. Conventional surrogate testing methods also require the accurate production, storage, transport and handling of 10 thousand to 100 million pure bacteria spores, proper control of growth medias, extended period of growth of the specific spores and careful protection of all growth materials for environmental contamination to qualify if all the test surrogate organisms were completely eliminated. If any component of the process is not vigorously controlled, the sterilization qualification could give false positive or false negative results. False positive results will trigger extensive effort to unnecessarily repair sterilization equipment as well as the recall of days or weeks of sterilized medical equipment and the patients treated with such equipment. False negative results are worse because they will result in defective sterilization equipment being used and the resulting contaminated medical equipment endangering patients.

For other infectious organisms, such as members of the bacterial genera *Clostridium, Staphylococcus* or fungal genera *Trichophyton* or *Candida*, and the like, specific tests for each genera may be based on similar fundamentals. *See* Table 2 below for a list of common bacteria and fungus genera and species having an impact on human medicine.

Genus	Example Species	Health Care Application - Problem pathogen or BL1/sterilization testing organism.
Bacillus	*Bacillus subtilis*	Research organism, used as an "indicator organism" during disinfection testing. BL1.
	Bacillus atrophaeus	Used as an "indicator organism" during gas (EtO) sterilization procedure. BL1.

	Bacillus pumilis	Used as an "indicator organism" during radiation sterilization procedure. BL1.
	Geobacillus stearothermophilus (formerly B. stearothermophilus)	Used as an "indicator organism" during steam sterilization procedure. BL1.
	Bacillus anthracis	Causes anthrax.
	Bacillus cereus	Causes food poisoning similar to that caused by Staphylococcus.
Clostridium	*Clostridium sporogenes*	Used as a surrogate for *C. botulinum* when testing the efficacy of commercial sterilization.
	Clostridium tetani	Causes tetanus.
	Clostridium botulinum	Causes botulism poisoning.
	Clostridium perfringens	Causes gas gangrene
	Clostridium difficile	Causes C. dif GI infection.
	Clostridium novyi	Causes a wide range of human and animal infections depending on type.
Mycobacterium	*Mycobacterium tuberculosis*	Major cause of human tuberculosis.
	Mycobacterium africanum	Slow growing form of tuberculosis.
	Mycobacterium caprae	More rare form of human tuberculosis.
	Mycobacterium kansasii	Chronic human pulmonary disease resembling tuberculosis (involvement of the upper lobe).
	Mycobacterium ulcerans	Infects the skin and subcutaneous tissues, giving rise to indolent nonulcerated and ulcerated lesions.
	Mycobacterium interjectum	Chronic lymphadenitis
	Mycobacterium leprae	Causes leprosy
	Mycobacterium lepromatosis	Causes leprosy
	Mycobacterium terrae	Causes serious skin infections that are relatively resistant to antibiotic therapy.
		Used to study effectiveness of disinfection processes for reusable medical instruments.

	Mycobacterium gastri	Casual resident of human stomachs, but not considered an etiologic agent of disease. (BL1).
Staphylococcus	*Staphylococcus aureus*	Causes a variety of infections in the body, including boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning.
		Substrain - Methicillin-resistant Staphylococcus aureus (MRSA).
		Sub-strain - Vancomycin-resistant Staphylococcus aureus (VRSA) - acquired gene from VRE.
	Staphylococcus capitis	Associated with prosthetic valve endocarditis, forms biofilms.
	Staphylococcus epidermidis	Hospital-acquired concern as it forms biofilms catheters or other surgical implants.
	Staphylococcus haemolyticus	Second-most frequently isolated hospital-acquired Infection, often associated with the insertion of medical devices; highly antibiotic-resistant phenotype and able to form biofilms.
	Staphylococcus lugdunensis	Wide variety of infections including cardiovascular infections, osteomyelitis and prosthetic/native joints infections, skin and soft-tissue infection, central nervous infections, peritonitis, endocephalitis, and urinary tract infections.
	Staphylococcus saccharolyticus	May cause of infective endocarditis.
	Staphylococcus saprophyticus	Common cause of community-acquired urinary tract infections.
	Staphylococcus auricularis	Occasionally can be involved with human skin infections.
Salmonella	*Salmonella enterica*	Causes food poisoning.
Enterococcus	*Enterococcus faecalis*	Can cause endocarditis and septicemia, urinary tract infections, meningitis, and other infections.

		Substrain - Vancomycin-resistant Enterococcus (VRE).
	Enterococcus faecium	Neonatal meningitis or endocarditis.
		Substrain - Vancomycin-resistant Enterococcus (VRE).
	Enterococcus gallinarum	Known to cause outbreaks and spread in hospitals.
	Enterococcus hirae	Endocarditis and septicemia in humans.
	Enterococcus malodoratus	Frequently the cause of hospital-acquired noscomial infections, bloodstream infections, and urinary tract infections.
Escherichia	*Escherichia coli*	Some serotypes can cause serious food poisoning in their hosts.
		Substrain K-12 strain commonly used in recombinant DNA work (BL1).
		Substrain O157:H7 causes serious illness or death in the elderly, the very young, or the immunocompromised.
		Substrain O104:H4, can trigger major cause of foodborne illness and lead to hemolytic-uremic syndrome (HUS).
	Escherichia fergusonii	Known to infect open wounds and may also cause bacteraemia or urinary tract infections; highly resistant to the antibiotic ampicillin and some also resistant to gentamicin and chloramphenicol.
Helicobacter	*Helicobacter pylori*	Cause gastritis and ulcers.
	Helicobacter hepaticus	May be associated with Crohn's disease and ulcerative colitis.
	Helicobacter bilis	May be associated with Crohn's disease and ulcerative colitis.
	Helicobacter ganmani	May be associated with Crohn's disease and ulcerative colitis.

Klebsiella	*Klebsiella pneumoniae*	Causes pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections; naturally resistant to many antibiotics.
		Substrain - CREs - carbapenem-resistant Klebsiella pneumoniae (CRKP).
	Klebsiella oxytoca	Cause colitis and sepsis.
Neisseria	*Neisseria gonorrhoeae*	Causes Gonorrhea.
	Neisseria meningitidis	Causes meningitis.
Pseudomonas	*Pseudomonas aeruginosa*	A multidrug resistant pathogen associated with hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. Common in CF patients.
	Pseudomonas mendocina	Occasionally causes hospital-acquired infections, such as infective endocarditis and spondylodiscitis.
	Pseudomonas fluorescens	Produces enzymes that cause milk to spoil and occasionally infects immunocompromised patients.
	Pseudomonas putida	Used in bioremediation, or the use of microorganisms to biodegrade oil.
Trichophyton (Fungus)	*Trichophyton rubrum*	Most common cause of athlete's foot, fungal infection of nail, jock itch, and ringworm.
	Trichophyton tonsurans	Causes ringworm infection of the scalp.
	Trichophyton interdigitale	One of three common fungi which cause ringworm.
	Trichophyton mentagrophytes	Causes tinea infections including athlete's foot, ringworm, jock itch, and similar infections of the nail, beard, skin and scalp.
	Trichophyton concentricum	Associated with the skin infection tinea imbricate.

Candida (Fungus)	*Candida albicans*	Dimorphic fungus that grows both as yeast and filamentous cells; Responsible for 50–90% of all cases of candidiasis in human. Important causes of morbidity and mortality in immunocompromised patients. Biofilms may form on the surface of implantable medical devices. Cause of 85-95% of vaginal infections cases are responsible for physician office visits every year.
	Candida dubliniensis	A fungal opportunistic pathogen originally isolated from AIDS patients. It is also occasionally isolated from immunocompetent individuals.
	Candida tropicalis	Common pathogen in neutropaenic hosts; research suggests that *C. tropicalis,* working synergistically with *Escherichia* coli and *Serratia marcescens*. May cause or contribute to Crohn's disease
	Candida auris	Causes candidiasis in humans; often acquired in the hospital when human immune systems are weakened; causes fungemia, yielding candidemia (systemic candidiasis); attracted clinical attention because of multidrug resistance.

Table 2. Common bacteria and fungus genera and species having an impact on human medicine.

Examples of standardized methods for sterilization or disinfection (A) and standardized testing methods protocols (B) used to determine the effectiveness of sterilization or disinfection, as shown in Table 3 below:

(A) Standard Methods for Preparing Healthcare Equipment	
Disinfection	Sterilization
Alcohol	Steam Sterilization
Chlorine and Chlorine Compounds	Flash Sterilization
Formaldehyde	Ethylene Oxide "Gas" Sterilization

Glutaraldehyde	Hydrogen Peroxide Gas Plasma
Hydrogen Peroxide	Peracetic Acid Sterilization
Iodophors	Ionizing Radiation
Ortho-phthalaldehyde	Dry-Heat Sterilizers
Peracetic Acid	Liquid Chemicals
Peracetic Acid and Hydrogen Peroxide	Performic Acid
Phenolics	Filtration
Quaternary Ammonium Compounds	Microwave
Radiation	Glass Bead "Sterilizer"
Pasteurization	Vaporized Hydrogen Peroxide
Flushing- and Washer-Disinfectors	Ozone
	Formaldehyde
	Gaseous Chlorine Dioxide
	Vaporized Peracetic Acid
	Infrared radiation
(B) Standard Test to Qualtify Healthcare Equipment	
Test Name	Example Test Species
BI (ethylene oxide (EtO) sterilization)	*B. atrophaeus*
BI (gamma radiation sterilization)	*B. pumilis*
BI (steam sterilization)	*G. stearothermophilus*
AOAC Sporicidal Efficacy Test Method	*Clostridium sporogenes*
	Bacillus subtilis
AOAC Tuberculosis Rate of Kill	*Mycobacterium terrae*
AOAC Use Dilution Test	*Pseudomonas aeruginosa*
	Staphylococcus aureus
	Salmonella enterica
AOAC Fungicidal Efficacy Test Method	*Trichophyton mentagrophytes*

Table 3. Approved Methods of Sterilization or Disinfection and Qualifying Test Protocols

For each standard test protocol, a define number of organisms are placed on a carrier, such as tube, filter paper, or coated on and in a test solid surface. The specific organisms may be a particular infectious species or could be a surrogate species of the same genus that is closely related to the infectious species. In all cases, the species, carrier and growth conditions are defined by the FDA and/or the Association of Analytical Communities (AOAC) protocol. Following treatment with sterilization or disinfection equipment, the carrier with the specific species sample is placed into

ideal growing conditions for the particular test species. After a required period in culture, usually 2 to 30 days, the culture is monitored. If no growth is observed, the sterilization or disinfection equipment is declared to be operating within required parameters.

In addition to enabling growth and infectivity, protein components of infectious organisms could trigger severe immunogenic or allergic reactions in susceptible individuals even at very low level. Examples include mold proteins that are able to trigger severe allergic reactions even if the mold has been rendered no longer able to grow. Immunogenic proteins can also occur in food such as gliadin, a highly immunogenic protein component of the seed storage protein gluten in wheat and related grains. Gliadin can trigger reactions in most individuals suffering from Crohn's disease. It is critical that immunogenic proteins are completely removed from any equipment that will be used in conjunction with susceptible individuals.

A wide range of pathogenic organisms use a multicopper oxidase with 3 cupredoxin superfamily domains for growth and survival. As disclosed herein, the loci *suf I* that contains a critical protein that confers different functions depending on the genus (bacteria or fungus) and this critical protein can be targeted. Depending on the genus, the *suf I* loci encoded protein can have different names. The functions of the protein encoded by *suf I* include cell division (FtsP), formation of spore coat proteins (CotA), chromosome partitioning, inorganic ion transport, and metabolism and cell wall, membrane, and envelope formation. As the protein product of the *suf I* loci are absolutely critical for the survival of the spores (in spore forming bacteria) and/or growth (all bacteria and fungus), if the protein product of the *suf I* loci is irreversibly fragmented into short polypeptides and amino acids, the bacteria or

fungus cannot survive. Additionally, it is likely that a sterilization method that clearly demonstrates fragmentation of the protein product of the *sufi I* loci would also fragment other proteins in the bacterium or fungus. Bacteria and fungus can be divided into distinct genus each containing multiple species. Many species also have subspecies that carry unique characteristics include multi-drug resistance. In human health situations, certain bacteria and fungus species and subspecies are of major concern because they are capable of causing disease. Related species may be used in medical research, e.g., *E. coli K12*, or as indicator species for qualification of sterilization, e.g., *B. atrophaeus* used to qualify gas sterilization. *See* Table 2 above for a list of common bacteria and fungus genera and species with impact on human medicine.

Prions are a unique category of a transmissible infectious agent that comprised only of protein, without DNA or RNA. Prions can cause a wide range of neurodegenerative diseases known as transmissible spongiform encephalopathies (TSE) or prion diseases including the new variant Creutzfeldt-Jakob disease (nvCJD). *See* Table 4 below. Infectious prions are in fact an abnormally folded brain protein. This brain protein (Protease resistant Proteins, PrP) can be folded into two different structural (tertiary) forms, the normal brain protein, PrP_c, and the abnormal, disease triggering form, PrP_{sc}. The disease triggering form, PrP_{sc}, is found in high quantity in the brain of infected humans and animals and can be transferred to a new host with the transfer of infected material. Once in the new host, the abnormally folded protein (PrP_{sc}) causes disease symptoms by promoting the unfolding of the normal host protein (PrP_c) and refolding into the disease causing form (PrP_{sc}). PrP proteins can also be partially cleave and still retain their infectious characteristics. Full length

mature PrP protein (both PrP_c and PrP_{sc}) is 209 amino acids long. Limited proteolysis of PrP_{sc} will cleave amino acids from the amino terminus resulting in another infectious protein form PrP 27-30 that is approximately 142 amino acids long. Additional cleavage that significantly reduces the 142 amino acid long PrP 27-30 is needed to render the PrP protein irreversibly non-infectious. Although most infectious agents can be permanently rendered non-infectious by heat or steam, these methods are not sufficient to eliminate infectious prions from medical equipment.

Disease	Species	Potential Origin	Disease	Species	Potential Origin
Creutzfeldt-Jakob disease (CJD)	Human	Inherited	Scrapie	Sheep and Goat	Inherited/ environmental
New Variant Creutzfeldt-Jakob disease (CJD)	Human	Consumption, Medical Contamination	Bovine Spongiform Encephalopathy (BSE)	Cattle	Consumption
Fatal Familial Insomnia (FFI)	Human	Inherited	Transmissible Mink Encephalopathy (TME)	Mink	Environmental
Gerstmann-Straussler disease (GSD)	Human	Inherited	Chronic Wasting Disease (CWD)	Mule Deer and Elk	Environmental
Huntington disease-like type 1 (HDL1)	Human	Inherited	Feline Spongiform Encephalopathy (FSE)	Cats	Consumption
Kuru	Human	Consumption of Human Brains	Exotic Ungulate Encephalopathy (EUE)	Nyala and Greater Kudu	Environmental

Table 4: Example of Prion Diseases in Different Species and Potential Origin of the Infectious Protein.

As discussed above, prions are abnormally folded protease resistant proteins (PrP_{sc}) that cause disease symptoms by promoting the unfolding of normal proteins (PrP_c) and refolding into the disease causing protein form (PrP_{sc}). As the level of the PrP_{sc} rises in the patient's brain, symptoms of progressive dementia, myoclonic seizures, abnormalities of high cortical function, cerebellar and corticospinal

disturbances develop. The period between infection and development of disease can extend for years to decades. The duration of disease symptoms is variable but is typically 8 to 18 months.

Once prion proteins fold into the infectious form (PrP_{sc}), they are extremely difficult to render non-infectious. Conventional methods to sterilize medical equipment contaminated with prions, such as high heat to promote loss of function of other protein types by triggering loss of tertiary structure, are ineffective because unlike most proteins, the denatured prion proteins, both infectious and non-infectious, will spontaneously refold by themselves back to their pre-treatment forms. In some cases, conventional methods may actually result in refolded into infectious form promoting the conversion of the non-infection prion protein into the infectious prion protein.

To render infectious proteins such as prions irreversibly non-infectious, all infectious proteins must be fragmented into small polypeptides, amino acids or components. The only currently approved conventional method for this process is harsh treatment of medical equipment and supplies with caustic soda, an extremely harsh process that frequently damages and/or destroys medical equipment.

Determining whether or not an infectious prion (PrP_{sc}) sample has been permanently destroyed can be extremely difficult and time consuming. Conventional methods for determining whether an infectious prion has been permanently destroyed require that after attempted deactivation, the PrP_{sc} sample is injected into a matched susceptible animal that is then followed for an extended time to see if the animal develops disease. In larger animals, the process can take years, but even in a small animal such as a mouse, the test can take months. As there is a potential for inter-

animal variation and poor test accuracy, a large animal test pool is required to obtain relatively accurate results.

Immunogens may include a wide range of molecules including proteins that can trigger dramatic immunologic responses in susceptible individuals. The responses can trigger serious allergic reactions on the skin (e.g., poison ivy rash), in the gut (e.g., triggering a flare-up in Crohn's disease), in the lung (e.g., asthma) or a systemic response (e.g., anaphylaxis). Protein immunogens are a special class of immunogens produced by a wide range of bacteria, fungus (e.g. mold) or plants and can be difficult to destroy. An example of a common plant immunogen is gluten. Common grains such as various strains of wheat, farro, rye and spelt are derived from wild and domesticated grains of the *Triticum, Aegilops and Secale* genera. Common to all these species is the seed storage protein complex called gluten, When seeds are ground into flour, the gluten protein complex gives bread dough its elastic quality and bread its spongy texture. Unfortunately gluten is comprised of several proteins including *Gliadin* (also called *Prolamin*) which triggers severe T cell attack on the gut of patients with the autoimmune disease Coeliac disease (CD). Gliadins can be typed as α, γ, and ω with a small protease resistant fragment (p57-73) of α- gliadins triggering the most severe destructive T cell response. As a results CD patients must not only avoid products containing gluten, but also need to be extremely careful to avoid small amounts of residual α-gliadin that may contaminate food preparation utensils.

Protease resistant proteins like α-gliadin are resistant to destruction so it is critical that devices and methods used to destroy them and other immunogens (also called allergens) can be easily checked to ensure they are operating at peak efficiency.

If not, residual allergens can trigger life threatening responses in sensitive patients. The process of removing immunogens by deimmunization methods or devices is called deimmunization. The ability to test for the destruction of different immunogens on surfaces is not standardized. Usually affected patients are subjected to skin test regiments to determine their individual reactions to different candidate immunogens/allergens. The patient is then advised to avoid all immunogen contact and discard any materials potential contaminated with the specific immunogen or allergen. In cooking and manufacturing situations, extreme care must be taken to avoid potential cross contamination to the point that food packaging labels frequently carry warning labels about the potential issues.

Thus, a method is needed to determine irreversible destruction of proteins critical for the growth of infectious organisms, immunogenic proteins, and/or infectious proteins (e.g., prions) and thus rapidly and accurately determines the effectiveness of sterilization, deimmunization, and/or disinfection of equipment or supplies by a device. With such a method for rapidly determining effective sterilization, deimmunization, and/or disinfection, medical personnel and patients can have confidence that the medical equipment used for patient treatment is not contaminated with potentially lethal or immunogenic proteins. Without such a method, medical personnel may believe they are using properly sterilized equipment and then later discover that they have accidentally exposed their patients to lethal infections and harmful immune reactions

SUMMARY OF THE INVENTION

In one aspect, a method for rapidly determining effective sterilization,

deimmunization, and/or disinfection of equipment and/or supplies by a device is featured. The method includes providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating the equipment and/or the supplies to be sterilized, deimmunized, and/or disinfected by the device. The defined surrogate protein having the predetermined sequence is subjected to sterilization, deimmunization, or disinfection. The effectiveness of the sterilization, deimmunization, or disinfection is rapidly determined by determining if the defined surrogate protein having the predetermined sequence has been destroyed.

In one embodiment, the defined surrogate protein may include proteins critical for stability, growth and/or infectious capacity of infectious agents. The defined surrogate protein may include a protein critical for stability, growth and/or infectious capacity of surrogate organisms of infectious agents. The infectious agent may include one or more of: an infectious protein, an infectious spore forming bacteria, an infectious vegetative bacteria, an infections fungus, and an infectious virus. The defined surrogate protein may include pathogenic proteins, proteins critical for the growth of infectious agents, and immunogenic proteins. The predetermined sequence may be defined by the sequence:

```
        10         20         30         40         50
MYNYTSAKYE VPIAIQDRSF NEDGSLNFPS EGDNPTIHPY WQPEFFGDTI
MVNGRVWPNM NVDMTRYRFR LLNGSNARFY NLKFSNGMQF WQIGTDGGYL
NKPVPLTSLL ISPGERADIL VDFTEIPAGT RIILNNDANA PYPTGDAPDK
DTTGQIMQFT VQHNDHHHHH H (SEQ ID NO. 1)
```

The defined surrogate protein for SEQ ID NO. 1 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The predetermined sequence may be defined by the sequence:

```
       10         20         30         40         50
MTLEKTYYEV TMEECTHQLH RDLPPTRLWG YNGLFPGPTI EVKRNENVYV
KWMNNLPSTH FLPIDHTIHH SDSQHEEPEV KTVVHLHGGV TPDDSDGYPE
AWFSKDFEQT GPYFKREVYH YPNQQRGAIL WYHDHAMALT RLNVYAGLVG
AYIIHDPKEK RLKHHHHHH  (SEQ ID NO. 2)
```

The defined surrogate protein for SEQ ID NO. 2 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The predetermined sequence may be defined by the sequence:

```
       10         20         30         40         50
MTGMPEGEGV DSNLLGGDGG DIAYPYYLIN GRIPVAATSF KAKPGQRIRI
RIINSAADTA FRIALAGHSM TVTHTDGYPV IPTEVDALLI GMAERYDVMV
TAAGGVFPLV ALAEGKNALA RALLSTGAGS PPDHHHHHH  (SEQ ID NO. 3)
```

The defined surrogate protein for SEQ ID NO. 3 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.. The predetermined sequence may be defined by the sequence:

```
       10         20         30         40         50
MTGYKNYTLK AQKGKTEFYK NNFSNTLGYN GNLLGPTLKL KKGDKVKIKL
INNLDENTTF HWHGLEVNGK VDGGPSQVIK PGKEKTIKFE VNQDSATLWY
HPHPSPNTAK QVYNGLSGLL YIEDSKKNHH HHHH  (SEQ ID NO. 4)
```

The defined surrogate protein SEQ ID NO. 4 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

. The the predetermined sequence may be defined by the sequence:

```
         10         20         30         40         50
MTGFRHEKVL CLKTWHVDEQ GAFTPFSVPR QAAREGTRGR YSTINGKHVP
TIDLPAGQIV RVRLLNVDNT VTYRLNPNGE ARIYAVDGHP VEPRGFEGQY
WIGPGMRLEL ALKVPEAGTE LSLRDGPVRL ATIRSVAHHH HHH (SEQ ID NO. 5)
```

The defined surrogate protein SEQ ID NO. 5 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The predetermined sequence may be defined by the sequence:

```
         10         20         30         40         50
MTITLEWSVT TGYRRLDGVK KRVYLINGLF PGPTIEARSG DSLQVQVTNN
IQDEGLVIHW HGLHMRGANH MDGVTGVTQC PIVPGDSMLY NFTISQSQSG
TFWYHAHSAL QRAEGLYGGF VVHKPSTHHH HHH (SEQ ID NO. 6)
```

The defined surrogate protein SEQ ID NO. 6 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The predetermined sequence may be defined by the sequence:

```
         10         20         30         40         50
MTAETHTWYF KTSWVDANPD GVFPRKMIGF NDSWPLPTLR VKKGDTVNLY
LINGFDDRNT SLHFHGLFQH GTNQMDGPEM VTQCPIPPGE TFLYNFTVDD
QVGSYWYHSH TSGQYGDGMR GVFIIEDHHH HHH (SEQ ID NO. 7)
```

The defined surrogate protein SEQ ID NO. 7 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The predetermined sequence may be defined by the sequence:

10 20 30 40 50

MKTVRVPVPQ PQPQNPSQPQ PQRQVPLVQQ QQFPGQQQQF PPQQPYPQPQ

PFPSQQPYLQ LQPFPQPQPF PPQLPYHHHH HH (SEQ ID NO. 8)

The defined surrogate protein SEQ ID NO. 8 may be at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence. The rapidly determining may include a sensitive protein analysis procedure. The sensitive protein analysis procedure may include one or more of: a Western Blot analysis, a protein assay analysis, a magnetic separation analysis, a peptide analysis, a mass spectrometry analysis, and a gas chromatography analysis. The sensitive protein analysis procedure may include fluorescence analysis of proteins covalently crosslinked on a solid surface. The sensitive protein analysis procedure may include fluorescence analysis of proteins covalently crosslinked on magnetic beads. The defined surrogate protein having the predetermined sequence may be disposed on a surface, disposed on a test strip, disposed in or on a vessel, on a tube, or in or on a holder. The holder may be disposed to receive a flow of a sterilization agent, a deimmunization agent or a disinfection agent.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Other objects, features and advantages will occur to those skilled in the art from the following description of a preferred embodiment and the accompanying drawings, in which:

Fig. 1 is a schematic block diagram showing the primary steps of one embodiment of the method for rapidly determining effective sterilization,

deimmunization, and/or disinfection of this invention;

Fig. 2 is a schematic diagram showing examples of multi-well glass slides having samples of one or more of the defined surrogate protein having a predetermined sequence in wells which are subjected to sterilization, deimmunization, or disinfection to provide a visual depiction of the effectiveness of sterilization, deimmunization, and/or disinfection; Fig. 3 is a schematic block diagram showing one example of the multi-well glass slides shown in Fig. 2 placed in a holder disposed at the end of a flow of sterilization, deimmunization, or disinfection agent in accordance with one embodiment of this invention;

Fig. 4 shows an example of an amino acid comparison of human PrP proteins with a selection of other species of PrP proteins;

Fig. 5 shows an example of a Western gel where the recombinant protein runs approximately 28 kDa inside;

Fig. 6 shows an example of a Western Blot where the absence of bands indicates successful sterilization, deimmunization, or disinfection;

Fig. 7 is a homology diagram comparing protein sequences of a research *Clostridium* species and two pathogenic *Clostridium* species;

Fig. 8 shows an example of a Western Blot for a defined *Clostridium* surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 9 is a homology diagram comparing protein sequences of a three research *Bacillus* species;

Fig. 10 shows an example of a Western Blot for a defined *Bacillus* surrogate protein having a predetermined sequence that has been subjected to sterilization,

deimmunization, or disinfection;

Fig. 11 is a homology diagram comparing protein sequences of three pathogenic *Mycobacterium* species;

Fig. 12 shows an example of a Western Blot for a defined *Mycobacterium* surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 13 is a homology diagram comparing protein sequences of three pathogenic *Staphylococcus* species;

Fig. 14 shows an example of a Western Blot for a defined *Staphylococcus* surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 15 is a homology diagram comparing protein sequences of a research *Pseudomonas* species and two pathogenic *Pseudomonas* species;

Fig. 16 shows an example of a Western Blot for a defined *Pseudomonas* surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 17 is a homology comparing protein sequences of two pathogenic *Trichophyton* species;

Fig. 18 shows an example of a Western Blot for a defined *Trichophyton* surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 19 is a homology comparing protein sequences of four pathogenic *Candida* species;

Fig. 20 shows an example of a Western Blot for a defined *Candida* surrogate

protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection;

Fig. 21 is a homology comparing protein sequences of α-Gliadin from many species of commonly consumed grains;

Fig. 22 shows an example of a Western Blot for a defined α-Gliadin surrogate protein having a predetermined sequence that has been subjected to sterilization, deimmunization, or disinfection.

DETAILED DESCRIPTION OF THE INVENTION

Aside from the preferred embodiment or embodiments disclosed below, this invention is capable of other embodiments and of being practiced or being carried out in various ways. Thus, it is to be understood that the invention is not limited in its application to the details of construction and the arrangements of components set forth in the following description or illustrated in the drawings. If only one embodiment is described herein, the claims hereof are not to be limited to that embodiment. Moreover, the claims hereof are not to be read restrictively unless there is clear and convincing evidence manifesting a certain exclusion, restriction, or disclaimer.

The method for rapidly determining effective sterilization, deimmunization, and/or disinfection of equipment or supplies of one or more embodiments of this invention may be utilized to qualify sterilization, deimmunization, and/or disinfection by a device, e.g., a sterilization device, a deimmunization device, or a disinfection device and provide improvements to the conventional methods discussed above. In one example, the method for rapidly detecting effective sterilization, deimmunization, and/or disinfection of equipment or supplies of one or more embodiments of this

invention may be based on a specific measuring the complete destruction of a specific protein critical for an organism's growth and requires only a few hours to return absolute results. The method for rapidly detecting effective sterilization, deimmunization, and/or disinfection of equipment or supplies of one or more embodiments of this invention also contains multiple layers of internal controls that enable a clear determination if either false positive and false negative results have occurred. This allows the avoidance of unnecessary repairs to sterilization, deimmunization, or disinfection equipment and eliminates false negative tests and more dangerous exposure of patients to improperly sterilized, deimmunized, or disinfected medical equipment or supplies.

The method for rapidly detecting effective sterilization, deimmunization, and/or disinfection of equipment or supplies of one or more embodiments of this invention may be used to rapidly determine if a sterilization, deimmunization, and/or disinfection device is effectively destroying specific infectious or immunogenic agents, defined herein as infectious or pathogenic proteins, infectious spore forming bacteria, infectious vegetative bacteria, infectious fungus, infectious viruses, and immunogenic proteins.

In one embodiment, the method or rapidly determining effective sterilization, deimmunization and/or disinfection of equipment and/or supplies by a device, such as a sterilization device, e.g., by a device that applies cycles of a solvent and electromagnetic radiation, e.g., microwaves, such as disclosed in U.S. Application Serial No. 15/330,469 by the assignee hereof, hereinafter the '469 Patent Application, any of the sterilization devices discussed in the Background section above, the devices for the methods of sterilization shown in Table 3 above, an autoclave, of

similar type sterilization device known to those skilled in the art, a deimmunization device, e.g., , a device that applies cycles of a solvent and electromagnetic radiation, e.g., microwaves, such as disclosed in the '469 Patent Application and/or disinfection device, e.g., a device that applies cycles of a solvent and electromagnetic radiation, e.g., microwaves, such as disclosed in the '469 Patent Application, or the devices for the methods of disinfection shown in Table 3 above, includes providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating the equipment and/or supplies to be sterilized, deimmunized and/or disinfected by the device, step 100, Fig. 1. The defined surrogate protein having the predetermined sequence is then subjected to sterilization, deimmunization, or disinfection, step 102. The effectiveness of the sterilization, deimmunization, and/or disinfection by the device is then rapidly determined by determining if the defined surrogate protein having the predetermined sequence has been destroyed, step 106, as discussed further below.

The method preferably directly measures the irreversible destruction of the defined surrogate proteins having the predetermined sequence that are critical for survival and/or growth of such infectious agents.

In one embodiment, the method utilizes one or more prion detection indicator samples configured as a defined surrogate protein. In this example, the defined surrogate protein has the following predetermined sequence:

```
KKRPKPGGWN TGGSRYPGQG SPGGNRYPPQ GGTWGQPHGG GWGQPHGGSW 50
GQPHGGSWGQ PHGGGWGQGG GTHNQWNKPS KPKTNLKHVA GAAAAGAVVG 100
GLGGYMLGSA MSRPMIHFGN DWEDRYYREN MYRYPNQVYY RPVDQYSNQN 150
NFVHDCVNIT IKQHTVTTTT KGENFTETDV KMMERVVEQM CVTQYQKESQ 200
AYYDGRRS
208
```

The defined prion surrogate protein having the predetermined sequence above

for prion detection is then subjected to sterilization, deimmunization, or disinfection, by a device, e.g., a sterilization device, a deimmunization device or a disinfection device, e.g., by conventional methods discussed in the Background section above, or by applying cycles of a solvent and electromagnetic radiation, e.g., microwaves, such as disclosed in the '469 Patent Application.

In this example, to rapidly determine the effectiveness of sterilization, deimmunization, or disinfection of equipment or supplies by the device, a determination is made if the defined surrogate protein has been destroyed using a sensitive protein analysis procedure, such as Western Blot analysis, or similar protein analysis techniques, such as fluorescence analysis of proteins covalently crosslinked to solid surfaces used in protein array analysis which are extremely sensitive processes that measure both the amount of full length intact defined surrogate protein having a predetermined sequence and the amount of the destroyed or degraded defined surrogate protein, protein array analysis, magnetic separation analysis, peptide analysis, mass spectrometry analysis, gas chromatography analysis, or similar type analysis. In one example, the defined surrogate protein includes a protein critical for stability, growth, and/or infectious capacity of an infectious agent. The defined surrogate protein may include a protein critical for stability, growth, and/or infectious capacity of a surrogate organism of the infectious agent.

One embodiment of the method for rapidly detecting effective sterilization, deimmunization, and/or disinfection of this invention includes providing the defined surrogate protein having a predetermined sequence that is based on the development of a synthetic recombinant test protein sequence with high homology to a section of a protein encoded by the *suf I* loci in the targeted pathogenic genus, e.g., one or more or

all of the genus shown in Table 2 above, and the development of a monoclonal or polyclonal antibody that is able to detect the defined surrogate recombinant protein using Western Blot or other similar protein analysis techniques discussed above. In genera that form spores, such as *Bacillus* and *Clostridium*, the target protein encoded in the *suf I* loci or defined surrogate protein having the predetermined sequence of the tests forms the spore coat protein *CotA*. In non-spore forming genus such as the bacteria *Mycobacterium, Staphylococcus and Pseudomonas,* or the fungus *Candida*, the target protein encoded in the *suf I* loci or defied surrogate protein of the tests forms the cell division protein (*FtsP*). In other genera of bacteria and fungus, the target protein or encoded defined surrogate protein having the predetermined sequence in the *suf I* loci may have additional names but all carry the same protein structure of multicopper oxidase with 3 cupredoxin superfamily domains.

For each method of rapidly determining effective sterilization, deimmunization, or disinfection, specific for its unique genus, a predetermined quantity of the defined surrogate having the predetermined protein sequence is placed on a carrier, such as filter paper, or a tube, or on the surface of an object, such as a glass or slide, a microtiter plate, a flexible membrane, magnetic beads, e.g., magnetic beads used for magnetic bead separation or similar type object or surface. For Western analysis, the defined surrogate protein having the predetermined sequence is not covalently linked to the carrier or surface. In other protein analysis processes such as protein array or magnetic bead separation, the defined surrogate protein having the predetermined sequence is covalently linked to the carrier or solid surface. After treatment with the sterilization, deimmunization or disinfection equipment of process and Western analysis, this involves recovering the recombinant defined

surrogate protein having the predetermined sequence, both in its intact and fragmented forms, from the carrier, surface tube, or object, treating the sample with denaturing loading buffer and running the sample on an acrylamide gel. A control sample that was not subjected to sterilization, deimmunization, or disinfection is also included. In one example, the samples are transferred nylon membrane and the intact and fragmented samples are visualized using the specific antibody. If the sterilization, deimmunizing or disinfection equipment is operating correctly, the control sample will have protein indicator bands that are easily visualized but the treated sample will be absent any type protein indicator bands indicating the sterilization, deimmunizing or disinfection equipment was able to irreversibly fragment the recombinant defined surrogate protein. If other protein analysis processes such as protein array or magnetic bead separation are used, the solid surface to which defined surrogate protein having the predetermined sequence was covalently linked will be tested such that intact defined surrogate protein having the predetermined sequence can be visualized using the specific antibody and fragment and/or destroyed defined surrogate protein having the predetermined sequence is no longer detected by the process. As the amino acid sequence and structure of the defined surrogate protein having a predetermined sequence is highly homologous to the target protein in the pathogenic members of the genus, irreversible destruction of the defined surrogate protein indicates that the sterilization, deimmunizing or disinfection equipment will have also destroyed the target proteins, resulting in destruction of all members of the genus that may be on the sterilization, deimmunizing or disinfection equipment or supplies.

In accordance with one or more embodiments of the method for rapidly

determining effective sterilization, deimmunization, and/or disinfection, the predetermined sequence of the defined surrogate protein and the corresponding peptide used for development of a polyclonal antibody for Western Blot analysis include one or more of the following predetermined sequences:

For *Clostridium*:

```
        10         20         30         40         50
MYNYTSAKYE VPIAIQDRSF NEDGSLNFPS EGDNPTIHPY WQPEFFGDTI
MVNGRVWPNM NVDMTRYRFR LLNGSNARFY NLKFSNGMQF WQIGTDGGYL
NKPVPLTSLL ISPGERADIL VDFTEIPAGT RIILNNDANA PYPTGDAPDK
DTTGQIMQFT VQHNDHHHHH H (SEQ ID NO. 1)
```

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

KYEVPIAIQDRSFNEDGSLNFPSE and YLNKPVPLTSLLISPGERADILVD

For *Bacillus*:

```
        10         20         30         40         50
MTLEKTYYEV TMEECTHQLH RDLPPTRLWG YNGLFPGPTI EVKRNENVYV
KWMNNLPSTH FLPIDHTIHH SDSQHEEPEV KTVVHLHGGV TPDDSDGYPE
AWFSKDFEQT GPYFKREVYH YPNQQRGAIL WYHDHAMALT RLNVYAGLVG
AYIIHDPKEK RLKHHHHHH (SEQ ID NO. 2)
```

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

QRGAILWYHDHAMALTRLNVYAGL and QLHRDLPPTRLWGYNGLFPGPTIE

For *Mycobacterium*:

```
        10         20         30         40         50
MTGMPEGEGV DSNLLGGDGG DIAYPYYLIN GRIPVAATSF KAKPGQRIRI
```

RIINSAADTA FRIALAGHSM TVTHTDGYPV IPTEVDALLI GMAERYDVMV
TAAGGVFPLV ALAEGKNALA RALLSTGAGS PPDHHHHHH (SEQ ID NO. 3)

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

DTAFRIALAGHSMTVTHTDGYPVIPTEVD and
VFPLVALAEGKNALARALLSTGAGS

For *Staphylucoccus*:

10 20 30 40 50
MTGYKNYTLK AQKGKTEFYK NNFSNTLGYN GNLLGPTLKL KKGDKVKIKL
INNLDENTTF HWHGLEVNGK VDGGPSQVIK PGKEKTIKFE VNQDSATLWY
HPHPSPNTAK QVYNGLSGLL YIEDSKKNHH HHHH (SEQ ID NO. 4)

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

NFSNTLGYNGNLLGPTLKLKKGDKVKIKL and KFEVNQDSATLWYHPHPSPNTAK

For *Pseudomonas*:

10 20 30 40 50
MTGFRHEKVL CLKTWHVDEQ GAFTPFSVPR QAAREGTRGR YSTINGKHVP
TIDLPAGQIV RVRLLNVDNT VTYRLNPNGE ARIYAVDGHP VEPRGFEGQY
WIGPGMRLEL ALKVPEAGTE LSLRDGPVRL ATIRSVAHHH HHH (SEQ ID
NO. 5)

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

DLPAGQIVRVRLLNVDNTVTYRLN and QYWIGPGMRLELALKVPEAG

For *Trichophyton*:

10 20 30 40 50
MTITLEWSVT TGYRRLDGVK KRVYLINGLF PGPTIEARSG DSLQVQVTNN
IQDEGLVIHW HGLHMRGANH MDGVTGVTQC PIVPGDSMLY NFTISQSQSG
TFWYHAHSAL QRAEGLYGGF VVHKPSTHHH HHH (SEQ ID NO. 6)

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

YRRLDGVKKRVYLINGLFPGPTIE and TQCPIVPGDSMLYNFTISQSQSG

For *Candida*:

```
        10          20          30          40          50
MTAETHTWYF KTSWVDANPD GVFPRKMIGF NDSWPLPTLR VKKGDTVNLY
LINGFDDRNT SLHFHGLFQH GTNQMDGPEM VTQCPIPPGE TFLYNFTVDD
QVGSYWYHSH TSGQYGDGMR GVFIIEDHHH HHH  (SEQ ID NO. 7)
```

The peptides used for the development of polyclonal and monoclonal antibodies for use by Western Blot analysis for the above sequence are:

GFNDSWPLPTLRVKKGDTVNLYL and WYFKTSWVDANPDGVFPRKMIG

For α-Gliadin:

```
        10          20          30          40          50
MKTVRVPVPQ PQPQNPSQPQ PQRQVPLVQQ QQFPGQQQQF PPQQPYPQPQ
PFPSQQPYLQ LQPFPQPQPF PPQLPYHHHH HH (SEQ ID NO. 8)
```

The peptide used for the development of monoclonal or polyclonal antibody used by Western Blot analysis for the above sequence is:

FPPQQPYPQPQPFPSQQPYLQLQPFPQPQ

Western Blot analysis typically utilizes equipment, e.g., acrylamide gel, a power supply to create an electric field to trigger protein migration where smaller fragments move faster than larger fragments to separate intact proteins from fragmented or degraded proteins, a membrane, transfer equipment, and visualization equipment. Western Blot analysis also preferably utilizes specific reagents, e.g., a

positive control protein to show the location of a full length defined surrogate protein and specific antibody for the defined surrogate protein, e.g., any of the defined surrogate proteins having the associated predetermined sequences and the corresponding antibody above. The specific antibody binds to the associated defined surrogate protein, both full length, and fragments, to provide a visualization if the defined surrogate protein was destroyed by sterilization, deimmunization, or disinfection.

Western Blot analysis for infectious organisms may also typically include a defined number of colony forming units (CFU) or spores of the test pathogen which are added to a stable substrate such as filter paper (dried) or other sample holder. The number of CFU or spores will contain a defined quantity of each indication protein to be followed.

Western Blot analysis may also be conducted on defined surrogate proteins having the associated predetermined sequences samples placed on indicator strips or other small sample holders that maybe subjected to sterilization, deimmunization or disinfection. After sterilization, deimmunization or disinfection, the defined surrogate proteins from the indicator strips or other small sample holders are extracted from the filter paper or other sample holders into a loading dye and denatured (eliminating any tertiary protein structure). The samples are then run on the gel, including control wells with (1) size marker, (2) positive control protein, (3) other controls if needed. After transfer to membrane, defined surrogate proteins and protein fragments are visualized with unique antibodies preferably having a high affinity and specific binding to an indicator region of the protein being tested. Successful sterilization, deimmunization or disinfection will result in the loss of all indicator proteins of

defined length. The development process may require side by side Western Blots and standard growth studies to demonstrate sufficient equivalence.

Western Blot analysis may also require defining the defined surrogate proteins having the associated predetermined sequence, positive control proteins, and/or a negative control proteins and may require the development of a polyclonal or monoclonal antibodies that specifically bind to specific defined surrogate proteins discussed above in Western Blot analysis or other protein analysis format, both full-length and fragments, and the positive control protein. The antibody will not bind to the negative control protein. It is also possible that a secondary antibody that is labeled with an enzyme or other visualization marker will be needed to visualize the detection antibody.

Protein array analysis can be used as a substitution for Western blot analysis in circumstances in which less sensitivity can be tolerated, but faster results are needed. In a Western Blot analysis, a substrate such as a piece of filter paper or tube is used to temporarily hold the recombinant protein sample during testing of sterilization equipment. After sterilization, deimmunization and/or disinfection, the recombinant protein sample is removed from the substrate, denatured, separated on an acrylamide gel and transferred to nylon membrane before visualizing with antibodies. In protein array analysis, the recombinant protein sample, e.g., one or more of the defined surrogate proteins having the associated predetermined sequence discussed above, is covalently crosslinked to solid surfaces such as glass, plastics or metal beads. After treatment by sterilization, deimmunization or disinfection, the solid surfaces, with the covalently crosslinked recombinant proteins are directly visualized with antibodies. Unlike Western blot analysis that can visualize the amount of protein fragmentation

has occurred, protein array analysis can indicate how much of the protein sample has lost regions that are recognized by visualizing antibodies protein assay analysis but is unable to determine what other areas of the recombinant proteins were not destroyed. Thus, protein array analysis is highly suitable for testing the presence or absence of intact test proteins, such as the defined surrogate proteins having the associated predetermined sequence homologous to proteins critical for spore coats or bacteria or fungus survival or growth, e.g., one or more of the defined surrogate proteins having the predetermined sequences for *Clostridium*, *Bacillus*, *Myocbacterium*, Staphylucoccus, *Pseudomonas*, *Trichophyton, Candida,* and *α-Gliadin* shown above. Because protein assay analysis cannot determine the absolute level of protein fragmentation that has occurred, protein array analysis may not be sensitive enough for quantifying the complete destruction of infectious proteins like prions although it is not excluded as a test method if less sensitivity is tolerated.

To establish a protein array analysis, defined quantities of the defined surrogate proteins for *Clostridium*, *Bacillus*, *Myocbacterium*, *Staphylucoccus, Pseudomonas*, *Trichophyton, Candida*, and *α-Gliadin* above are covalently linked to a solid surface. The solid surface may include a wide support surface, such as a microscope slide made of glass or silicon, a flexible membrane, a magnetizable bead, a microtiter plate, or other similar solid surface to which a selection of the defined surrogate proteins having the associated predetermined sequence can be permanently attached. The surface used is determined by the type detector that will be used to determine the quantity of protein left on the support after sterilization, deimmunization or disinfection. After sterilization, deimmunization or disinfection, the solid surface is visualized with antibodies or other specific ligands that will

specifically bind to the intact defined surrogate protein but not to destroyed defined surrogate proteins. The antibodies or other ligands can contain fluorescent dyes or other detection enabling attachments. Control slides that were not subjected to sterilization, deimmunization and/or disinfection are included as a positive control to enable estimations for the relative amount of test protein destroyed by sterilization, deimmunization and/or disinfection. Fig. 2 shows an example of multi-well glass slide 130 which has the control defined surrogate protein having the associated predetermined sequence in the wells exemplarily, indicated at 132 and multi-well glass slide 134 which has one of the defined surrogate proteins having the predetermined sequence for *Clostridium*, *Bacillus*, *Myocbacterium*, Staphylucoccus, *Pseudomonas*, *Trichophyton, Candida*, and *α-Gliadin* in the wells, exemplarily indicated at 136. In this example, control multi-well glass slide 130 was not subject to sterilization, deimmunization, or disinfection and visualization of the antibodies is indicated by the shading as shown which indicates the defined surrogate protein having the associated predetermined sequence was not sterilized, deimmunized, or disinfected. In this example, multi-well glass slide 134 was subject to sterilization, deimmunization and/or disinfection and visualization shows no presence of antibodies and, therefore, the defined surrogate proteins having the predetermined sequence were effectively sterilized. Thus, in this embodiment, the method for rapidly determining effective sterilization was able to quickly determine effective sterilization, deimmunization and/or disinfection in as little as two hours depending on the type of detection equipment used to rapidly determine effective sterilization, deimmunization or disinfection discussed above.

One key benefit of using protein array analysis in accordance with one

embodiment of the method for rapidly determining effective sterilization, deimmunization, and/or disinfection of this invention is the covalently linked surrogate proteins on the surface of an object can be used for testing sterilization, deimmunization or disinfection by a sterilization, deimmunization or disinfection device that may use radiation or heat, as well as devices that use flowing disinfectants or gases to sterilize, deimmunize or disinfect medical equipment or supplies. In one example, holder 150, Fig. 3, may be used to hold multiwall glass slide 152 having one or more wells, exemplarily indicated at 154, which each holds one or more samples of the defined surrogate protein having the predetermined sequence discussed above, e.g., one of *Clostridium*, *Bacillus*, *Myocbacterium*, Staphylucoccus, *Pseudomonas*, *Trichophyton, Candida* or *α-Gliadin.* Holder 150 with multi-well glass slide 152 including one or more samples of the defined surrogate protein having the predetermined sequence discussed above may then be placed below flow 156 of sterilization, deimmunization, or disinfection agent at the end of a medical device undergoing sterilization, deimmunization, or disinfection, indicated at 158. After the test is complete, holder 150 is then removed, indicated at 160, and multi-well glass slide 152 can be easily transferred for complete protein analysis and for rapid detection of sterilization, deimmunization, or disinfection as discussed above.

If magnetizable beads are used as the solid surface, one or more of the defined surrogate proteins having the associated predetermined sequence could also be mixed into liquid sterilization, deimmunization and/or disinfection steam and later harvested with a strong magnet. Flowing disinfectants or gases would result in the complete loss of the defined surrogate protein having the associated predetermined sequence samples that are not covalently linked to a support surface and the protein array

analysis may be used to rapidly determine effective sterilization, deimmunization or disinfection.

Both Western Blot Analysis and protein array analysis are an indirect measurement of sterilization, deimmunization and/or disinfection. Sterilization is the complete destruction of spore forming infectious organisms so the standard direct measurements are long growth studies to determine the number of organisms that survived the sterilization process. Deimmunization is the complete destruction of immunogenic proteins such that no protein fragments capable of triggering an immune reaction in human or animal remain. Disinfection is the complete destruction of vegetative bacteria, fungus and/or viruses. As disclosed herein, the defined surrogate proteins are considered to be in the form of isolated fragments and destroyed if proteins critical for the survival of the infectious agents are used in quantities 10,000 to 1,000,000 times as much as would occur in a standard sample of intact infectious agents. To correctly establish the scale to determine success ratio between the direct and indirect tests, multiple conditions are preferably utilized to determine the Western Blot analysis and protein array analysis conditions that perfectly align with standard sterilization, deimmunization and/or disinfection studies using intact infectious organisms and post sterilization growth conditions.

The samples prepared for Western Blot analysis and protein array analysis may use a wide range of circumstances. If the sterilization, deimmunization and/or disinfection equipment uses flowing gases or liquid to wash over the surfaces to be sterilized holder 150, Fig. 3, and multi-well glass slide 152 or similar type device, may be used to ensure the defined surrogate protein samples remain in the flowing stream of disinfecting gas or liquid. For example, if the sterilization, deimmunization

or disinfecting gas or liquid is washed through medical equipment to sterilize, deimmunize, or disinfect the inside of a lumen, holder 150 with the multi-well glass slide 150 having one or more defined surrogate proteins having the associated predetermined sequence therein, e.g., *Clostridium*, *Bacillus*, *Myocbacterium*, Staphylucoccus, *Pseudomonas*, *Trichophyton*, *Candida*, and *α-Gliadin* analysis tests could be connected to the end of the stream to better measure the quality of sterilization, deimmunization, or disinfection that occurred through the entire length of the medical equipment lumen.

The following examples are exemplary and explanatory only and do not limit or restrict this invention.

EXAMPLES

Example 1: Comparing Amino Acid Sequence of Human PrP Protein to Other Species

It is important to qualify the ability of a sterilization device to destroy prions that may be contaminating medical equipment. For the specific test described herein in accordance with one or more embodiments of the method for rapidly determining effective sterilization, deimmunization, or disinfection of this invention, a defined quantity of the defined surrogate prion protein discussed above is provided and then evaluated using Western Blot analysis and an antibody specific for the defined prion surrogate protein.

To protect the human operators of the test, infectious human prions cannot be used. Instead, the defined surrogate protein is used that incorporates all the characteristics of human prion (PrP) proteins, with the critical exception that it cannot infect humans. In this example, to select the defined surrogate PrP protein to be used, a protein analysis was conducted comparing the amino acid protein sequence of

human PrP protein against a selection of the protein sequence databases of other species including a primate, two companion animals, two food animals and two research animals. From this analysis, the mouse PrP protein was determined to be the best candidate surrogate protein. Structurally, mouse PrP protein is as robust as the human PrP protein and thus will be equivalently resistant to a wide range of destructive methods but is also sufficiently different as to be unable to infect humans. Arrow 168, Fig 4, indicates the initiation location for Pr27-30, the smallest PrP fragment that retains infectivity. If a PrP protein is cleaved anywhere from this initiation point to the end of the protein sequence, the resulting fragments can no longer cause disease.

To evaluate additional surrogate PrP proteins that could be used, the mouse PrP protein sequence was compared against the protein sequence database. Table 5 below shows the wide diversity of a predetermined list of species PrP proteins that could be used as well as each protein's sequence ID and its homology to Mouse PrP. The human sequence is less than 90% homologous to mouse PrP. The search also demonstrates how conserved the PrP protein is across a wide range of mammalian species contained within the sequence database and any one of these could be used as the surrogate protein in the Protein Indicator Test. The database also contains the sequence for chicken PrP but as it is less than 50% homologous to other mammalian PrP proteins, it is possible that it or other related proteins could be used in the Protein Indicator Test but its divergence could impact its resistance to destruction. This would make it a less suitable surrogate for the test than other proteins, especially any mammalian PrP protein.

Species	Sequence ID Number	Homology to Mouse (%)
Mouse (Mus musculus)	sp|P04925.2|PRIO_MOUSE	100
Rat (Rattus norvegicus)	sp|P13852.2|PRIO_RAT	98
Cotton Rat (Sigmodon hispidus)	sp|Q9Z0T3.1|PRIO_SIGHI	97
Chinese Hamster (Cricetulus griseus)	sp|Q60506.1|PRIO_CRIGR	97
Grey Dwarf Hamster (Cricetulus migratorius	sp|Q60468.1|PRIO_CRIMI	95
Golden Hamster (Mesocricetus auratus)	sp|P04273.1|PRIO_MESAU	95
Greater Kudu (Tragelaphus strepsiceros)	sp|P40243.1|PRIO2_TRAST	90
Red-bellied Titi (Callicebus moloch)	sp|P40248.1|PRIO_CALMO	96
Three-striped Night Monkey (Aotus trivirgatus)	sp|P40245.1|PRIO_AOTTR	94
Black-capped capuchin (Sapajus apella)	sp|P40249.1|PRIO_CEBAP	95
Common Marmoset (Callithrix jacchus)	sp|P40247.1|PRIO_CALJA	95
Red-faced Spider Monkey (Ateles paniscus)	sp|P51446.1|PRIO_ATEPA	94
Geoffroy's Spider Monkey (Ateles geoffroyi)	sp|P40246.1|PRIO_ATEGE	91
Nilgai (Boselaphus tragocamelus)	sp|Q5UJG7.1|PRIO_BOSTR	86
Alpine Musk Deer (Moschus chrysogaster)	sp|Q68G95.1|PRIO_MOSCH	88
Common Squirrel Monkey (Saimiri sciureus)	sp|P40258.1|PRIO_SAISC	91
Gelada Baboon (Theropithecus gelada)	sp|Q95270.1|PRIO_THEGE	90
Black Crested Mangabey(Lophocebus aterrimus)	sp|P67990.1|PRIO_LOPAT	90
Mona Monkey(Cercopithecus mona)	sp|P61761.1|PRIO_CERMO	91
Patas Monkey(Erythrocebus patas)	sp|Q95174.1|PRIO_ERYPA	91
Grivet(Chlorocebus aethiops)	sp|P67988.1|PRIO_CHLAE	90
Mantled Guereza(Colobus guereza)	sp|P40251.1|PRIO_COLGU	91
Bornean orangutan(Pongo pygmaeus)	sp|P40256.1|PRIO_PONPY	91
François' Langur (Trachypithecus francoisi)	sp|P40257.2|PRIO_TRAFR	91
Sooty Mangabey (Cercocebus atys)	sp|Q95176.1|PRIO_CERAT	91
Crab-eating Macaque (Macaca fascicularis)	sp|P67992.1|PRIO_MACFA	91
Mandrill(Mandrillus sphinx)	sp|P40255.1|PRIO_MANSP	91
Gorilla (Gorilla gorilla gorilla)	sp|P40252.1|PRIO_GORGO	90
Cat (Felis catus)	sp|O18754.3|PRIO_FELCA	87
Human (Homo sapiens)	sp|P04156.1|PRIO_HUMAN	89
Lar Gibbon(Hylobates lar)	sp|P61766.1|PRIO_HYLLA	89
Bighorn Sheep (Ovis canadensis)	sp|Q7JIH3.1|PRIO_OVICA	88
Goat(Capra hircus)	sp|P52113.1|PRIO_CAPHI	88
Blackbuck(Antilope cervicapra)	sp|Q5UJG1.1|PRIO_ANTCE	85
Takin(Budorcas taxicolor)	sp|Q95M08.1|PRIO_BUDTA	87
Sheep (Ovis aries)	sp|P23907.1|PRIO_SHEEP	87
Rabbit (Oryctolagus cuniculus)	sp|Q95211.1|PRIO_RABIT	90
Water Buffalo (Bubalus bubalis)	sp|Q5UJH8.1|PRIO_BUBBU	84
Rocky Mountain Elk (Cervus canadensis nelsoni)	sp|P67986.1|PRIO_CEREN	87
Mule Deer (Odocoileus hemionus)	sp|P47852.1|PRIO_ODOHE	87
Lesser Kudu (Tragelaphus imberbis)	sp|Q5UJG3.1|PRIO_TRAIM	85
Greater Kudu(Tragelaphus strepsiceros)	sp|P40242.1|PRIO1_TRAST	84
Cow (Bos taurus)	sp|P10279.2|PRIO_BOVIN	84
Common Brushtail Possum (Trichosurus Vulpecula)	sp|P51780.1|PRIO_TRIVU	77
Giant Panda(Ailuropoda melanoleuca)	sp|Q6EH52.1|PRIO_AILME	84

American Mink (Neovison vison)	sp|P40244.1|PRIO_NEOVI	79
Dromedary Camel (Camelus dromedaries)	sp|P79141.1|PRIO_CAMDR	88
Pig (Sus scrofa)	sp|P49927.1|PRIO_PIG	79
Ferret(Mustela putorius furo)	sp|P52114.1|PRIO_MUSPF	78
Artic Fox(Vulpes lagopus)	sp|B0FYL5.1|PRIO_VULLA	78
Dog (Canis lupus familiaris)	sp|O46501.1|PRIO_CANFA	85
Chicken (Gallus gallus)	sp|P27177.2|PRIO_CHICK	43

Table 5. Potential PrP Protein that could be used as Surrogate Protein in Sterilization Indication Tests. Information about each PrP protein includes species, Sequence ID number and homology to Mouse recombinant PrP protein.

Example 2: Developing Method for Testing the Ability to Destroy PrP Proteins:

One purpose of developing the defined prion surrogate protein test is to provide a rapid method for determining effective sterilization equipment. The method preferably has multiple steps including at least: 1) preparing defined prion surrogate proteins having a predetermined sequence, 2) subjecting the prion surrogate protein samples to sterilization, and 3) using Western Blot analysis to visualize the effects of sterilization of defined prion surrogate protein samples. Successful sterilization has occurred when all of the defined prion surrogate protein sample was fragmented and, as a result of the protein fragmentation, none remains to bind to the visualization antibodies. If sterilization was not successful, protein bands will be see on the Western Blot analysis.

A defined surrogate protein having a sequence above for the prions, recombinant mouse PrP full-length protein (208 amino acids long) was obtained from Abcam, Cambridge Massachusetts. On a Western gel, the recombinant protein runs at about approximated 28 kDa in size, indicated at 290, Fig. 5.

The Western Blot analysis discussed above is well-established and is an extremely sensitive method to determine the presence and/or absence of a specific protein. The first step in Western Blot analysis involves preparing the defined prion

surrogate protein samples for separation by size. In accordance with one embodiment of the method for rapidly determining effective sterilization, deimmunization, or disinfection of the defined prion surrogate proteins will be run as single denatured proteins and thus must be denatured through the use of a denaturing loading buffer, boiled for 10 minutes and then run on a denaturing gel. Once loaded on to an appropriate acrylamide gel, the samples are subjected to electric current that results in the protein fragments traveling at different rates depending on size with smaller fragments moving faster than larger ones. In one example, the gel contained 8% acrylamide. To be able to monitor the separation of the defined prion surrogate protein samples, a standard sample with a mix of proteins of defined sizes was also included. For some experiments discussed herein, additional protein samples may be included to provide for controls of the sample handling conditions.

After separation, the defined prion surrogate protein samples are transferred to a special nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody available from Abcam, Cambridge Massachusetts that specifically binds to the defined surrogate protein of interest. In the experiments discussed herein, the primary anti-PrP antibody was a rabbit monoclonal antibody that was raised against a synthetic peptide corresponding to residues near the C-terminus of human Prion protein PrP, as indicated by underlined region indicated at 170, Fig 4. In addition to binding to both PrP_c and PrP_{sc}, the antibody will bind to the infectious protein form PrP 27-30. The primary antibody binds to Human, Rat and Mouse PrP proteins. The secondary antibody is a HRP-labelled goat anti-rabbit available from Abcam, Cambridge Massachusetts to enable visualization of the infectious PrP fragments,

both intact and fragmented. If the defined prion surrogate proteins have been fragmented into non-infectious fragments that eliminate the C-terminus region of the PrP, the antibody will not bind them. Without this C-terminus region, the degraded PrP protein is no longer infectious. Very small fragments and amino acids which are no longer infectious will be too small to be retained on the gel.

The first tests involved carefully drying defined quantities of recombinant mouse PrP protein onto filter papers to assist in storage, transport to and from location of sterilization equipment and handling during sterilization tests. To create, test strips of filter paper were cut 4 mm wide by 20 mm long and the bottoms were squared. Serial dilutions (1:3) of mouse PrP protein were created to contain protein solutions 0.1ug/ul to 0.0037 ug/ul. The protein preps were added dropwise to the filter paper strips until 10 ul were added. The filter paper strips were then air dried. Filter paper strips were prepared in duplicate with 1 ug, 0.333 ug, 0.111 ug, or 0.033ug applied respectively to a pair of strips. When dry, the strips were placed in Eppendorf tubes and 50 ul 1X Loading Buffer where BME was added. The strips and loading buffer were incubated at 95°C for 5 minutes then given a quick spin in a micro centrifuge. For one of each paired sample, the filter paper strip was taken out of the Eppendorf tube and inserted into the well of the SDS-PAGE gel. For these samples, electroporation was used to extract any residual defined prion surrogate protein that was retained on the filter paper after extraction by boiling and centrifugation. Half of the loading buffer from these tubes was also loaded into the same well. For the other samples of the pair only boiling and centrifugation was used to extract the defined prion surrogate protein from the filter strips. From these, half of the loading buffer was added to the wells without also adding the filter paper. A control sample of 0.5

ug protein (equivalent to half of the 1ug concentration samples) was also loaded on the gel for comparison. The gel was run and the Western Blot Analysis performed using the procedure described above.

The results demonstrated that there was no significant difference between the protein concentrations that were dried onto the filter paper and the control sample. There was also no significant difference between the extraction process using only boiling and centrifugation and the more difficult process of using boiling, centrifugation and electroporation for extraction. In addition, the Western Blot analysis demonstrated that the Western Blot analysis was sensitive to below 0.05 ug. Hence, a 1 ug of recombinant mouse PrP protein should be used for all sterilization tests. The samples should be extracted from filter paper using boiling in loading the buffer with BME and centrifugation and half of the sterilized samples used for Western Bolt analysis.

Example 3: Evaluation of Destruction of Prion Proteins Using Sterilization Equipment

Experiments were conducted to demonstrate a combination of one or more of heat, a vaporizing solvent, and electromagnetic radiation, e.g., microwaves, e.g., as disclosed in the '469 Patent Application, could irreversibly destroy the defined prion surrogate protein shown with the sequence shown above. The stable PrP protein was selected for the experiments as it cannot be irreversibly destroyed using a standard sterilization autoclave device. For the experiments, samples were created that each contained 1 ug of a structurally robust mouse PrP protein and wrapped in 100% cotton paper to avoid extraneous contamination. This containment was placed in a second layer of 100% cotton paper to increase stability during treatment. The samples were

treated with different temperatures and for differing numbers of moisture saturation and microwave cycles, e.g., as disclosed in the '469 Patent Application. After treatment, the samples were prepared in loading buffer and boiled. Half of each sample was then run on a denaturing protein gel. After Western Blot analysis, it was possible to see that certain combinations of temperature and treatment cycles completely destroyed the stable protein samples, shown by the absence of protein bands of gel indicated at 294, 296, Fig. 6. Other treatment conditions did not destroy the stable proteins.

Example 4: Evaluation of Destruction of Prion Proteins Contained within Polypropylene Tubes

Additional experiments were conducted to demonstrate that with the combination of one or more of heat, saturating moisture and microwaves as disclosed in the '469 Patent Application could destroy the defined surrogate stable PrP proteins if the samples were contained within a vessel such as a polypropylene tube. In this example, samples of the defined prion surrogate proteins were created that each contained about 1 ug of a structurally robust mouse protein. However, instead of drying the protein samples onto filter paper, the 1 ug samples were dried within polypropylene tubes of different lengths including 0.75 cm and 3.5 cm in length. The tubes were closed at one end (on to which the samples were dried) and open at the other. The tubes were contained within 2 layers of 100% cotton paper to prevent cross contamination during treatment. The samples were subjected to sterilization by a combination of one or more of heat, a solvent, e.g., reverse osmois (RO) filtered water, and electromagnetic radiation with differing numbers of moisture saturation

and radiation cycles, e.g., as disclosed in the '469 Patent Application. After treatment, the samples were prepared in loading buffer and boiled. Half of each sample was then run on a denaturing protein gel. After Western blot analysis, it was possible to see that certain combinations of temperature and treatment cycles completely destroyed the stable protein samples (as demonstrated by the absence of protein bands of gel). Other treatment conditions did not destroy the stable proteins. The results of the sterilization treatment of PrP samples dried onto filter paper and dried within a polypropylene tube were identical indicating that the method of containing the PrP sample did not alter the results.

Example 5: Comparison of Prion Protein Indicator Test Results and Mouse Model Prion Tests

All mice were kept in an AAALAC-accredited facility and handled in compliance with guidelines provided by the US Guide for the Care and Use of Laboratory Animals.

Creating Prion Infected Brain Homogenate: Brains from terminally ill C57BL/6 mice infected with 22L prions were prepared as follows: each brain was homogenized (10%, w/v) in phosphate-buffered saline (PBS) by repeatedly passing the material first through an 18-gauge needle and then repeatedly through a 26 gauge needle. The brain homogenates were combined to make a stock preparation, diluted with PBS (1/10), aliquoted into 100 ul preps in 2 ml polypropylene freezing vials and frozen at -80°C until use.

Division into treated and non-treated preps: frozen prion preps having the defined prion surrogate protein were allowed to thaw and the caps removed. In duplicate, samples were sterilized by a combination of one or more of heat,

microwaves and saturating moisture, e.g., as disclosed in the '469 Patent Application. The used conditions were similar to the process disclosed in Example 4 above. In one example, the sterilization preferably includes: (A) 140°C, 100 cycles of microwave; (B) 100°C, 100 cycles of microwave; and (C) room temperature, 100 cycles with no microwaves. The Prion Protein Indicator Test having the defined surrogate protein samples were analyzed by Western Blot analysis as described in Example 4 above. Depending on treatment conditions, the test sample was completely destroyed (A), partially destroyed (B), or completely intact (C).

Testing infectiveness of Prion Prep: C57BL/6 mice aged 4-5 weeks were divided into 4 cohorts, 10 mice per cohort, to receive the samples that correlated with the following Prion Protein Indicator Test samples having the defined prion surrogate protein: (A) complete destruction; (B) partial destruction; (C) no treatment and (D) PBS control. After anesthesia each mouse was intracerebrally inoculated with a 20 ul-aliquot of the designated inoculum. The mice were observed up to one year after inoculation, unless they displayed terminal symptoms of PrP infection including persistent signs of ataxia, kyphosis, somnolence, and hind leg weakness. Terminally-ill mice were euthanized and their brains divided sagittally along the midline and place formalin fixation for histological analysis or flash-frozen in liquid nitrogen for protein analysis. At one year, all remaining mice (showed no obvious signs of neurologic disease) were euthanized and their brains also divided for histological analysis or protein analysis.

Results: Over the observation period, none of the mice that received either the PBS control (D) or the brain homogenate treated with the conditions that demonstrated complete destruction on the Prion Protein Indicator Test having the

defined prion surrogate protein (A) demonstrated any symptoms of disease. After euthanization, none of the brains demonstrated any signs of prion disease. Western Blot analysis of the brains showed no increase in concentrations of PrP proteins over normal levels. The mice that received the brain homogenate and also received no sterilization treatment (C) or partial destruction (B) as indicated by the Prion Protein Indicator Test all demonstrated terminal symptoms of PrP infection before the completion of the 1 year observation period. Their brains demonstrated obvious signs of prion disease and by Western analysis, the concentration of PrP proteins were greatly increased over normal levels. Together the results of the mouse study indicated a clear correlation between the results from the Prion Protein Indicator Test results and the mouse model results.

Example 6: Comparing Amino Acid Sequence of Multiple members of the *Clostridium* Genus

It is important to qualify the ability of a sterilization device, a deimmunization device, or a disinfection device to destroy bacteria of any *Clostridium* species that may be contaminating medical equipment or supplies. In this example, a defined quantity of the defined surrogate protein having the predetermined SEQ ID NO. 1 discussed above was subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis, and the antibody for the defined *Clostridium* surrogate protein shown above. In this example, to protect the human operators of the test, the defined surrogate protein needs to incorporate critical characteristics of *Clostridium* proteins that are critical for the survival and growth of members of the *Clostridium* genus while

avoiding organisms that can infect humans.

To design the synthetic defined surrogate, *Clostridium* protein having the predetermined sequence, SEQ ID NO. 1, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Clostridium* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci proteins, multiple *Clostridium* species, used for the comparative are shown in Table 5 below:

Species	Sequence ID
Clostridium sporogenes	WP_061905762.1
Clostridium botulinum	WP_011948579.1
Clostridium novyi	WP_039217212.1

Table 5 (Clostridium). Sequences used to determine regions of high homology in *suf I* locus of multiple Clostridium Species.

The protein produced by the *suf I* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Clostridium* genus, the protein product of the *suf I* loci is called *cotA* and is critical for many live stages including strongly contributing to the stability of the spore coat.

To design the defined *Clostridium* surrogate protein having SEQ ID NO. 1, the specific amino acids from the proteins listed in Table 5 were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Clostridium* species as shown in Fig. 7. The protein encoded by the *suf I* loci includes three cupredoxin domains that are indicated at 200 for domain 1, 202 for domain 2, and 204 for domain 3. In the *Clostridium* genus, domain 2, indicated at 202, shows high homology between *Clostridium* species so was used to for the design of the synthetic surrogate protein SEQ ID NO. 1 above to be created for the *Clostridium* test and the

corresponding peptides discussed above were used to develop the polyclonal and monoclonal antibodies for use by Western Blot analysis.

Example 7: Developing Western Test to Qualify Ability to Destroy *Clostridium* Test Protein:

One purpose of developing the synthetic defined *Clostridium* surrogate protein is to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as sterilization device, deimmunization device, or disinfection device. In this example, the method for rapidly determining effective sterilization, deimmunization, and/or disinfection includes multiple steps including at least: 1) preparing the synthetic defined *Clostridium* surrogate protein test samples, 2) subjecting the defined *Clostridium* surrogate protein test samples to sterilization, deimmunization, or disinfection, and 3) using Western Blot or similar type analysis to visualize the effects of sterilization, deimmunization, or disinfection of defined *Clostridium* surrogate protein test samples. Successful sterilization, deimmunization, or disinfection has occurred when all the defined *Clostridium* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Clostridium* surrogate protein was destroyed. If sterilization, deimmunization, or disinfection was not successful, protein bands will be seen on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the *Clostridium* sterilization, deimmunization, and/or disinfection test. First, to create the sample for the test using the defined *Clostridium* surrogate protein, DNA encoding the amino acid SEQ ID NO. 1 for *Clostridium*

above was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length defined *Clostridium* surrogate protein (171 amino acids long) was isolated. To create the samples having the defined *Clostridium* surrogate protein to qualify sterilization, deimmunization, or disinfection of the samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, or disinfection e.g., using cycles of applying a solvent and microwave energy as disclosed in the '469 Patent Application.

After sterilization, deimmunization, or disinfection, the treated *Clostridium* samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the defined *Clostridium* surrogate protein of interest. In the experiments discussed herein, the primary anti-*suf I* loci encoded protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 1 residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot Analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the defined *Clostridium* surrogate proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, or disinfection occurred, the visualized Western blot has a dark ban in the untreated

control sample, e.g., indicated at 210, Fig. 8, and a complete absence of any bands for the defined *Clostridium* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, e.g., indicated at 212.

Example 8: Comparing Amino Acid Sequence of Multiple members of the *Bacillus* Genus

It is important to qualify the ability of a sterilization device, a deimmunization device or disinfection device to destroy bacteria of any *Bacillus* species that may be contaminating medical equipment or supplies. In this example, a defined quantity of the defined *Bacillus* surrogate protein having the predetermined SEQ ID NO. 2 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis, and the antibody for the defined *Bacillus* surrogate protein shown above. In this example, to protect the human operators of the test, the defined surrogate *Bacillus* surrogate protein needs to incorporate critical characteristics of *Bacillus* proteins that are critical for the survival and growth of members of the *Bacillus* genus while avoiding organisms that can infect humans.

To design the synthetic defined surrogate *Bacillus* protein having SEQ ID NO. 2, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Bacillus* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci encoded proteins, multiple *Bacillus* species, used for the comparative are shown in Table 6 below.

Species	Sequence ID
Bacillus subtilis	AAB62305.1
Bacillus atrophaeus	WP_011948579.1
Bacillus pumilus	WP_039217212.1

Table 6 (Bacillus). Sequences used to determine regions of high homology in *suf I* locus of multiple *Bacillus* Species.

The protein produced by the *suf I* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Bacillus* genus, the protein product of the *suf I* loci is called *cotA* and is critical for many live stages including strongly contributing to the stability of the spore coat.

To design the synthetic defined *Bacillus* surrogate protein for SEQ ID NO. 2, the specific amino acids from the proteins listed in Table 6 were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Bacillus* species as shown in Fig. 9. The protein encoded by the *suf I* loci includes three cupredoxin domains, indicated at 214 for domain 1, 216 for domain 2, and 218 for domain 3. In the *Bacillus* genus, domain 2, indicated at 216, shows high homology between *Bacillus* species, so SEQ ID NO. 2 above was used as the synthetic defined *Bacillus* surrogate protein to be used for the *Bacillus* test and the corresponding peptide discussed above was used to raise a polyclonal antibody for use by Western Blot analysis. Additional polyclonal and monoclonal antibodies were created as needed.

Example 9: Developing Western Test to Qualify Ability to Destroy *Bacillus* Test Protein:

One purpose of developing the synthetic defined *Bacillus* surrogate protein is

to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as a sterilization device, deimmunization device, or disinfection device. The method for rapidly determining effective sterilization, deimmunization, and/or disinfection preferably includes multiple steps including at least: 1) preparing synthetic defined *Bacillus* surrogate protein test samples, 2) subjecting the *Bacillus* surrogate protein test samples to sterilization, deimmunization, or disinfection, and 3) using Western Blot or similar analysis to visualize the effects of sterilization, deimmunization, or disinfection of defined *Bacillus* surrogate protein test samples. Successful sterilization, deimmunization, or disinfection has occurred when all the defined *Bacillus* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Bacillus* surrogate protein was destroyed. If sterilization, deimmunization, or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the *Bacillus* sterilization, deimmunization, and/or disinfection test. First, to create the sample for the test using the synthetic defined *Bacillus* surrogate protein, DNA encoding the amino acid for SEQ ID NO. 2 above was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of the defined *Bacillus* surrogate protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length defined *Bacillus* surrogate protein (171 amino acids long) was isolated. To create the samples having the synthetic defined *Bacillus* surrogate protein to qualify sterilization, deimmunization, and/or disinfection the

samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, or disinfection, e.g., using cycles of applying a solvent and microwave energy, e.g., as disclosed in the '469 Patent Application.

After sterilization deimmunization and/or disinfection, the treated *Bacillus* samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the defined *Bacillus* surrogate proteins. In the experiments discussed herein, the primary anti-*suf I* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 2 residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot Analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the defined *Bacillus* surrogate proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, or disinfection occurred, the visualized Western blot has a dark ban in the untreated control sample, e.g., indicated at 220, Fig. 10, and a complete absence of any bands for defined *Bacillus* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, indicated at 222.

Example 10: Comparing Amino Acid Sequence of Multiple members of the

Mycobacterium Genus

It is important to qualify the ability of a sterilization device, a deimmunization device, or a disinfection device to destroy bacteria of any *Mycobacterium* species that may be contaminating medical equipment or supplies. In this example, a defined quantity of the defined surrogate protein having a SEQ ID NO. 3 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis and using an antibody specific for the protein. In this example, to protect the human operators of the test, the defined *Mycobacterium* surrogate protein needs to incorporate critical characteristics of *Mycobacterium* proteins that are critical for the survival and growth of members of the *Mycobacterium* genus while avoiding organisms that can infect humans.

To design the synthetic defined *Mycobacterium* surrogate protein SEQ ID NO. 3 above, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Mycobacterium* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci proteins, multiple *Mycobacterium* species, used for the comparative are shown in Table 7 below:

Species	Sequence ID
Mycobacterium tuberculosis	WP_003404392.1
Mycobacterium africanum	KBG17039.1
Mycobacterium kansasii	WP_023367763.1

Table 7 (Mycobacteria). Sequences used to determine regions of high homology in *suf I* locus of multiple *Mycobacterium* Species.

The protein produced by the *suf I* loci is fundamental to survival and growth of

a wide range of spores, bacteria and fungus. In species of the *Mycobacterium* genus, the protein product of the *suf I* loci is called *cumA* and is critical for many live stages including cell survival and growth.

To design the synthetic defined *Mycobacterium* surrogate protein having SEQ ID NO. 3, the specific amino acids from the proteins listed in Table 7 were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Mycobacterium* species as shown in Fig. 11. The protein encoded by the *suf I* loci includes three cupredoxin domains that are indicated at 230 for domain 1, 232 for domain 2, and 234 for domain 3. In the *Mycobacterium* genus, domain 2, indicated at 232, shows high homology between *Mycobacterium* species so SEQ ID NO. 3 above was used to for the design for synthetic defined *Mycobacterium* surrogate protein to be created for the *Mycobacterium* test and the corresponding peptide for the defined *Mycobacterium* surrogate protein discussed above was used for a polyclonal antibody for use by Western Blot analysis.

Example 11: Developing Western Test to Qualify Ability to Destroy *Mycobacterium* Test Protein:

One purpose of developing the defined *Mycobacterium* surrogate protein is to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as a sterilization device, a deimmunization device, or a disinfection device. The method for rapidly determining effective sterilization, deimmunization, and/or disinfection preferably includes multiple steps including at least: 1) preparing the defined *Mycobacterium* surrogate protein test samples, 2) subjecting the defined *Mycobacterium* surrogate protein test samples to sterilization, deimmunization, or disinfection, and 3) using Western Blot or

similar analysis to visualize the effects of sterilization, deimmunization, and/or disinfection of synthetic defined *Mycobacterium* surrogate protein test samples. Successful sterilization, deimmunization, and/or disinfection has occurred when all the synthetic defined *Mycobacterium* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Mycobacterium* protein was destroyed. If sterilization, deimmunization, and/or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the *Mycobacterium* sterilization, deimmunization, and/or disinfection test. First, to create the sample for the test using the defined *Mycobacterium* surrogate protein, DNA encoding the amino acid SEQ ID NO. 3 above was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length synthetic *Mycobacterium* surrogate protein (171 amino acids long) was isolated. To create the samples having the *Mycobacterium* surrogate protein to qualify sterilization, deimmunization, and/or disinfection the samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, and/or disinfection, e.g., using cycles of applying a solvent and microwave energy as disclosed in the '469 Patent Application.

After sterilization, deimmunization, and/or disinfection the treated *Mycobacterium* surrogate protein samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-

linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the protein of interest. In the experiments discussed herein, the primary anti-*suf I* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 3 for *Mycobacterium* above residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot Analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the *Mycobacterium* surrogate proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, and/or disinfection has occurred, the visualized Western blot has a dark ban in the untreated control sample, e.g., indicated at 236, Fig. 12, and a complete absence of any bands for the *Mycobacterium* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, e.g., indicated at 238.

Example 12: Comparing Amino Acid Sequence of Multiple members of the *Staphylococcus* Genus

It is important to qualify the ability of a sterilization device, a deimmunization device, or a disinfection device to destroy bacteria of any *Staphylococcus* species that may be contaminating medical equipment or supplies. In this example, a defined quantity of the defined *Staphylococcus* surrogate protein having the predetermined SEQ ID NO. 4 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization,

deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis and the antibody specific for the defined *Staphylococcus* surrogate protein. In this example, to protect the human operators of the test, the synthetic defined *Staphylococcus* surrogate protein needs to incorporate critical characteristics of *Staphylococcus* proteins that are critical for the survival and growth of members of the *Staphylococcus* genus while avoiding organisms that can infect humans.

To design the synthetic defined *Staphylococcus* surrogate protein having SEQ ID NO. 4, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Staphylococcus* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci proteins, multiple *Staphylococcus* species, used for the comparative are shown in Table 8 below:

Species	Sequence ID
Staphylucoccus aureus	WP_000282432.1
Staphylucoccus epidermidis	WP_023567454.1
Staphylucoccus saprophyticus	OEK13316.1

Table 8 (*Staphylucoccus*). Sequences used to determine regions of high homology in *Suf I* locus of multiple *Staphylucoccus* Species.

The protein produced by the *suf I* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Staphylococcus* genus, the protein product of the *suf I* loci is called *cueO* and is critical for many live stages including strongly contributing to cell survival and growth

To design the synthetic defined *Staphylococcus* surrogate protein, the specific amino acids from the proteins listed in Table 8 were aligned to determine amino acid

sequence regions that are highly homologous in all evaluated *Staphylococcus* species as shown in Fig. 13. The protein encoded by the *suf I* loci includes three cupredoxin domains that are indicated at 240 for domain 1, 242 for domain 2, and 244 for domain 3. In the *Staphylococcus* genus, domain 2 indicated at 242 shows high homology between *Staphylococcus* species, so SEQ ID NO. 4 above was used to for the design of the synthetic defined *Staphylococcus* surrogate protein to be created for the *Staphylococcus* test and the corresponding peptide discussed above was used for a polyclonal antibody for use by Western Blot analysis.

Example 13: Developing Western Test to Qualify Ability to Destroy *Staphylococcus* Test Protein:

One purpose of developing the defined *Staphylococcus* surrogate protein is to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection device, such as sterilization device, deimmunization device, or disinfection device and/or supplies. The method for rapidly determining effective sterilization, deimmunization, and/or disinfection includes multiple steps including at least: 1) preparing synthetic defined *Staphylococcus* surrogate protein test samples, 2) subjecting the defined *Staphylococcus* surrogate protein test samples to sterilization, deimmunization, and/or disinfection, and 3) using Western Blot or similar analysis to visualize the effects of sterilization, deimmunization, and/or disinfection of defined *Staphylococcus* surrogate protein test samples of the defined surrogate protein. Successful sterilization has occurred when all the defined *Staphylococcus* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Staphylococcus* surrogate protein was

destroyed. If sterilization, deimmunization, and/or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the *Staphylococcus* sterilization, deimmunization, or disinfection test. First to create the sample for the test using the synthetic defined *Staphylococcus* surrogate protein, DNA encoding the amino acid SEQ ID NO. 4 above was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of the defined *Staphylococcus* surrogate protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length defined *Staphylococcus* surrogate protein (171 amino acids long) was isolated. To create the samples having the synthetic defined *Staphylococcus* surrogate protein to qualify sterilization, deimmunization, and/or disinfection the samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, and/or disinfection e.g., using cycles of applying a solvent and microwave energy as disclosed in the '469 Patent Application.

After sterilization, deimmunization, and/or disinfection, the treated defined *Staphylococcus* surrogate protein samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the *Staphylococcus* surrogate protein of interest. In the experiments discussed herein, the primary anti-*suf I* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 4 residues. For added sensitivity, addition

antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the defined *Staphylococcus* surrogate proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, and/or disinfection occurred, the visualized Western blot has a dark ban in the untreated control sample, indicated at 250, Fig. 14, and a complete absence of any bands for defined *Staphylococcus* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, indicated at 252.

Example 14: Comparing Amino Acid Sequence of Multiple members of the *Pseudomonas* genus

It is important to qualify the ability of a sterilization device, a deimmunization device or disinfection device to destroy bacteria of any *Pseudomonas* species that may be contaminating medical equipment or supplies. In this example, a defined *Pseudomonas* surrogate protein quantity of the defined surrogate protein having a predetermined SEQ ID NO. 5 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis, and using an antibody specific for the defined *Pseudomonas* surrogate protein shown above. In this example, to protect the human operators of the test, the defined *Pseudomonas* surrogate protein needs to incorporate critical characteristics of *Pseudomonas* proteins that are critical for the survival and growth of members of the *Pseudomonas* genus while avoiding organisms that can

infect humans.

To design the synthetic defined *Pseudomonas* surrogate protein having SEQ ID NO. 5, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Pseudomonas* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci Table 9 below:

Species	Sequence ID
Pseudomonas aeruginosa	WP_023096478.1
Pseudomonas fluorescens	WP_003227851.1
Pseudomonas putida	WP_019750583.1

Table 9 *Pseudomonas*. Sequences used to determine regions of high homology in *suf 1* locus of multiple *Pseudomonas* Species.

The protein produced by the *suf 1* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Pseudomonas* genus, the protein product of the *suf 1* loci is called *cumA* and is critical for many live stages including strongly contributing to the cell survival and growth

To design the defined *Pseudomonas* surrogate protein, the specific amino acids from the proteins listed in Table 9 were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Pseudomonas* species as shown in Fig. 15. The protein encoded by the *suf 1* loci includes three cupredoxin domains indicated at 260 for domain 1, 262 for domain 2, and 264 for domain 3. In the *Pseudomonas* genus, domain 2, indicated at 262, shows high homology between *Pseudomonas* species so SEQ ID NO. 5 above was used to for the design of the synthetic surrogate protein to be created for the defined *Pseudomonas* surrogate protein test and the corresponding peptide shown above was used for a polyclonal

antibody for use by Western Blot analysis.

Example 15: Developing Western Test to Qualify Ability to Destroy *Pseudomonas* Test Protein:

One purpose of developing the defined *Pseudomonas* surrogate protein test is to create a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as a sterilization device, a deimmunization device, or a disinfection device. The method for rapidly determining effective sterilization, deimmunization, and/or disinfection preferably includes multiple steps including at least: 1) preparing synthetic defined *Pseudomonas* surrogate protein test samples, 2) subjecting the synthetic *Pseudomonas* surrogate protein test samples to sterilization, deimmunization, and/or disinfection, and 3) using Western Blot or similar analysis to visualize the effects of sterilization, deimmunization, and/or disinfection of synthetic defined *Pseudomonas* surrogate test samples. Successful sterilization has occurred when all the defined *Pseudomonas* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Pseudomonas* surrogate protein was destroyed. If sterilization, deimmunization, and/or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the defined *Pseudomonas* sterilization, deimmunization and/or disinfection test. First, to create the sample for the test using the defined *Pseudomonas* surrogate protein, DNA encoding the amino acid SEQ ID NO. 5 defined above was synthesized and cloned into standard vectors both for *E. coli* and

yeast expression. Using standard techniques, large quantities of the defined *Pseudomonas* surrogate protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length synthetic defined *Pseudomonas* surrogate protein (171 amino acids long) was isolated. To create the samples having the synthetic defined surrogate protein to qualify sterilization, deimmunization, and/or disinfection of the samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, and/or disinfection, e.g., using cycles of applying a solvent and microwave energy as disclosed in the '469 Patent Application.

After sterilization, deimmunization, and/or disinfection, the treated defined *Pseudomonas* surrogate protein samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the defined *Pseudomonas* surrogate protein. In the experiments discussed herein, the primary anti-*suf I* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 5 residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot Analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the synthetic defined *Pseudomonas* surrogate proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, or disinfection occurred, the visualized

Western blot has a dark ban in the untreated control sample, e.g., indicated at 266, Fig. 16, and a complete absence of any bands for the defined *Pseudomonas* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, indicated at 268.

Example 16: Comparing Amino Acid Sequence of Multiple members of the *Trichophyton* Genus

It is important to qualify the ability of a sterilization device, a deimmunization device, and/or a disinfection device to destroy bacteria of any *Trichophyton* species that may be contaminating medical equipment. In this example, a defined quantity of the defined *Trichophyton* surrogate protein having a predetermined SEQ ID NO. 6 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis and using the antibody specific for the defined *Trichophyton* surrogate protein. In this example, to protect the human operators of the test, the synthetic defined *Trichophyton* surrogate protein needs to incorporate critical characteristics of *Trichophyton* proteins that are critical for the survival and growth of members of the *Trichophyton* genus while avoiding organisms that can infect humans.

To design the synthetic defined *Trichophyton* surrogate protein having the SEQ ID NO. 6, a protein analysis was conducted comparing the amino acid sequences of the *suf I* loci gene from multiple species of the *Trichophyton* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf I* loci proteins, multiple *Trichophyton* species, used for the comparative are shown in Table 10 below:

Species	Sequence ID
Trichophyton rubrum	XP_003236812.1
Trichophyton tonsurane	EGD95875.1

Table 10. *Trichophyton*. Sequences used to determine regions of high homology in *suf 1* locus of *Trichophyton* Species.

The protein produced by the *suf 1* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Trichophyton* genus, the protein product of the *suf 1* loci is called laccase and is critical for many live stages including strongly contributing to cell survival and growth

To design the synthetic defined *Trichophyton* surrogate protein, the specific amino acids from the proteins listed in Table 10 above were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Trichophyton* species as shown in Fig. 17. The protein encoded by the *suf 1* loci includes three cupredoxin domains that are indicated at 270 for domain 1, 272 for domain 2, and 274 for domain 3. In the *Trichophyton* genus, domain 2, indicated at 272, shows high homology between *Trichophyton* species so SEQ ID NO. 6 above was used to for the design of the synthetic defined *Trichophyton* surrogate protein to be created for the *Trichophyton* test and the corresponding peptide discussed above was used for a polyclonal antibody for use by Western Blot analysis.

Example 17: Developing Western Test to Qualify Ability to Destroy *Trichophyton* Test Protein:

One purpose of developing the defined *Trichophyton* surrogate protein is to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as sterilization device, a

deimmunization device, or a disinfection device. The method for rapidly determining effective sterilization, deimmunization, or disinfection preferably includes multiple steps including at least: 1) preparing synthetic defined *Trichophyton* surrogate protein test samples, 2) subjecting the defined *Trichophyton* surrogate protein test samples to sterilization, deimmunization, or disinfection, and 3) using Western Blot or similar analysis to visualize the effects of sterilization, deimmunization, or disinfection of defined *Trichophyton* surrogate protein test samples. Successful sterilization, deimmunization, or disinfection has occurred when all the defined *Trichophyton* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Trichophyton* surrogate protein was destroyed. If sterilization, deimmunization and/or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the *Trichophyton* sterilization, deimmunization and/or disinfection test. First, to create the sample for the test using the synthetic defined *Trichophyton* surrogate protein, DNA encoding the amino acid SEQ ID NO. 7 above was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of defined *Trichophyton* surrogate protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length synthetic defined *Trichophyton* surrogate protein (171 amino acids long) was isolated. To create the samples having the synthetic defined *Trichophyton* surrogate protein to qualify sterilization, deimmunization, or disinfection, the defined *Trichophyton* surrogate

protein samples were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization and/or disinfection, e.g., using cycles of applying a solvent and microwave energy as disclosed in the '469 Patent Application.

After sterilization, deimmunization, or disinfection, the treated *Trichophyton* samples were transferred to tubes, denatured and separated by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the protein of interest. In the experiments discussed herein, the primary anti-*suf 1* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 6 residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, or disinfection has occurred, the visualized Western blot has a dark ban in the untreated control sample, e.g., indicated at 276, Fig. 18, and a complete absence of any bands for the defined *Trichophyton* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, indicated at 278.

Example 18: Comparing Amino Acid Sequence of Multiple members of the *Candida* Genus

It is important to qualify the ability of a sterilization device, a deimmunization device, or disinfection device, to destroy bacteria of any *Candida* species that may be contaminating medical equipment. In this example, a defined quantity of the defined *Candida* surrogate protein having a predetermined SEQ ID NO. 7 discussed above is subjected to sterilization, deimmunization, or disinfection to rapidly determine the effectiveness of the sterilization, deimmunization, or disinfection using Western Blot analysis, protein array analysis, or similar type analysis and the antibody specific for the defined *Candida* surrogate protein. In this example, to protect the human operators of the test, the defined *Candida* surrogate protein needs to incorporate critical characteristics of *Candida* proteins that are critical for the survival and growth of members of the *Candida* genus while avoiding organisms that can infect humans.

To design the synthetic defined *Candida* surrogate protein having the SEQ ID NO. 7, a protein analysis was conducted comparing the amino acid sequences of the *suf 1* loci gene from multiple species of the *Candida* genus shown in Table 2 above. In this example, the Sequence IDs (found in Pubmed, www.ncbi.nlm.nih.gov/Pubmed/) for the *suf 1* loci proteins, multiple *Candida* species, used for the comparative are shown in Table 11 below:

Species	Sequence ID
Candida albicans	KHC71512.1
Candida dubliniensis	XP_002420841.1
Candida tropicalis	XP_002548698.1
Candida auris	XP_018169615.1

Table 11. (*Candida*). Sequences used to determine regions of high homology in *Suf I* locus of *Candida* Species.

The protein produced by the *suf I* loci is fundamental to survival and growth of a wide range of spores, bacteria and fungus. In species of the *Candida* genus, the protein product of the *suf I* loci is called laccase and is critical for many live stages including strongly contributing to cell survival and growth

To design the synthetic defined *Candida* surrogate protein, the specific amino acids from the proteins listed in Table 11 were aligned to determine amino acid sequence regions that are highly homologous in all evaluated *Candida* species as shown in Fig. 19. The protein encoded by the *suf I* loci includes three cupredoxin domains, indicated at 280 for domain 1, 282 for domain 2, and 284 for domain 3. In the *Candida* genus, domain 2, indicated at 284, shows high homology between *Candida* species so SEQ ID NO. 7 above was used to for the design of the synthetic defined *Candida* surrogate protein to be created for the *Candida* test and the corresponding peptide discussed above was used for a polyclonal antibody for use by Western Blot analysis.

Example 19: Developing Western Test to Qualify Ability to Destroy *Candida* Test Protein:

One purpose of developing the defined *Candida* surrogate protein test is to provide a method for rapidly determining the effectiveness of sterilization, deimmunization, and/or disinfection by a device, such as a sterilization device, a deimmunization device, or a disinfection device. The method for rapidly determining effective sterilization, deimmunization, and/or disinfection includes multiple steps preferably including at least: 1) preparing synthetic defined *Candida* surrogate protein test samples, 2) subjecting the defined *Candida* surrogate protein test samples to sterilization, deimmunization, or disinfection, and 3) using Western Blot or similar

analysis to visualize the effects of sterilization, deimmunization, or disinfection of defined *Candida* surrogate protein test samples. Successful sterilization, deimmunization, or disinfection has occurred when all the defined *Candida* surrogate protein test samples are fragmented and as a result of the protein fragmentation, none remains to bind to the visualization antibodies indicating the defined *Candida* surrogate protein was destroyed. If sterilization, deimmunization, or disinfection was not successful, protein bands will be see on the Western Blot analysis.

Following the process more fully described for the Prion test, a similar process was followed to create the defined *Candida* surrogate protein sterilization, deimmunization and/or disinfection test. First, to create the sample for the synthetic defined *Candida* surrogate protein test using the defined *Candida* surrogate protein, DNA encoding the amino acid SEQ ID NO. 7 was synthesized and cloned into standard vectors both for *E. coli* and yeast expression. Using standard techniques, large quantities of protein were produced in *E. coli* or yeast and isolated by standard recombinant methods. Using a nickel column, a full-length synthetic defined *Candida* surrogate protein (171 amino acids long) was isolated. To create the samples having the synthetic defined *Candida* surrogate protein to qualify sterilization, deimmunization, or disinfection, the samples having the defined *Candida* surrogate protein were dried onto small filter papers, dried inside small tubes or a surface of an object subjected to sterilization, deimmunization, and/or disinfection, e.g., using cycles of applying a solvent and microwave energy as discussed in the '469 Patent Application.

After sterilization, deimmunization, or disinfection, the treated defined *Candida* surrogate protein samples were transferred to tubes, denatured and separated

by size and transferred to nylon membrane before being permanently cross-linked to the membrane. The final steps include incubating the nylon membrane with a primary antibody that specifically binds to the defined *Candida* surrogate protein. In the experiments discussed herein, the primary anti-*suf 1* loci protein antibody was a rabbit polyclonal antibody that was raised against a synthetic peptide discussed above for SEQ ID NO. 7 residues. For added sensitivity, addition antibodies, both monoclonal and polyclonal, were raised against the other synthetic peptide(s). For the Western Blot analysis, a secondary antibody may be a HRP-labelled goat anti-rabbit to enable visualization of the protein fragments, both intact and fragmented. If the proteins have completely fragmented no bands will be visualize on the Western blot. Very small fragments and amino acids will be too small to be retained on the gel. When successful sterilization, deimmunization, or disinfection has occurred, the visualized Western blot has a dark ban in the untreated control sample, indicated at 286, Fig. 20, and a complete absence of any bands for the defined *Candida* surrogate protein sample subjected to sterilization, deimmunization, or disinfection indicates successful sterilization, deimmunization, or disinfection, indicated at 290.

Example 19 Immobilizing Proteins onto a Solid Surface

Many processes and solid substrates are well known in the art for immobilizing proteins onto a solid surface. In this example, glass slides were obtained having round wells created by printing the glass with highly water-repellent mark, e.g., as shown by multi-well glass slides 130, 134, Fig. 2, or multi-well glass slide 152, Fig. 3. In this example, the glass slides were washed with acetone and milli-Q water before soaking overnight in 1 M NaOH (room temperature). To create amino silane-treated slides, washed slides using milli-Q water and 99.5% ethanol,

were treated with 3-aminopropyltriethoxysilane for 2 hours at room temperature, washed with milli-Q water, and baked for 2 hr. at 100°C. The multi-well slides were soaked in 1% (v/v) glutaraldehyde overnight at 37°C to produce glutaraldehyde-treated slides. The slides were then incubated overnight at 37°C in chitosan (0.05% (w/v)) dissolved in 0.1 M acetic acid buffer (pH 5.0) supplemented with 0.25 mM sodium azide. The treated slides were rinsed twice with 0.1 M acetic acid buffer (pH 5) and then three times with milli-Q water before being soaked in 1% (v/v) glutaraldehyde overnight at 37°C. The slides were incubated overnight at 37°C in a 0.05% (w/v) solution of N-(5-Amino-1-carboxy pentyl) iminodiacetic acid (AB-NTA) in 0.1 M HEPES buffer (pH 8.0), then rinsed 3 times with milli-Q water. The slides were then soaked in blocking solution (1% (v/v) glycine) for 1 h at 37°C, then rinsed 3 times with milli-Q water, 3 times with 0.5 M NiCl2, and 3 times with milli-Q water to produce Ni-NTA immobilized slides ready for protein immobilization.

In this example, the defined *Clostridium* surrogate protein having SEQ ID NO. 1 was diluted in TG buffer (50 mM Tris-HCl (pH 8.0), 10% (v/v) glycerol) to 200 µg/ml. Different quantities of the defined *Clostridium* surrogate protein, e.g., 200 ng, 100 ng, 50 ng, 25 ng, 12.5 ng, 6.25 ng, 3.125 ng, 1.562 ng, 0.781 ng and 0 ng, were spotted in individual wells, e.g., the wells exemplarily indicated at 132 and 136, Fig. 2, and incubated for 60 min in a moist, dark chamber and finished by washing with TG buffer. After subjecting the slides 130 and 134 to sterilization, deimmunization, or disinfection using cycles of a solvent and electromagnetic radiation, e.g., microwaves as disclosed in the '469 Patent Application, slides 130 and 134 were incubated with anti-*Clostridium* antibodies discussed above raised against the peptide selected from SEQ ID NO. 1. To visualize the amount of the defined *Clostridium*

surrogate protein remaining after sterilization, deimmunization, or disinfection, the slides were then treated with anti-Ig antibody labeled with the fluorescence dye fluorescence isothiocynate (FITC). A matched slide that was not sterilized in this example, slide 130, was used as a control for the protein array analysis. Using a fluorescence microscope with camera, the loss of protein can be visualized that can be detected with the specific anti-Clostridium antibodies. With additional fluorescence detection equipment, such as automated readers, increasing sensitivity and titration of detection can be added. An example of successful sterilization, deimmunization and/or disinfection is indicated at 140, for slide 134, Fig. 2, when compared against control slide 130 which visibly shows color, indicated by the shading in wells 132. Confirmatory tests were conducted by including samples of intact *C. sporogenes* using cycles of a solvent and electromagnetic energy or microwaves, e.g., as disclosed in the '469 Patent Application. After treatment, the intact bacteria were processed following industry standard protocols. Similar methods may be used for any of Examples 6 to 18 above.

Example 20: Comparing Amino Acid Sequence of α-Gliadin from Appropriate Human Consumed Grains

To develop a test to qualify the ability of a sterilization device, deimmunization, or disinfection to destroy medically important immunogenic proteins, such as α-gliadin, that may be contaminating medical equipment, the following experiments were conducted. In this example, to rapidly detect effective deimmunization, a defined quality of an isolated protein needs to be deimmunized with a deimmunization device, e.g., using cycles of a combination of a solvent and electromagnetic microwaves radiation, e.g., microwaves, as disclosed in the '469

patent application and then evaluated by Western Blot analysis using an antibody specific for the protein. To design a candidate protein to serve as the representative defined surrogate α-gliadin protein having a predetermined sequence, the translated sequences of representative α-gliadin genes from many commonly consumed human grains were aligned. *See* Table 13 and Table 14 below. Gliadin protein is the immunogenic component of gluten and must be avoided by Celiac patients. Fig. 21 shows the amino acid numbering aligned with an example of Bread wheat α-Gliadin. The underline region, indicated at 400, is the polypeptide that acts as common immunogen in a majority of Celiac Patients. Regions at 402, 404 indicate protein region used to design test immunogen reagent.

Common Name	Species Name	Sequence ID Number w/ Link
Bread Wheat	Triticum aestivum	pir‖A27319
Common Wheat	Triticum sphaerococcum	ABQ45316.1
Durum wheat/ Pasta Wheat	Triticum turgidum subsp. durum	ADA83698.1
Farro/ Emmer Wheat	Triticum dicoccon	AKC91191.1
Macha Wheat	Triticum macha	AKC91223.1
Rye	Secale cereale	AFK32718.1
Spelt/ Dinkel Wheat	Triticum spelta	APU92351.1
Red Wild Einkor	Triticum urartu	AKC91171.1
Precursor □Gliadin *Bateman et al 2004*	Triticum aestivum	Q41545

Table 13. Sequence ID of α-Gliadin Proteins or Prolamins in Common Grains Used to Design Recombinant Protein Sequence.

Species	Common	Sequence ID	Species	Common Name	Sequence ID
Triticum aestivum	Bread Wheat	pir‖A27319	Triticum turgidum subsp. durum	Durum wheat, pasta wheat or macaroni wheat	ADA83698.1
		P04726.1			ADA83690.1
		AED99851.1	Triticum dicoccon	Emmer wheat or Farro	AKC91191.1

		SCW25751.1	Triticum urartu	red wild einkor	AKC91171.1
		AHY37812.1	Triticum macha	Macha wheat	AKC91223.1
		AHY37818.1	Secale cereale	Rye	AFK32718.1
		AFX69612.1			
		ABQ52118.1	Aegilops tauschii	Tausch's goatgrass	AFX69602.1
		AFX69616.1			XP_020186089.1
		AFX69586.1			AKC91337.1
Triticum sphaerococcum	Common wheat	ABQ45316.1			ABQ52112.1
Triticum spelta	Spelt or dinkel wheat	APU92351.1	Aegilops sharonensis	Sharon goatgrass	AMS25611.1
		APU92675.1			AMS25610.1
		APU92554.1			AMS25614.1
		APU92300.1	Aegilops searsii	Goatgrass	AKC91312.1
		APU92425.1			AKC91311.1
		APU92357.1	Aegilops speltoides	Goatgrass	AHN85624.1
		APU92415.1	Aegilops speltoides	Goatgrass	AHN85626.1
		APU92583.1	Aegilops uniaristata	Goatgrass	AEV55370.1
		APU92336.1	Thinopyrum bessarabicum	Wild Thinopyrum grasses	ADP94197.1
		APU92334.1			

Table 14. Additional α-Gliadin Protein Sequences that could be used to design Recombinant Protein.

In this example, the defined α-Gliadin surrogate protein has the following predetermined sequence:

For α-Gliadin:

```
         10          20          30          40          50
MKTVRVPVPQ PQPQNPSQPQ PQRQVPLVQQ QQFPGQQQQF PPQQPYPQPQ
PFPSQQPYLQ LQPFPQPQPF PPQLPYHHHH HH (SEQ ID NO. 8)
```

The peptide used for the development of monoclonal or polyclonal antibody used by

Western Blot analysis for the above sequence is:

FPPQQPYPQPQPFPSQQPYLQLQPFPQPQ

A fragment of this peptide (KLQPFPQPELPYPQPQ) in form is the medically important immunogen is CD.

If the defined α-Gliadin surrogate proteins have completely fragmented, no bands will be visualized on the Western Blot. Very small fragments in amino acid would be too small to be retained on the gel. When successful deimmunization, in this example, has occurred, the visualized Western Blot has a dark band in the untreated control sample, e.g., indicated at 420, Fig. 22, and a complete absence of any bands for the defined α-Gliadin surrogate protein sample subject to deimmunization indicates successful deimmunization, e.g., indicated at 422.

Confirmatory tests were conducted by including samples of intact wheat flour using a combination of cycles of a solvent and microwaves, e.g., as disclosed in the '469 Patent Application. After treatment, the intact wheat flour samples were processed following industry standard protocols to test for the presence of gluten in food stuffs.

SEQUENCE LISTING

NUMBER OF SEQ IDENTIFIER NOS: 8

SEQ ID NO. 1
LENGTH: 171
TYPE: PROTEIN
ORGANSIM: bacteria

SEQUENCE: 1

```
        10         20         30         40         50
MYNYTSAKYE VPIAIQDRSF NEDGSLNFPS EGDNPTIHPY WQPEFFGDTI
```

MVNGRVWPNM NVDMTRYRFR LLNGSNARFY NLKFSNGMQF WQIGTDGGYL

NKPVPLTSLL ISPGERADIL VDFTEIPAGT RIILNNDANA PYPTGDAPDK

DTTGQIMQFT VQHNDHHHHH H

SEQ ID NO. 2

LENGTH: 170
TYPE: PROTEIN
ORGANSIM: bacteria

SEQUENCE: 2

10 20 30 40 50

MTLEKTYYEV TMEECTHQLH RDLPPTRLWG YNGLFPGPTI EVKRNENVYV

KWMNNLPSTH FLPIDHTIHH SDSQHEEPEV KTVVHLHGGV TPDDSDGYPE

AWFSKDFEQT GPYFKREVYH YPNQQRGAIL WYHDHAMALT RLNVYAGLVG

AYIIHDPKEK RLKHHHHHH

SEQ ID NO. 3
LENGTH: 139
TYPE: PROTEIN
ORGANSIM: bacteria

SEQUENCE: 3

10 20 30 40 50

MTGMPEGEGV DSNLLGGDGG DIAYPYYLIN GRIPVAATSF KAKPGQRIRI

RIINSAADTA FRIALAGHSM TVTHTDGYPV IPTEVDALLI GMAERYDVMV

TAAGGVFPLV ALAEGKNALA RALLSTGAGS PPDHHHHHH

SEQ ID NO. 4
LENGTH: 134
TYPE: PROTEIN
ORGANSIM: bacteria

SEQUENCE: 4

10 20 30 40 50

MTGYKNYTLK AQKGKTEFYK NNFSNTLGYN GNLLGPTLKL KKGDKVKIKL

INNLDENTTF HWHGLEVNGK VDGGPSQVIK PGKEKTIKFE VNQDSATLWY

HPHPSPNTAK QVYNGLSGLL YIEDSKKNHH HHHH

SEQ ID NO. 5
LENGTH: 143
TYPE: PROTEIN
ORGANSIM: bacteria

SEQUENCE: 5

```
        10          20          30          40          50
MTGFRHEKVL CLKTWHVDEQ GAFTPFSVPR QAAREGTRGR YSTINGKHVP
TIDLPAGQIV RVRLLNVDNT VTYRLNPNGE ARIYAVDGHP VEPRGFEGQY
WIGPGMRLEL ALKVPEAGTE LSLRDGPVRL ATIRSVAHHH HHH
```

SEQ ID NO. 6
LENGTH: 133
TYPE: PROTEIN
ORGANSIM: fungus

SEQUENCE: 6

```
        10          20          30          40          50
MTITLEWSVT TGYRRLDGVK KRVYLINGLF PGPTIEARSG DSLQVQVTNN
IQDEGLVIHW HGLHMRGANH MDGVTGVTQC PIVPGDSMLY NFTISQSQSG
TFWYHAHSAL QRAEGLYGGF VVHKPSTHHH HHH
```

SEQ ID NO. 7

LENGTH: 133
TYPE: PROTEIN
ORGANSIM: fungus

SEQUENCE: 7

```
        10          20          30          40          50
MTAETHTWYF KTSWVDANPD GVFPRKMIGF NDSWPLPTLR VKKGDTVNLY
LINGFDDRNT SLHFHGLFQH GTNQMDGPEM VTQCPIPPGE TFLYNFTVDD
QVGSYWYHSH TSGQYGDGMR GVFIIEDHHH HHH
```

SEQ ID NO. 8
LENGTH: 82

TYPE: PROTEIN
ORGANSIM: plant

SEQUENCE: 8

```
        10          20          30          40          50

MKTVRVPVPQ PQPQNPSQPQ PQRQVPLVQQ QQFPGQQQQF PPQQPYPQPQ

PFPSQQPYLQ LQPFPQPQPF PPQLPYHHHH HH  (SEQ ID NO. 8)
```

Although specific features of the invention are shown in some drawings and not in others, this is for convenience only as each feature may be combined with any or all of the other features in accordance with the invention. The words "including", "comprising", "having", and "with" as used herein are to be interpreted broadly and comprehensively and are not limited to any physical interconnection. Moreover, any embodiments disclosed in the subject application are not to be taken as the only possible embodiments. Other embodiments will occur to those skilled in the art and are within the following claims.

In addition, any amendment presented during the prosecution of the patent application for this patent is not a disclaimer of any claim element presented in the application as filed: those skilled in the art cannot reasonably be expected to draft a claim that would literally encompass all possible equivalents, many equivalents will be unforeseeable at the time of the amendment and are beyond a fair interpretation of what is to be surrendered (if anything), the rationale underlying the amendment may bear no more than a tangential relation to many equivalents, and/or there are many other reasons the applicant cannot be expected to describe certain insubstantial substitutes for any claim element amended.

What is claimed is:

CLAIMS

1. A method for rapidly determining effective sterilization, deimmunization, and/or disinfection of equipment and/or supplies by a device, the method comprising:

providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating the equipment and/or the supplies to be sterilized, deimmunized, and/or disinfected by the device;

subjecting the defined surrogate protein having the predetermined sequence to sterilization, deimmunization, or disinfection; and

rapidly determining the effectiveness of the sterilization, deimmunization, and/or disinfection by determining if the defined surrogate protein having the predetermined sequence has been destroyed.

2. The method of claim 1 in which the defined surrogate protein includes proteins critical for stability, growth and/or infectious capacity of infectious agents.

3. The method of claim 1 in which the defined surrogate protein includes a protein critical for stability, growth and/or infectious capacity of surrogate organisms of infectious agents.

4. The method of claim 2 in which the infectious agent includes one or more of: an infectious protein, an infectious spore forming bacteria, an infectious vegetative bacteria, an infections fungus, and an infectious virus.

5. The method of claim 1 in which the defined surrogate protein includes pathogenic proteins, proteins critical for the growth of infectious agents, and immunogenic proteins.

6. The method of claim 1 in which the predetermined sequence is defined by the sequence:

```
        10          20          30          40          50
MYNYTSAKYE VPIAIQDRSF NEDGSLNFPS EGDNPTIHPY WQPEFFGDTI
MVNGRVWPNM NVDMTRYRFR LLNGSNARFY NLKFSNGMQF WQIGTDGGYL
NKPVPLTSLL ISPGERADIL VDFTEIPAGT RIILNNDANA PYPTGDAPDK
DTTGQIMQFT VQHNDHHHHH H (SEQ ID NO. 1)
```

7. The method of claim 6 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

8. The method of claim 1 in which the predetermined sequence is defined by the sequence:

```
        10          20          30          40          50
MTLEKTYYEV TMEECTHQLH RDLPPTRLWG YNGLFPGPTI EVKRNENVYV
KWMNNLPSTH FLPIDHTIHH SDSQHEEPEV KTVVHLHGGV TPDDSDGYPE
AWFSKDFEQT GPYFKREVYH YPNQQRGAIL WYHDHAMALT RLNVYAGLVG
AYIIHDPKEK RLKHHHHHH  (SEQ ID NO. 2)
```

9. The method of claim 8 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the

predetermined sequence.

10. The method of claim 1 in which the predetermined sequence is defined by the sequence:

```
        10         20         30         40         50
MTGMPEGEGV DSNLLGGDGG DIAYPYYLIN GRIPVAATSF KAKPGQRIRI
RIINSAADTA FRIALAGHSM TVTHTDGYPV IPTEVDALLI GMAERYDVMV
TAAGGVFPLV ALAEGKNALA RALLSTGAGS PPDHHHHHH  (SEQ ID NO. 3)
```

11. The method of claim 10 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

12. The method of claim 1 in which the predetermined sequence is defined by the sequence:

```
        10         20         30         40         50
MTGYKNYTLK AQKGKTEFYK NNFSNTLGYN GNLLGPTLKL KKGDKVKIKL
INNLDENTTF HWHGLEVNGK VDGGPSQVIK PGKEKTIKFE VNQDSATLWY
HPHPSPNTAK QVYNGLSGLL YIEDSKKNHH HHHH  (SEQ ID NO. 4)
```

13. The method of claim 12 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

14. The method of claim 1 in which the predetermined sequence is defined by the sequence:

10 20 30 40 50

MTGFRHEKVL CLKTWHVDEQ GAFTPFSVPR QAAREGTRGR YSTINGKHVP

TIDLPAGQIV RVRLLNVDNT VTYRLNPNGE ARIYAVDGHP VEPRGFEGQY

WIGPGMRLEL ALKVPEAGTE LSLRDGPVRL ATIRSVAHHH HHH (SEQ ID NO. 5)

15. The method of claim 14 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

16. The method of claim 1 in which the predetermined sequence is defined by the sequence:

10 20 30 40 50

MTITLEWSVT TGYRRLDGVK KRVYLINGLF PGPTIEARSG DSLQVQVTNN

IQDEGLVIHW HGLHMRGANH MDGVTGVTQC PIVPGDSMLY NFTISQSQSG

TFWYHAHSAL QRAEGLYGGF VVHKPSTHHH HHH (SEQ ID NO. 6)

17. The method of claim 16 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

18. The method of claim 1 in which the predetermined sequence is defined by the sequence:

10 20 30 40 50

MTAETHTWYF KTSWVDANPD GVFPRKMIGF NDSWPLPTLR VKKGDTVNLY

LINGFDDRNT SLHFHGLFQH GTNQMDGPEM VTQCPIPPGE TFLYNFTVDD

QVGSYWYHSH TSGQYGDGMR GVFIIEDHHH HHH (SEQ ID NO. 7)

19. The method of claim 18 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

20. The method of claim 1 in which the predetermined sequence is defined by the sequence:

```
        10          20          30          40          50
MKTVRVPVPQ PQPQNPSQPQ PQRQVPLVQQ QQFPGQQQQF PPQQPYPQPQ
```

PFPSQQPYLQ LQPFPQPQPF PPQLPYHHHH HH (SEQ ID NO. 8)

21. The method of claim 20 in which the defined surrogate protein is at least 95% homologous the predetermined sequence or substantial fragment of the predetermined sequence.

22. The method of claim 1 in which the rapidly determining including a sensitive protein analysis procedure.

23. The method of claim 22 in which the sensitive protein analysis procedure includes one or more of: a Western Blot analysis, a protein assay analysis, a magnetic separation analysis, a peptide analysis, a mass spectrometry analysis, and a gas chromatography analysis.

24. The method of claim 22 in which the sensitive protein analysis

procedure includes fluorescence analysis of proteins covalently crosslinked on a solid surface.

24. The method of claim 22 in which the sensitive protein analysis procedure includes fluorescence analysis of proteins covalently crosslinked on magnetic beads.

25. The method of claim 1 in which the defined surrogate protein having the predetermined sequence is disposed on a surface.

26. The method of claim 1 in which the defined surrogate protein having the predetermined sequence is disposed on a test strip.

27. The method of claim 1 in which the defined surrogate protein having the predetermined sequence is disposed in or on a vessel.

29. The method of claim 1 in which the surrogate protein having the predetermined sequence is disposed on a tube.

30. The method of claim 30 in which the surrogate protein having the predetermined sequence is disposed on a holder.

31. The method of claim 1 in which the holder is disposed to receive a flow of a sterilization agent, a deimmunization agent or a disinfection agent.

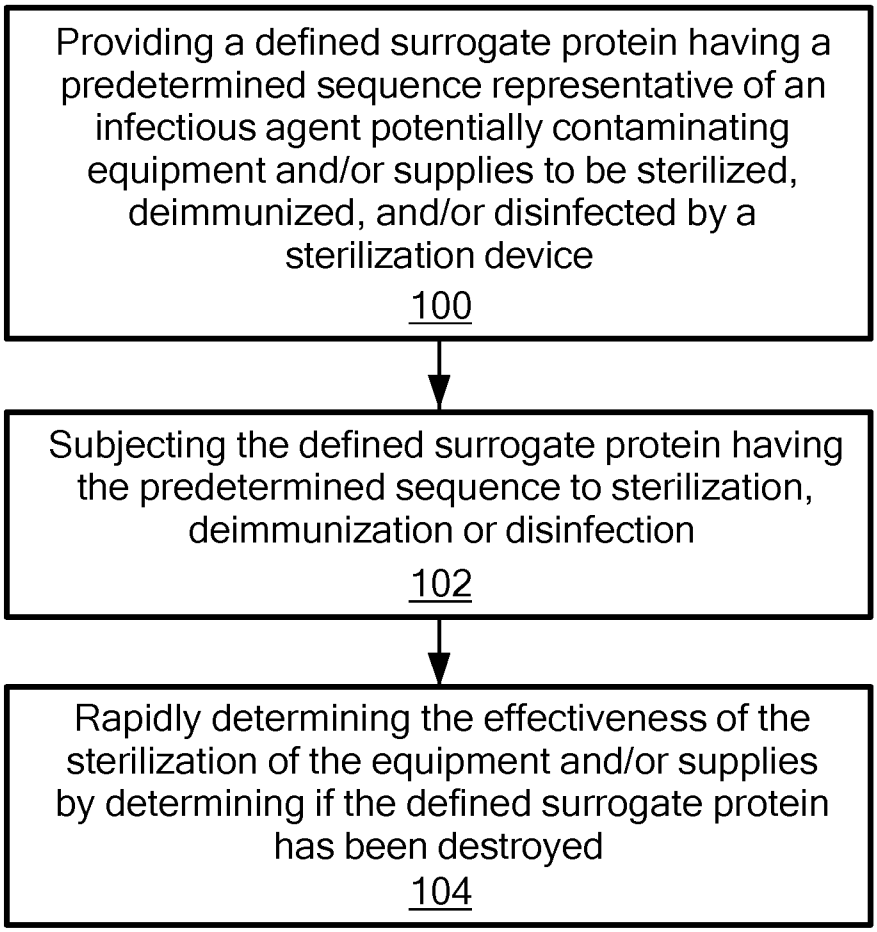

Providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating equipment and/or supplies to be sterilized, deimmunized, and/or disinfected by a sterilization device
100
Subjecting the defined surrogate protein having the predetermined sequence to sterilization, deimmunization or disinfection
102
Rapidly determining the effectiveness of the sterilization of the equipment and/or supplies by determining if the defined surrogate protein has been destroyed
104

FIG. 1

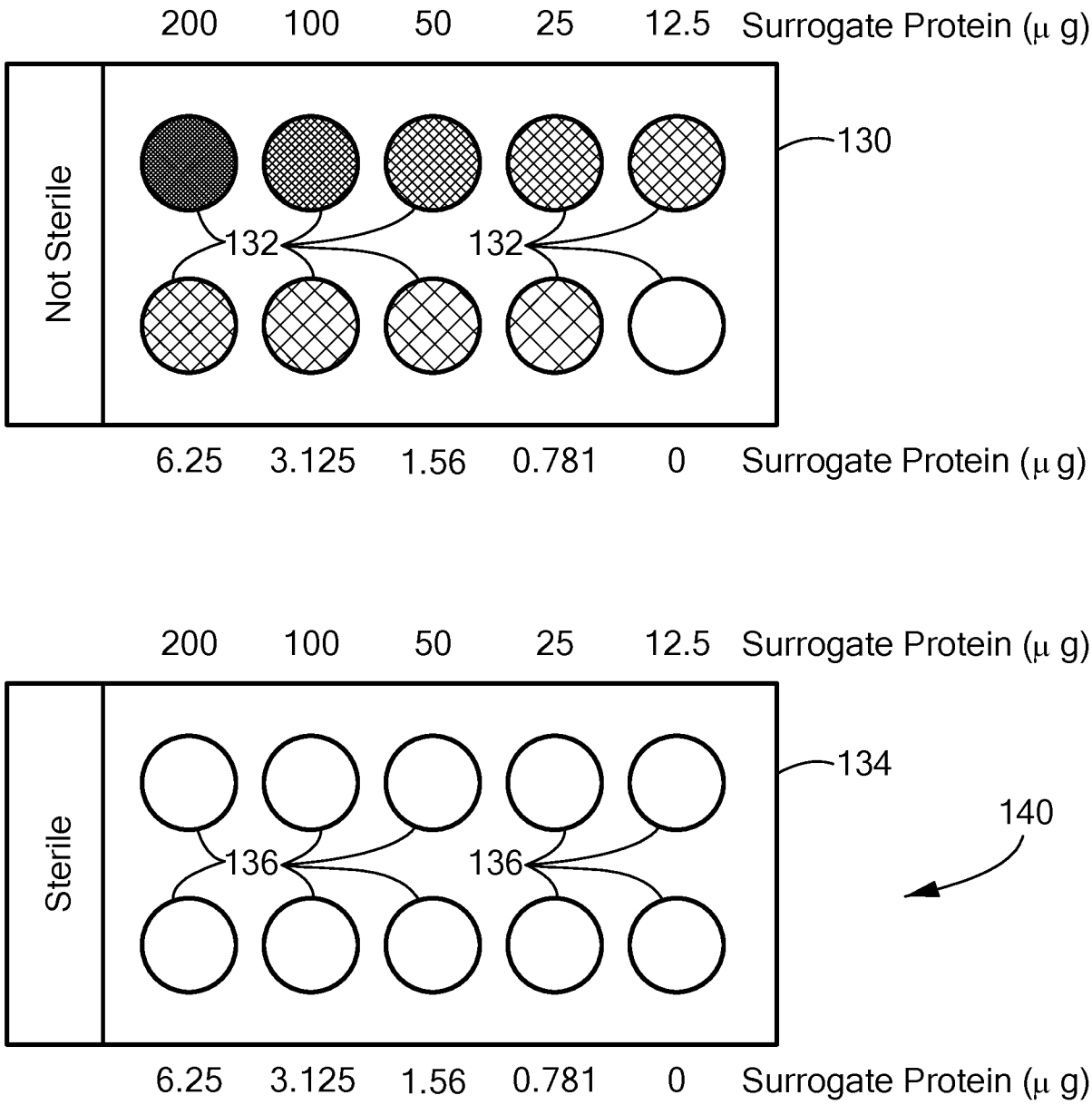

200
100
50
25
12.5
Surrogate Protein (μ g)
Not Sterile
130
132
132
6.25
3.125
1.56
0.781
0
Surrogate Protein (μ g)
200
100
50
25
12.5
Surrogate Protein (μ g)
Sterile
134
140
136
136
6.25
3.125
1.56
0.781
0
Surrogate Protein (μ g)

FIG. 2

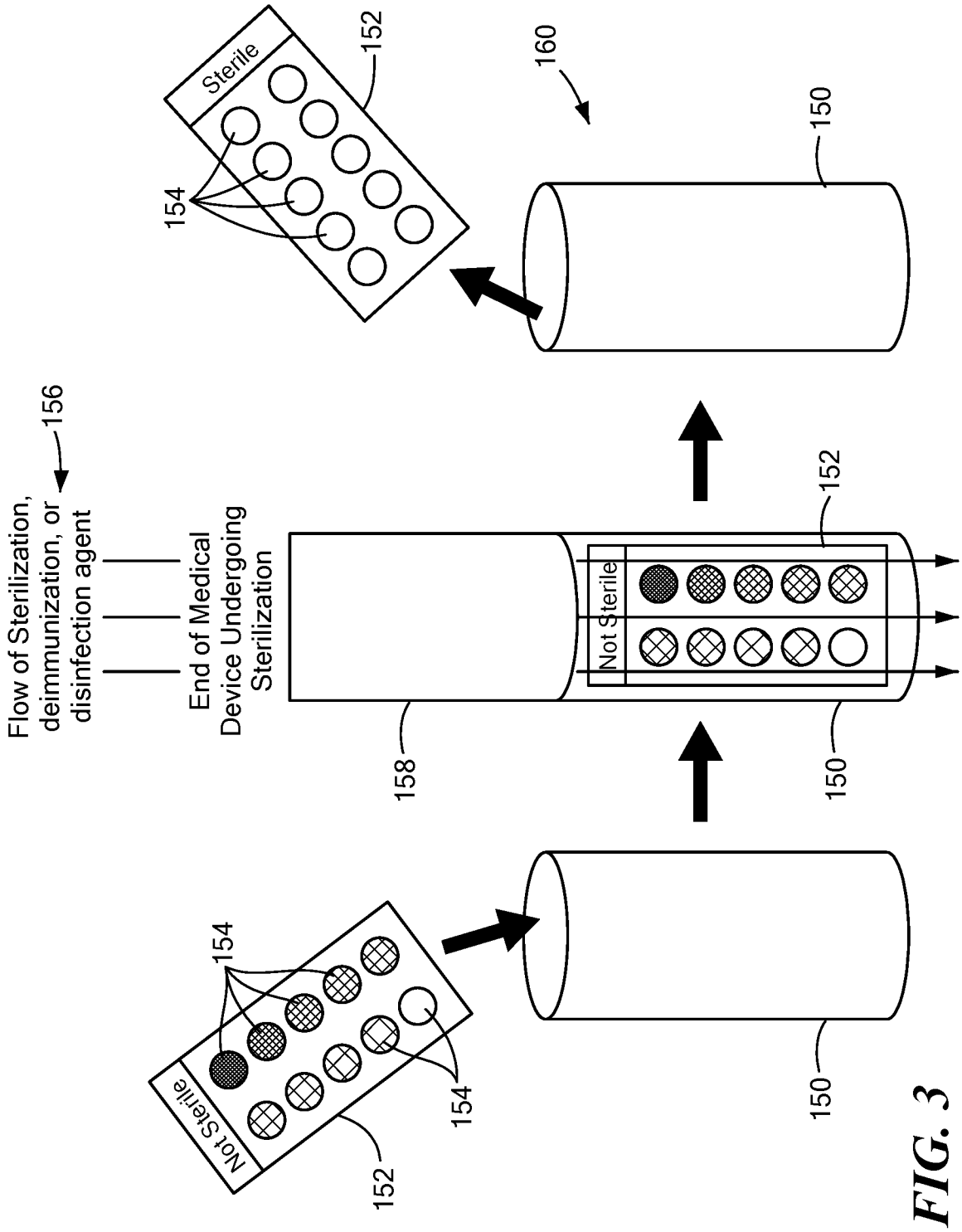
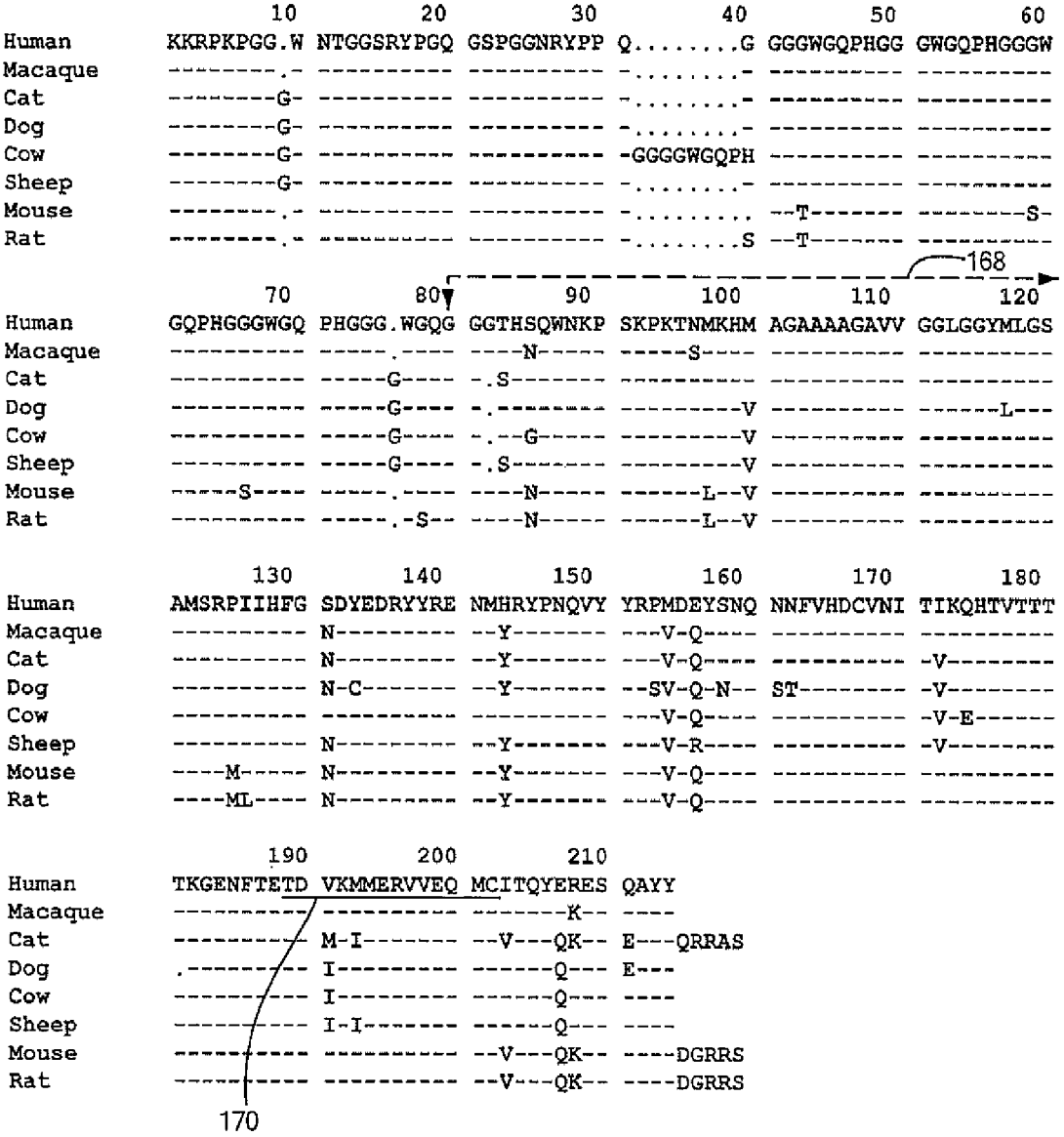

Sterile
152
154
160
150
156
Flow of Sterilization, deimmunization, or disinfection agent
End of Medical Device Undergoing Sterilization
Not Sterile
158
Not Sterile
154
152

FIG. 3



Amino Acid Sequence Comparison of Human PrP Proteins with a Selection of other Species PrP Proteins. Residues cover only standard mature protein sequences.

FIG. 4

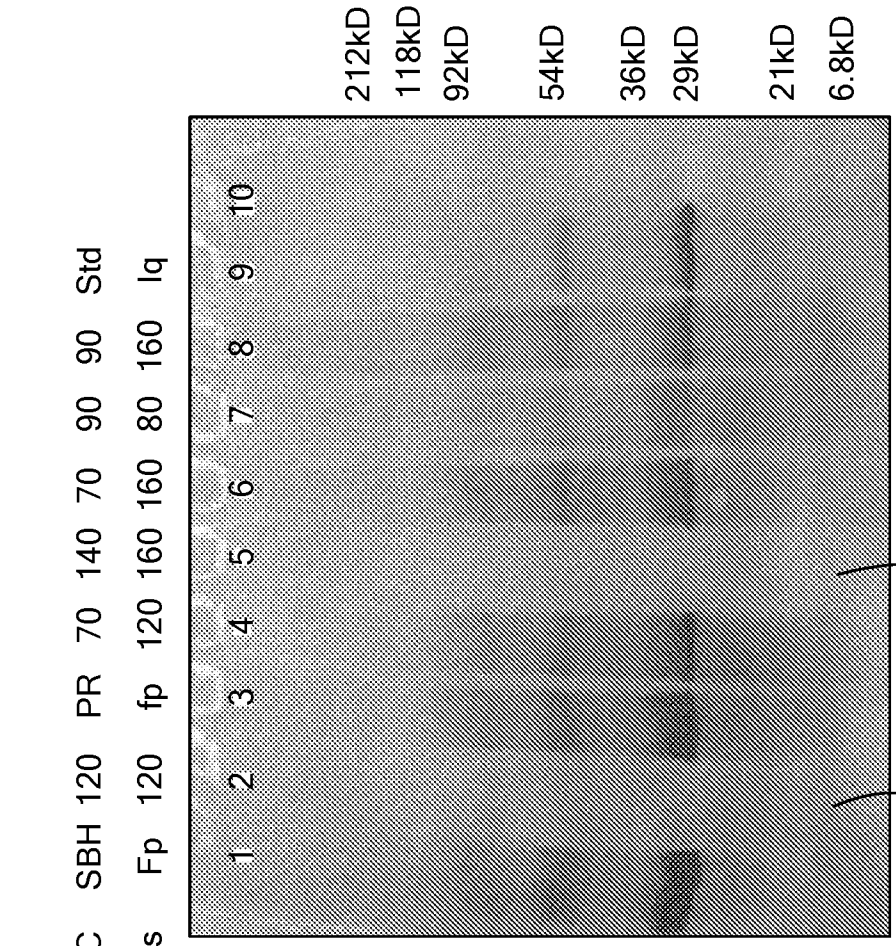

Temperature °C
Microwave Cycles
SBH 120
Fp 120
PR 70
fp 120
140 160
70 160
90 80
90 160
Std Iq
1 2 3 4 5 6 7 8 9 10
212kD
118kD
92kD
54kD
36kD
29kD
21kD
6.8kD
294
296

FIG. 6

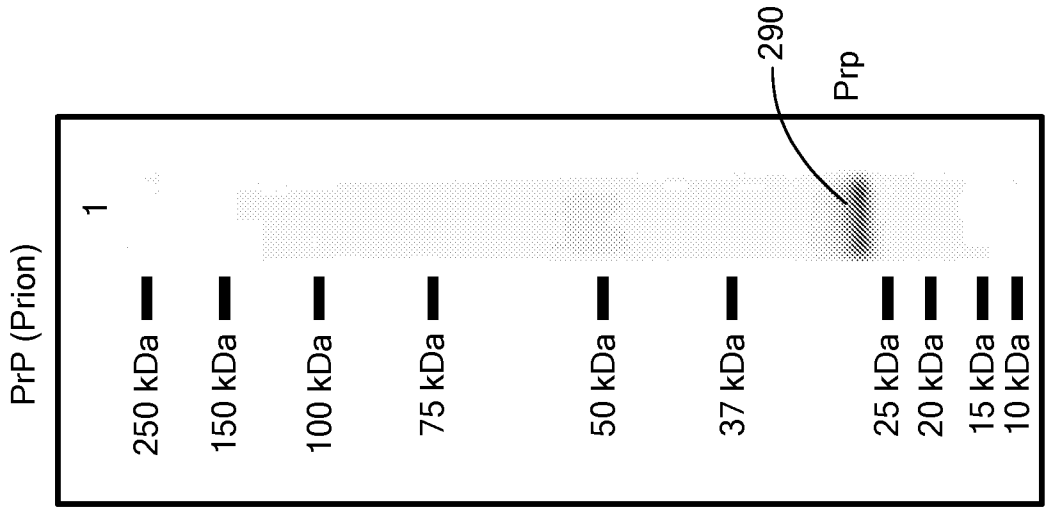

PrP (Prion)
1
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
290
Prp

FIG. 5

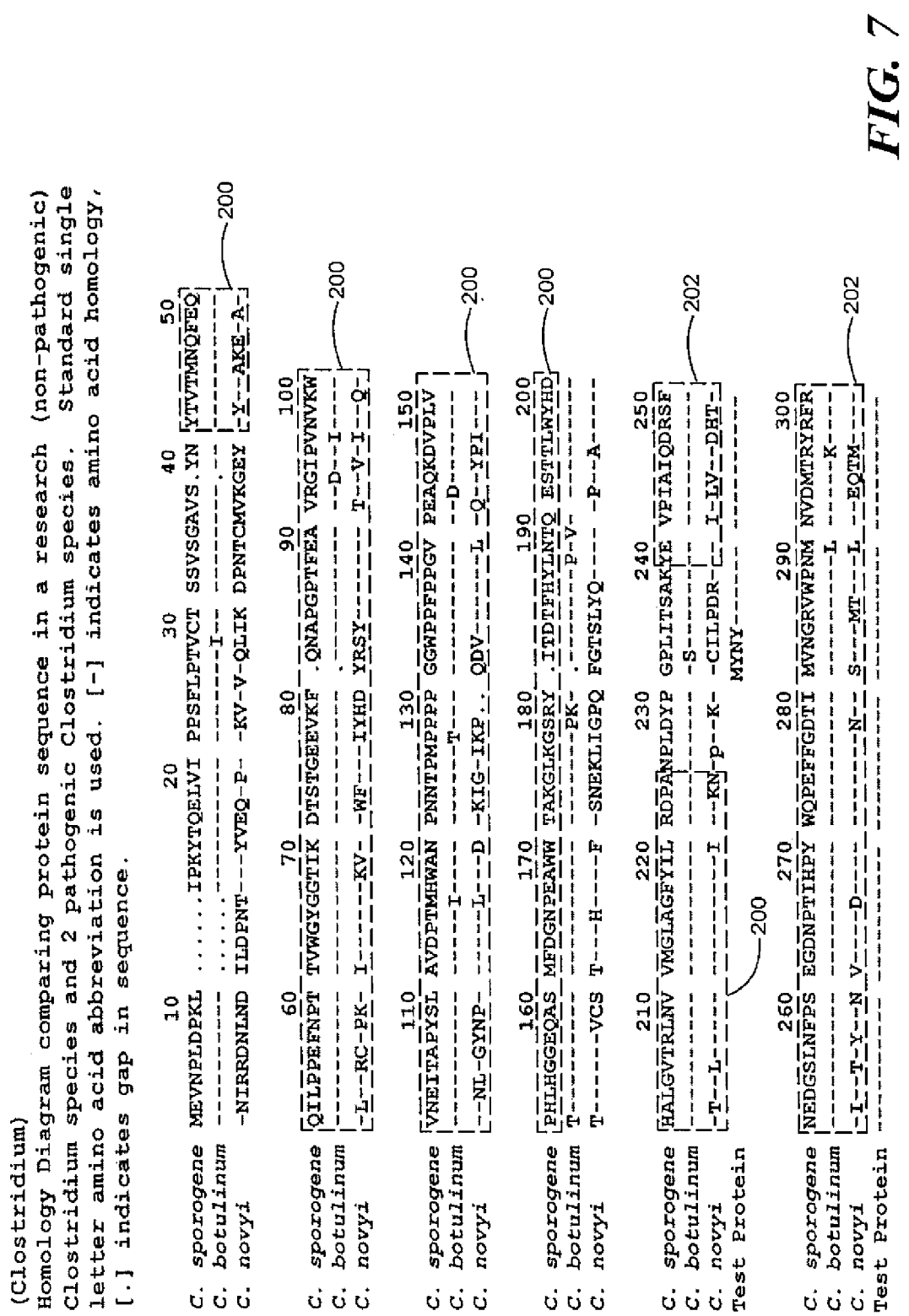

(Clostridium)
Homology Diagram comparing protein sequence in a research (non-pathogenic) Clostridium species and 2 pathogenic Clostridium species. Standard single letter amino acid abbreviation is used. [-] indicates amino acid homology, [.] indicates gap in sequence.
C. sporogene
C. botulinum
C. novyi
Test Protein

FIG. 7

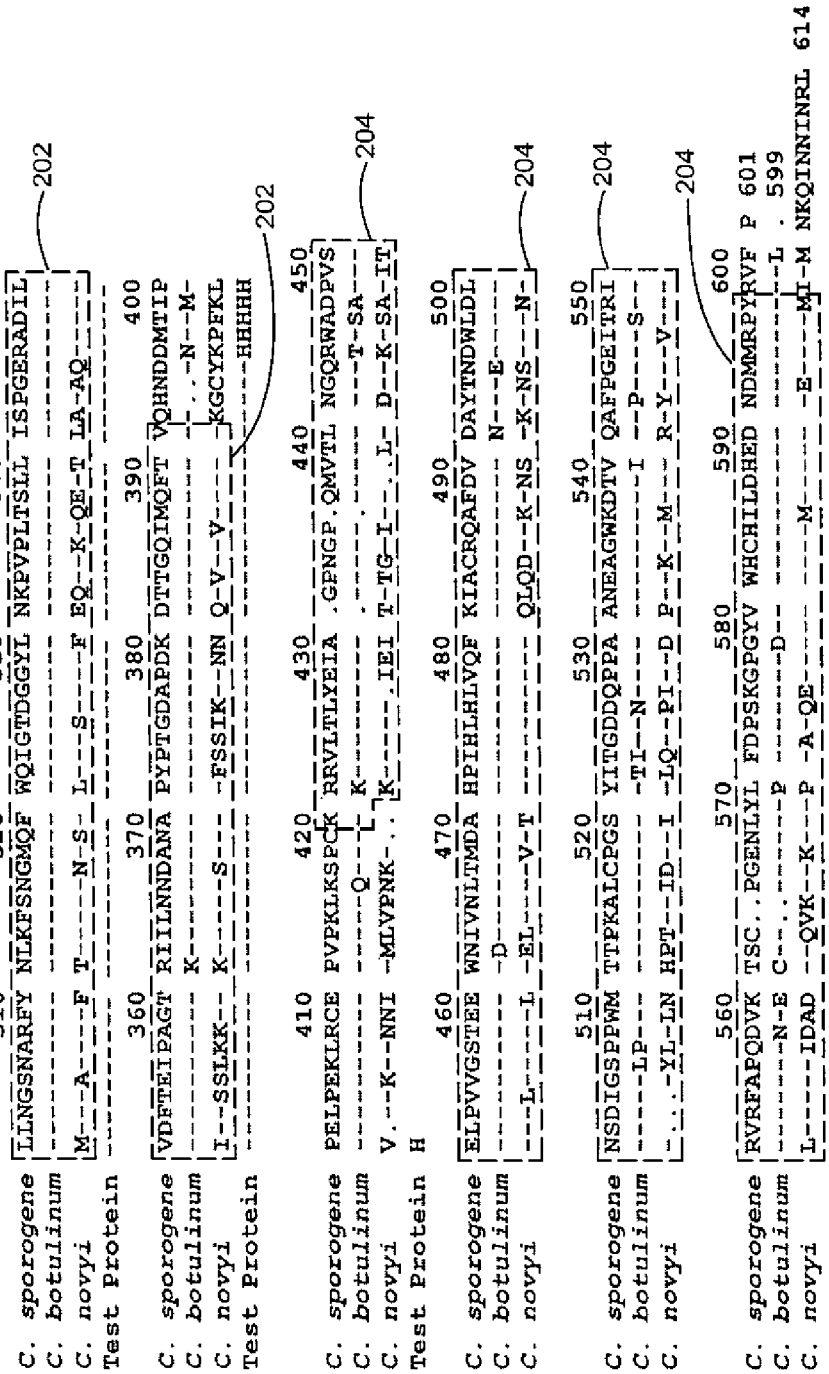


FIG. 7 Continued

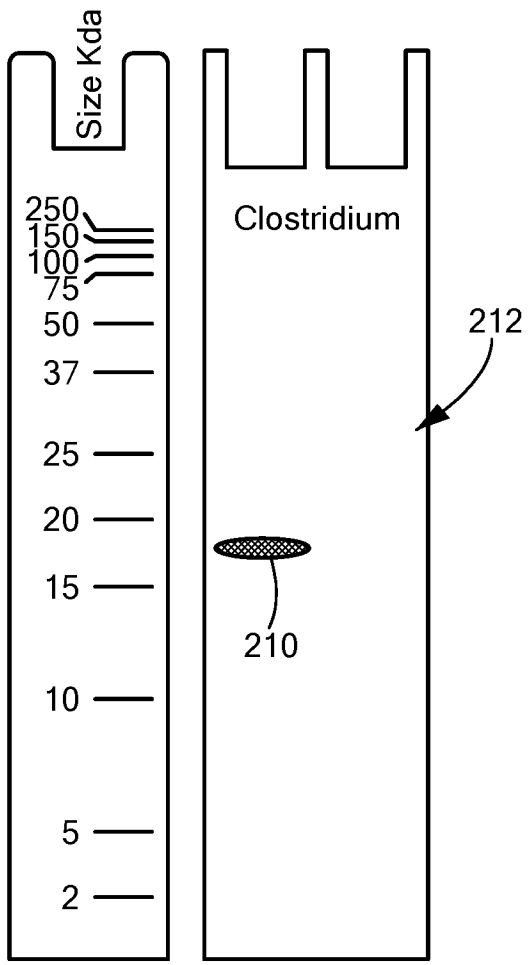


FIG. 8

(Bacillus)
Homology Diagram comparing protein sequence of three Bacillus species
that are used in approved sterilization equipment qualification.
Standard single letter amino acid abbreviation is used. [-] indicates
amino acid homology, [.] indicates gap in sequence.
B subtilis
B atrophae
B pumilis
Test Protein
214
216

FIG. 9

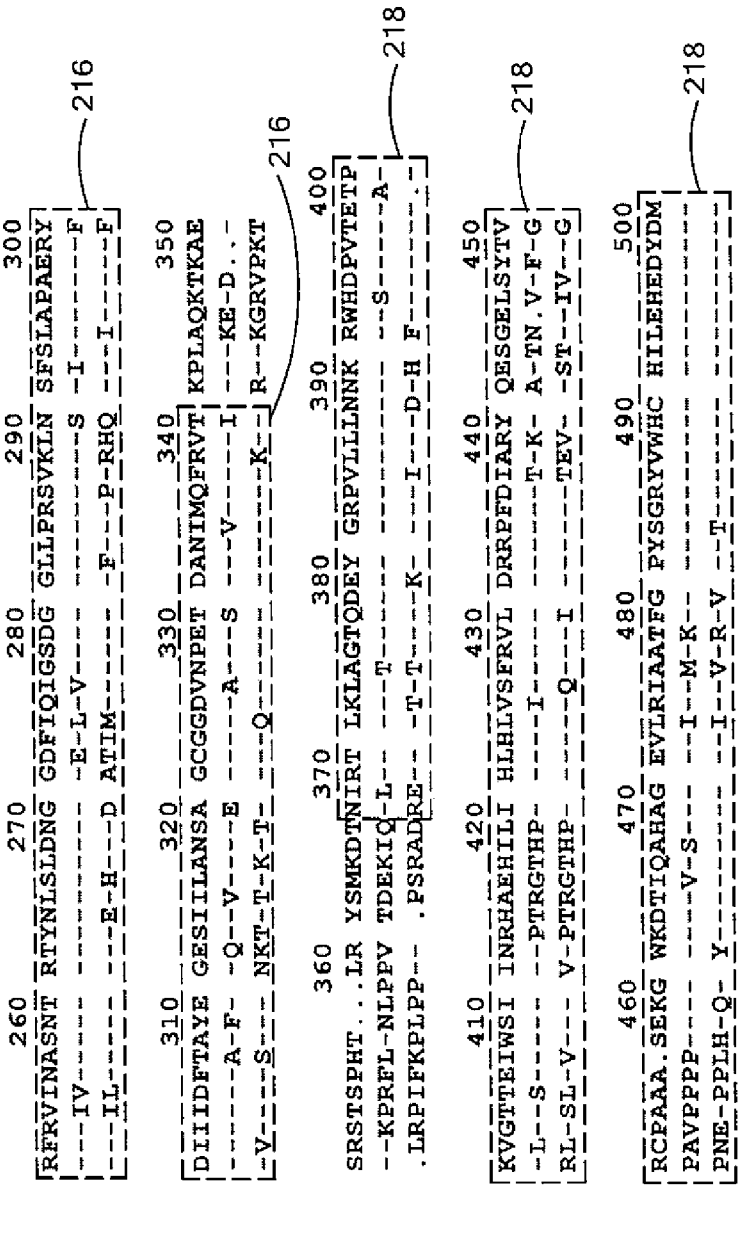


FIG. 9 Continued

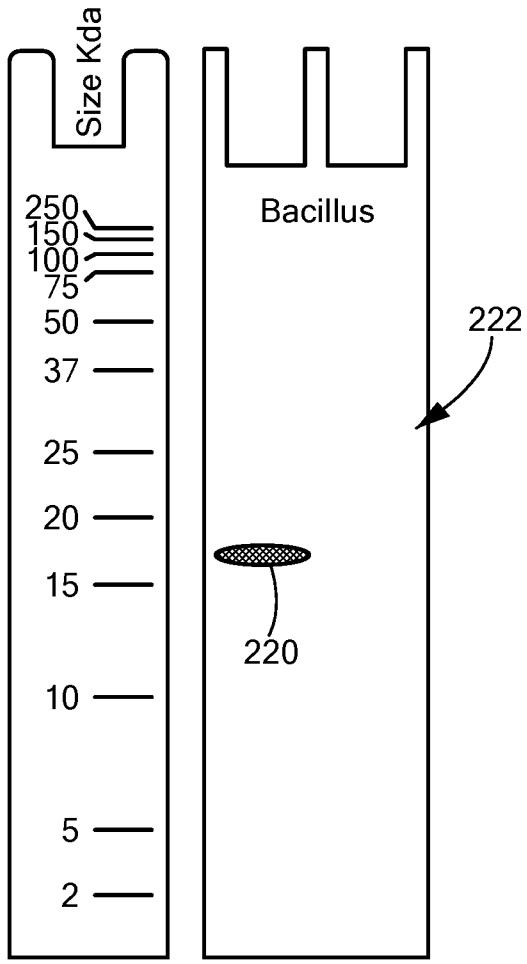


FIG. 10

(Mycobacterium)

Homology Diagram comparing protein sequence in three *Mycobacterium* species.
Standard single letter amino acid abbreviation is used. [-] indicates amino acid homology, [.] indicates gap in sequence.

```
                10        20        30        40        50
M tuberculosis  MPELATSGNA FDKRRFSRRG FLGAGIASGF ALAACASKPT ASGAAGMTAA
M africanum
M kansasii      --V-PA--HP LGGVQL---- -I-------L ---G-GHSQT H-AE-A-A--

                60        70        80        90        100
M tuberculosis  IDAAEAARPH SGRTVTATLT PQPARIDLGG PIVSTLTYGN TIPGFLIRAT  230
M africanum
M kansasii      -----R---- ----S-V --Q-E----- --AH--A--- -V------A  230

                110       120       130       140       150
M tuberculosis  VGDEIVVSVT NRLGDPTSVH WHGIALRNDM DGTEPATANI GPGGDFTYRF  230
M africanum       M       ---------- ---------- ---------- ----------
M kansasii      -------A-- ---DR----- ---------- --VV---P-- EA-H------

                160       170       180       190       200
M tuberculosis  SVPDPGTYWA HPHVGLQGDH GLYLPVVVDD PTEPGHYDAE WIIILDDWTD
M africanum     ---------- ---------- ---------- ---------- ----------
M kansasii      ---------- ------EE-M ------II-- -----R---- --VV------
                                                  230

                210       220
M tuberculosis  GIGKSPQQLY GELTDPNKPT .MQNTT.....................
M africanum     ---------- ---------- ----------.....................
M kansasii      -V------I- DA-V------ A-PT-TPPSTTPPTTTDTTSTTSATSTTTTTET

                230       240       250
M tuberculosis  .......GMPEG EGVDSNLLGG DGGDIAYPYY  232
M africanum     ........---- ---------- ----------
M kansasii      TPSSPMP---G- DVASSD---- -A--V-----
Test Protein      MT------- ---------- ----------
```

FIG. 11

FIG. 11 Continued

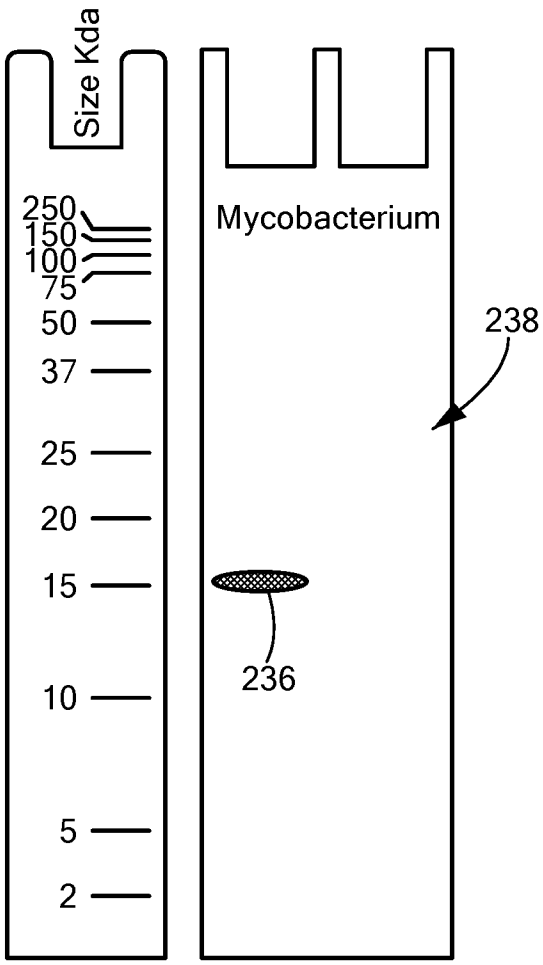

Size Kda
250
150
100
75
50
37
25
20
15
10
5
2
Mycobacterium
238
236

FIG. 12

(Staphylucoccus)
Homology Diagram comparing protein sequence in three Staphylucoccus species.
Standard single letter amino acid abbreviation is used. [-] indicates amino acid homology, [.] indicates gap in sequence.
10 20 30 40 50
S. aureus MYNKVFAILI IIFSITIIAS NDTFAESKND MMNMKEDKKS TMDMTNMKHH
S. epidermidis ---------- ---------- ---------- ----------N ----K-----
S. saprophyticus --K-M-T--- TL---MFMVP ------G-HN --D---NDQK RN--MD--S-
60 70 80 90 100
S. aureus DERKKLNSSQ GKNEIIFPEV AESKKDNNGY KNYTLKAQKG KTEFYKNNFS
S. epidermidis ---------- ---------- ---------- ----------E- ----------
S. saprophyticus ----N----- -----T--K- LDP------- ----------G ------G---
Test Protein MT-- ---------- ---------- ---------- ----------
110 120 130 140 150
S. aureus NTLGYNGNLL GPTLKLKKGD KVKIKLINNL DENTTFHWHG LEVNGKVDGG
S. epidermidis ---------- ---------- ---------- ---------- --T-------
S. saprophyticus ---------- ---------- ---------- ---------- --ID------
Test Protein ---------- ---------- ---------- ---------- ----------
160 170 180 190 200
S. aureus PSQVIKPGKE KTIKFEVNQD SATLWYHPHP SPNTAKQVYN GLSGLLYIED
S. epidermidis ---------- ---------- ---------- ---------- ----------
S. saprophyticus ---------- ------K-E A--------- ---------- ----------
Test Protein ---------- ---------- ---------- ---------- ----------
210 220 230 240 250
S. aureus SKKNNYPSNY GKNDLPIIIQ DKTFVSKKLN YSKTKDEDGT QGDTVLVNGI
S. epidermidis ---------- ---------- ---------- ---------- ----------
S. saprophyticus D--------- ---------- ---------- -T-------- ----------K
Test Protein ----HHHHHH ---------- ---------- ---------- ----------
240
240
240
242

FIG. 13

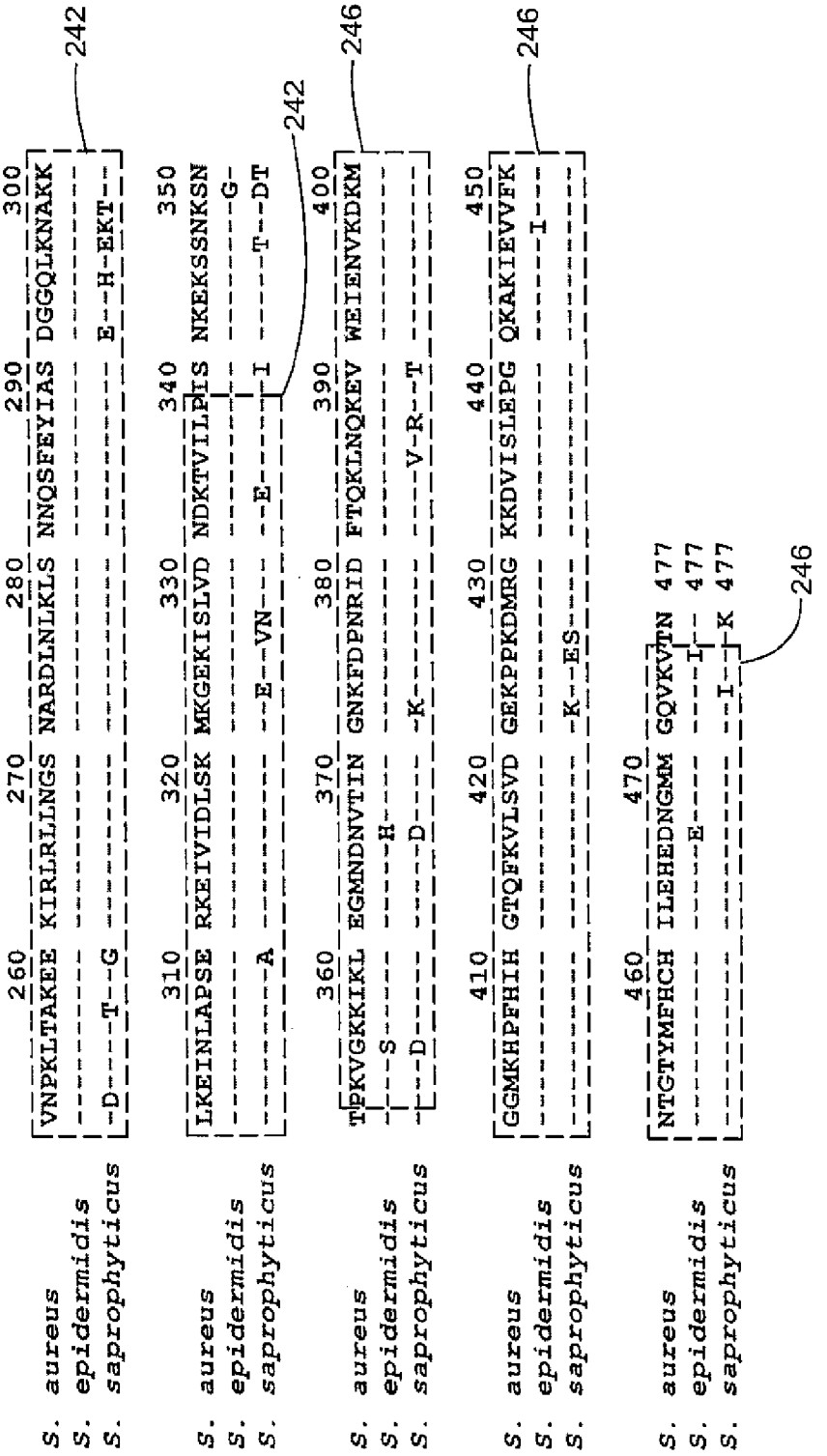


FIG. 13 Continued

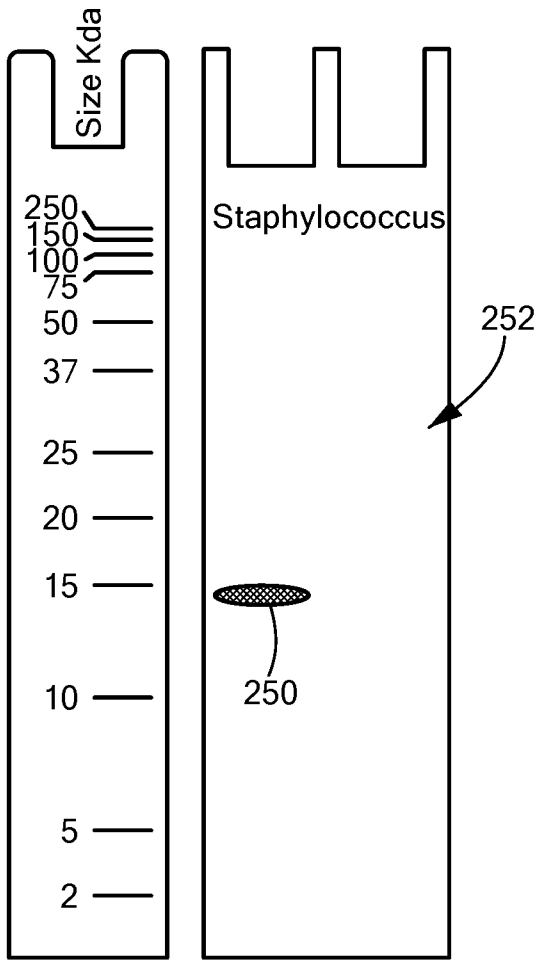

Size Kda
250
150
100
75
50
37
25
20
15
10
5
2
Staphylococcus
252
250

FIG. 14

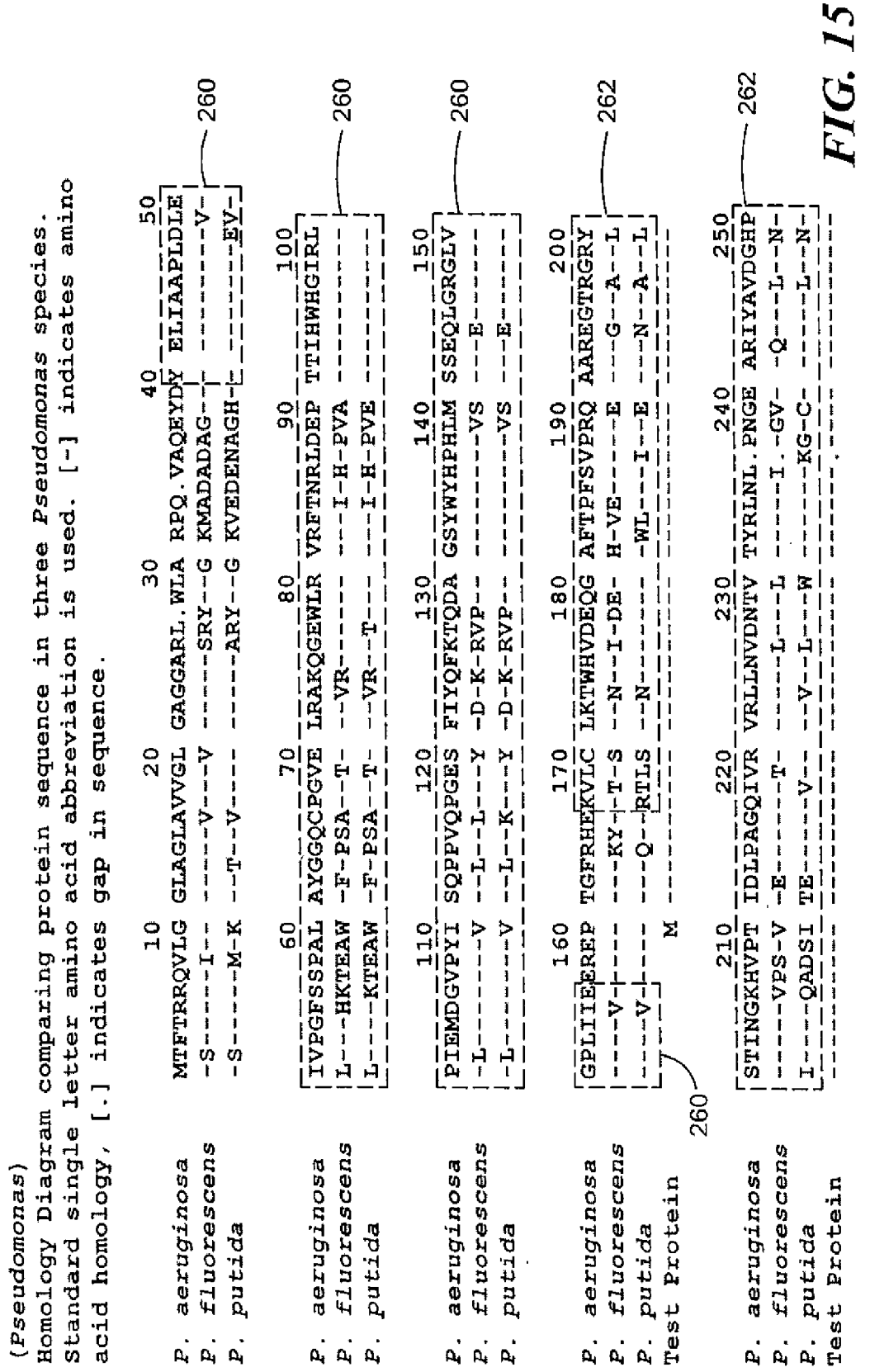

(Pseudomonas)
Homology Diagram comparing protein sequence in three Pseudomonas species.
Standard single letter amino acid abbreviation is used. [-] indicates amino acid homology, [.] indicates gap in sequence.
P. aeruginosa MTFTRRQVLG GLAGLAVVGL GAGGARL.WLA RPQ.VAQEYDY ELIAAPLDLE
P. fluorescens -S-----I-- -----V---V -----SRY--G KMADADAG-- ---------V-
P. putida -S-----M-K --T--V---- -----ARY--G KVEDENAGH- ---------EV-
P. aeruginosa IVPGFSSPAL AYGGQCPGVE LRAKQGEWLR VRFTNRLDEP TTIHWHGIRL
P. fluorescens L---HKTEAW -F-PSA--T- --VR------ ---I-H-PVA ----------
P. putida L---KTEAW -F-PSA--T- --VR--T--- ---I-H-PVE ----------
P. aeruginosa PIEMDGVPYI SQPPVQPGES FIYQFKTQDA GSYWYHPHLM SSEQLGRGLV
P. fluorescens -L---------V --L--L---Y -D-K-RVP-- ---------VS ---E------
P. putida -L---------V --L--K---Y -D-K-RVP-- ---------VS ---E------
P. aeruginosa GPLIIEEREP TGFRHEKVLC LKTWHVDEQG AFTPFSVPRQ AAREGTRGRY
P. fluorescens ----V----- ---KY--T-S --N--I-DE- H-VE------E ---G--A--L
P. putida ----V----- ---Q--RTLS --N--------- -WL---I--E ---N--A--L
Test Protein M
P. aeruginosa STINGKHVPT IDLPAGQIVR VRLLNVDNTV TYRLNL.PNGE ARIYAVDGHP
P. fluorescens -----VPS-V -E------T- -----L---L ----I.-GV- -Q---L--N-
P. putida I-----QADSI TE------V-- --V--L---W -------KG-C- -----L--N-
260
262

FIG. 15

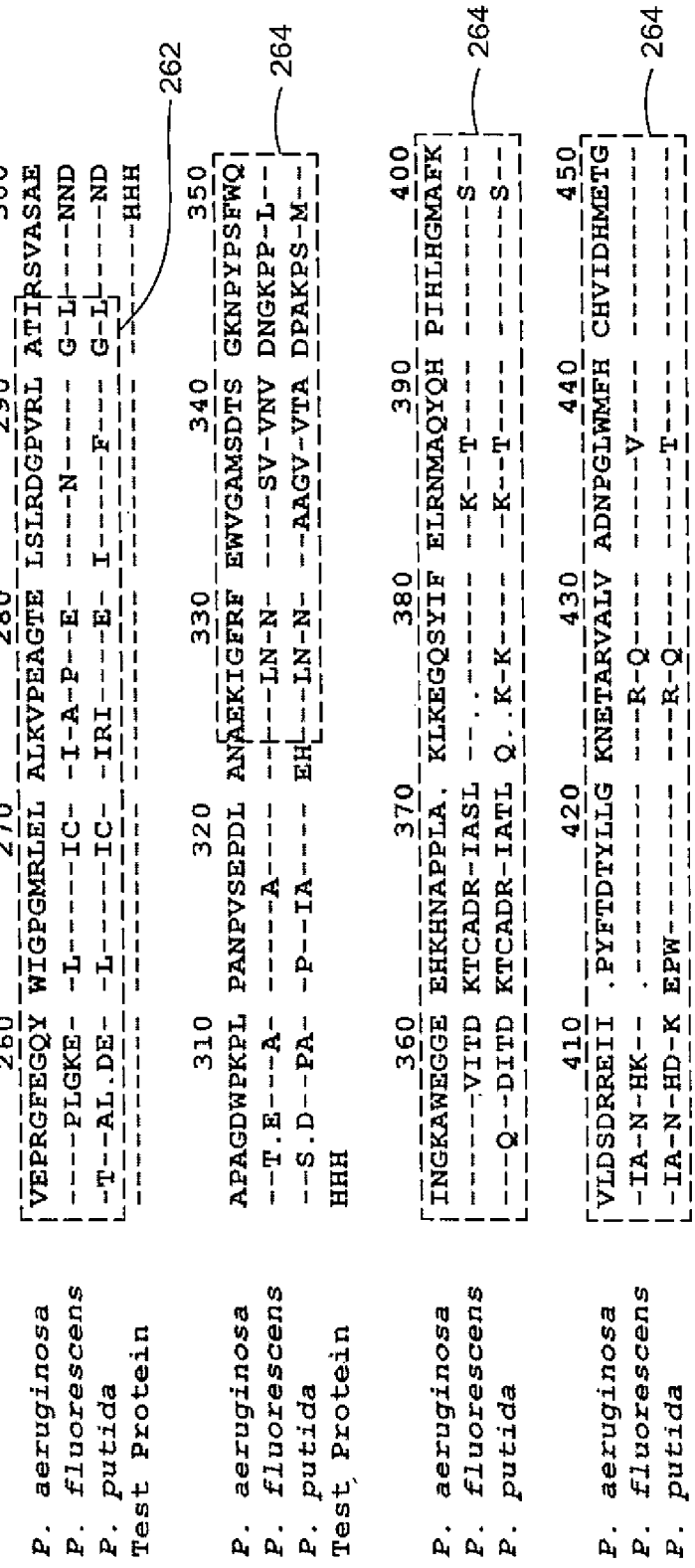


FIG. 15 Continued

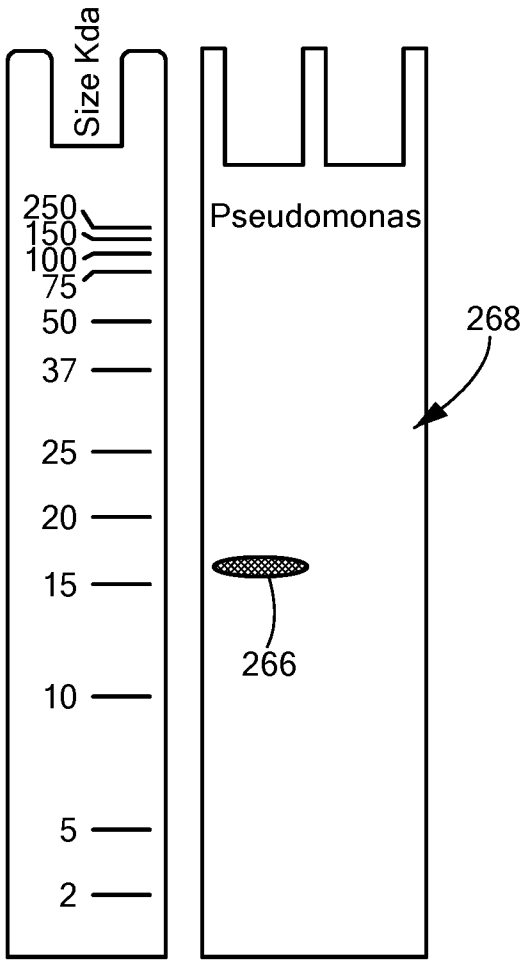


FIG. 16

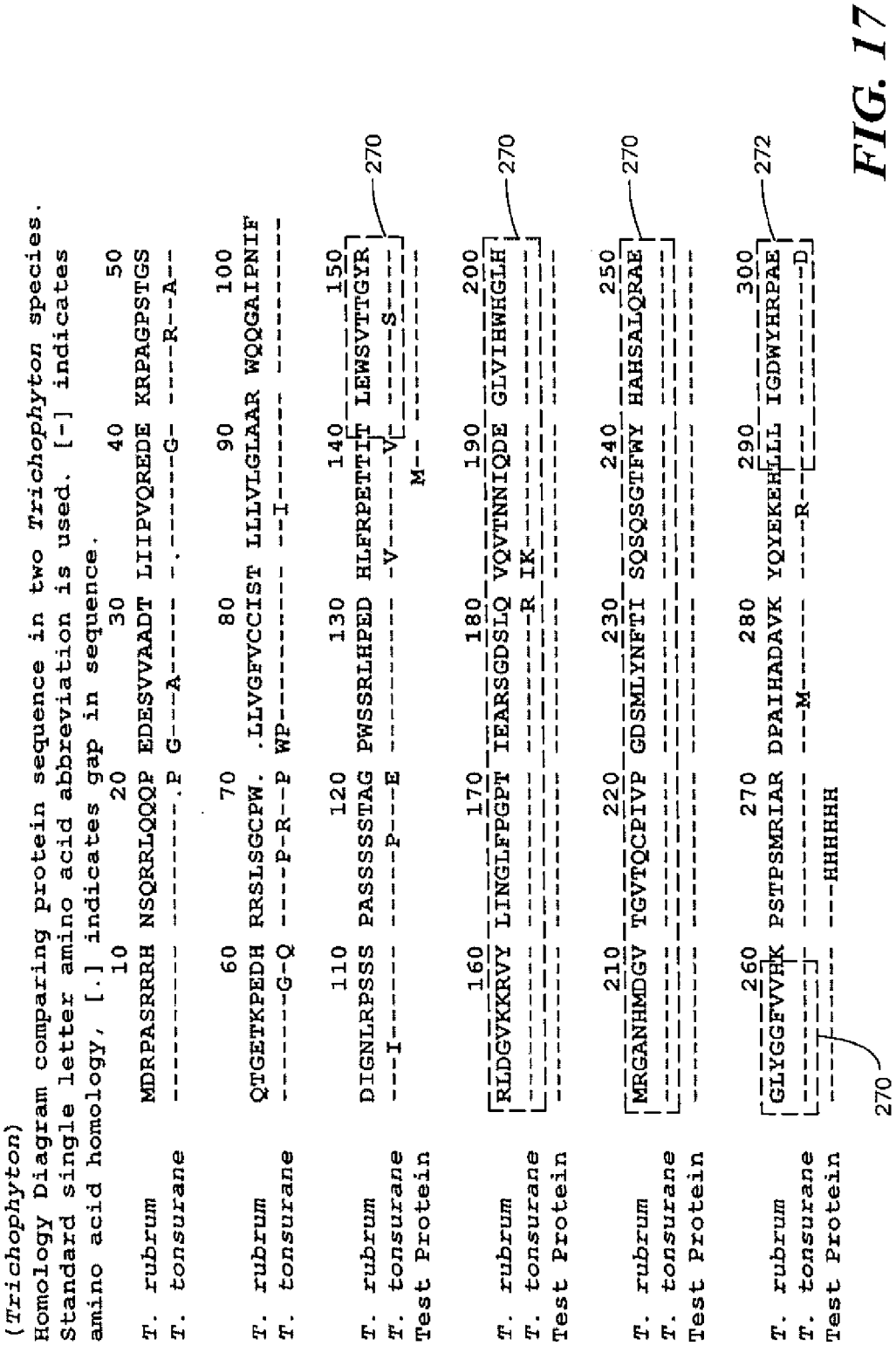

(Trichophyton)
Homology Diagram comparing protein sequence in two Trichophyton species.
Standard single letter amino acid abbreviation is used. [-] indicates
amino acid homology, [.] indicates gap in sequence.
T. rubrum MDRPASRRRH NSQRRLQQQP EDESVVAADT LIIPVQREDE KRPAGPSTGS
T. tonsurane ---------- ---------- .P G---A----- -.------G- ----R--A--
T. rubrum QTGETKPEDH RRSLSGCPW. .LLVGFVCCIST LLLVLGLAAR WQQGAIPNIF
T. tonsurane ------G-Q ----P-R--P WP---------- --I------- ----------
T. rubrum DIGNLRPSSS PASSSSSTAG PWSSRLHPED HLFRPETTIT LEWSVTTGYR
T. tonsurane ---I------ -----P---E ---------- -V------V- -----S----
Test Protein ---------- ---------- ---------- ---------- M-- ----------
T. rubrum RLDGVKKRVY LINGLFPGPT IEARSGDSLQ VQVTNNIQDE GLVIHWHGLH
T. tonsurane ---------- ---------- ---------- ---------R IK--------
Test Protein ---------- ---------- ---------- ---------- ----------
T. rubrum MRGANHMDGV TGVTQCPIVP GDSMLYNFTI SQSQSGTFWY HAHSALQRAE
T. tonsurane ---------- ---------- ---------- ---------- ----------
Test Protein ---------- ---------- ---------- ---------- ----------
T. rubrum GLYGGFVVHK PSTPSMRIAR DPAIHADAVK YQYEKEHLLL IGDWYHRPAE
T. tonsurane ---------- ---------- ---M------ ----R----- ---------D
Test Protein ---------- ---HHHHHH
10 20 30 40 50
60 70 80 90 100
110 120 130 140 150
160 170 180 190 200
210 220 230 240 250
260 270 280 290 300
270
270
270
270
272

FIG. 17

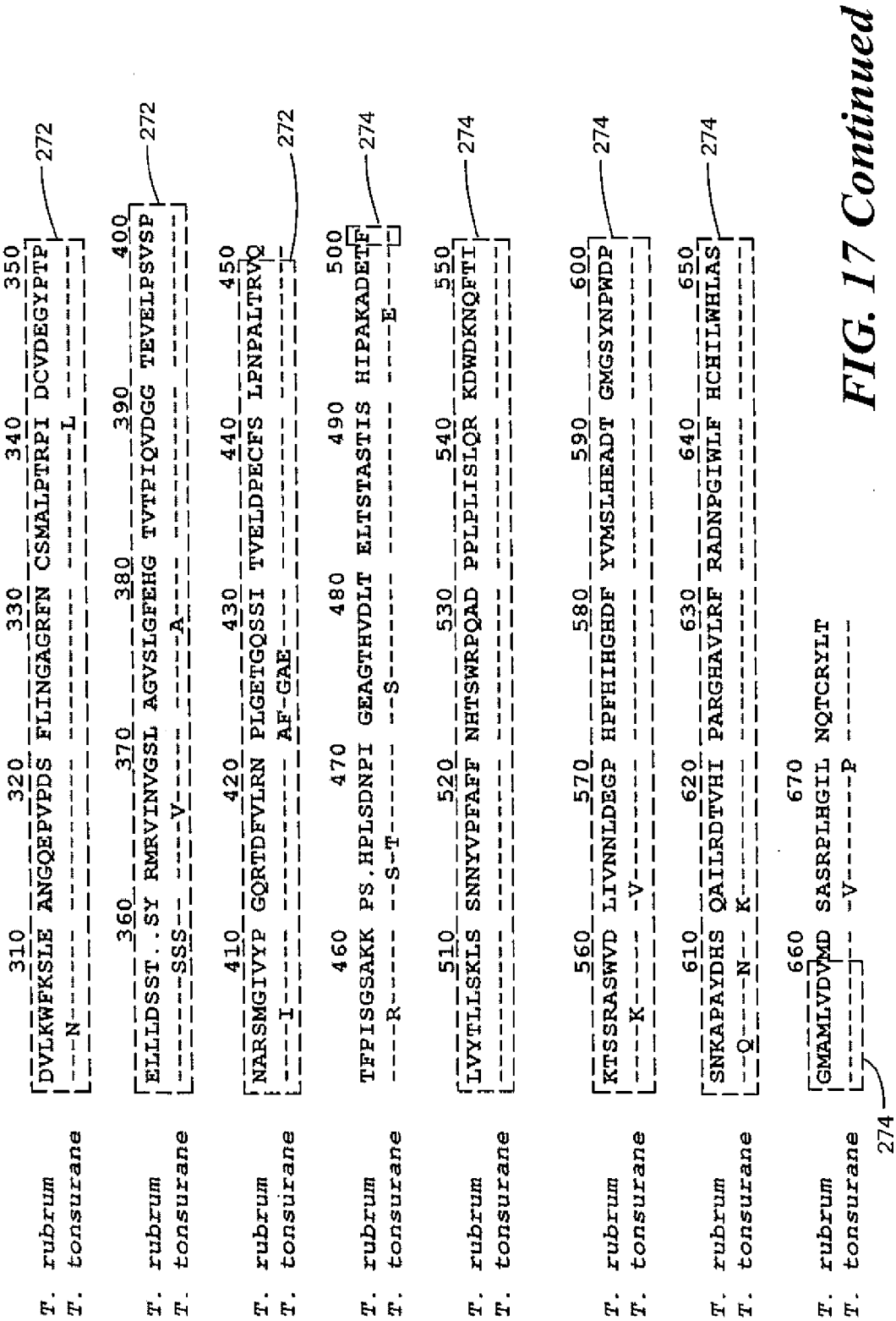


FIG. 17 Continued

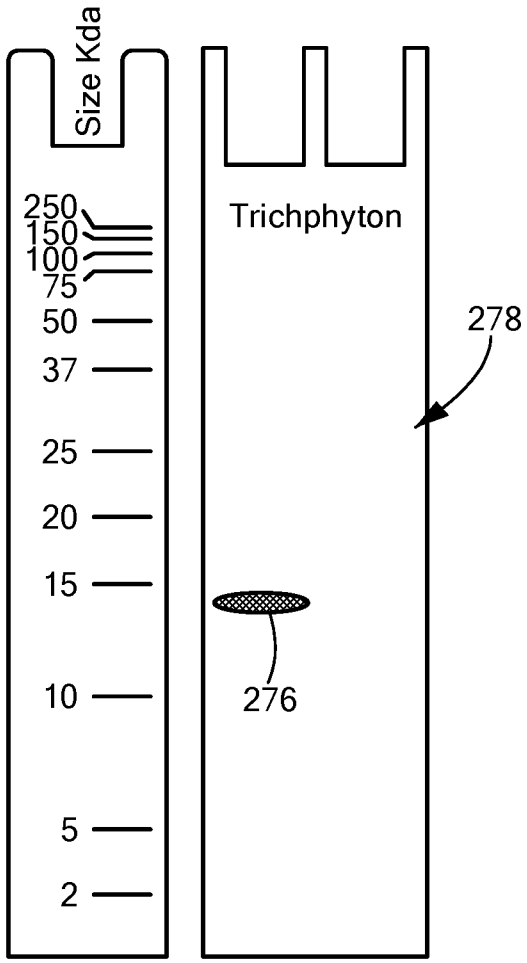


FIG. 18

(*Candida*)
Homology Diagram comparing protein sequence in four *Candida* species.
Standard single letter amino acid abbreviation is used. [-] indicates
amino acid homology, [.] indicates gap in sequence.

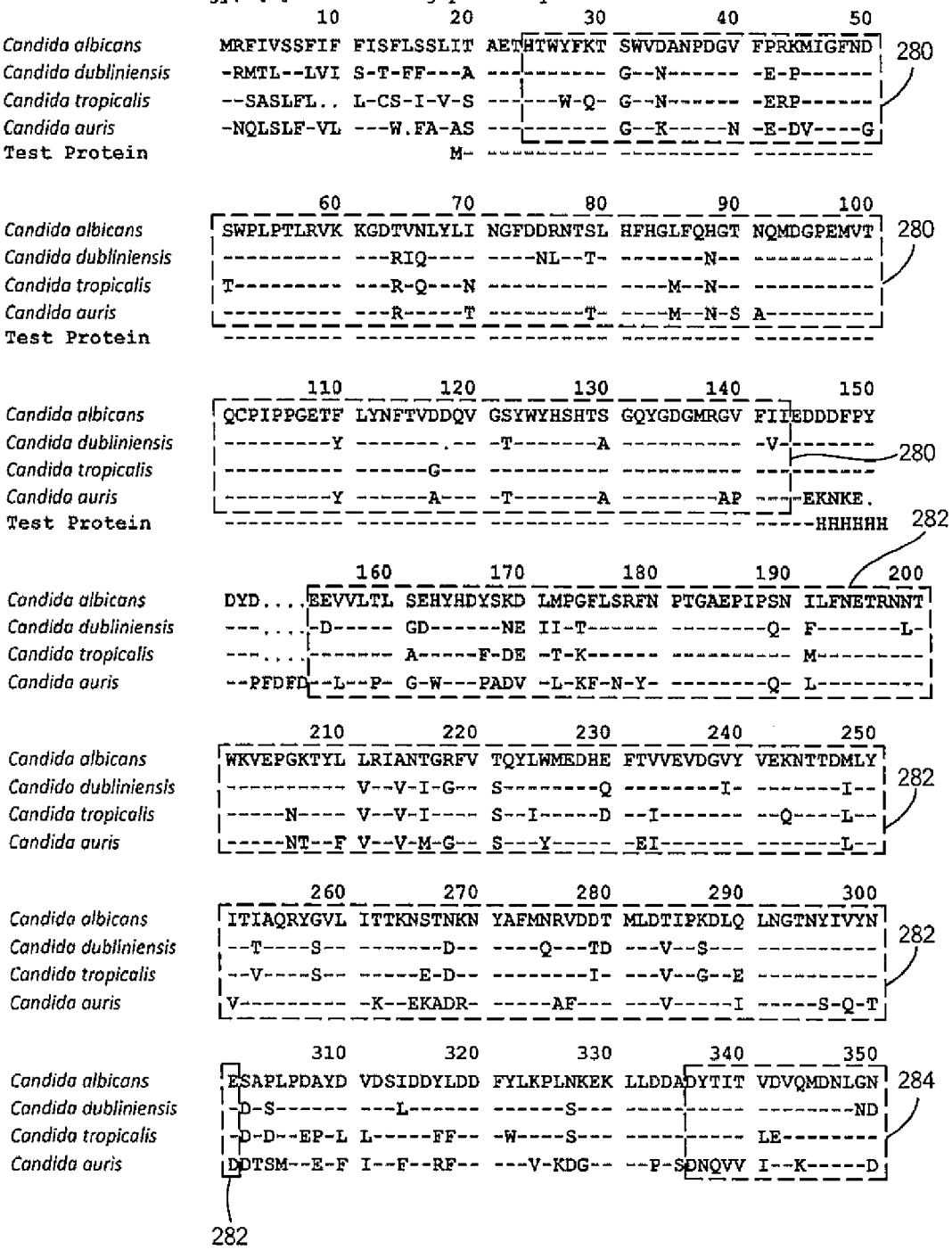


FIG. 19

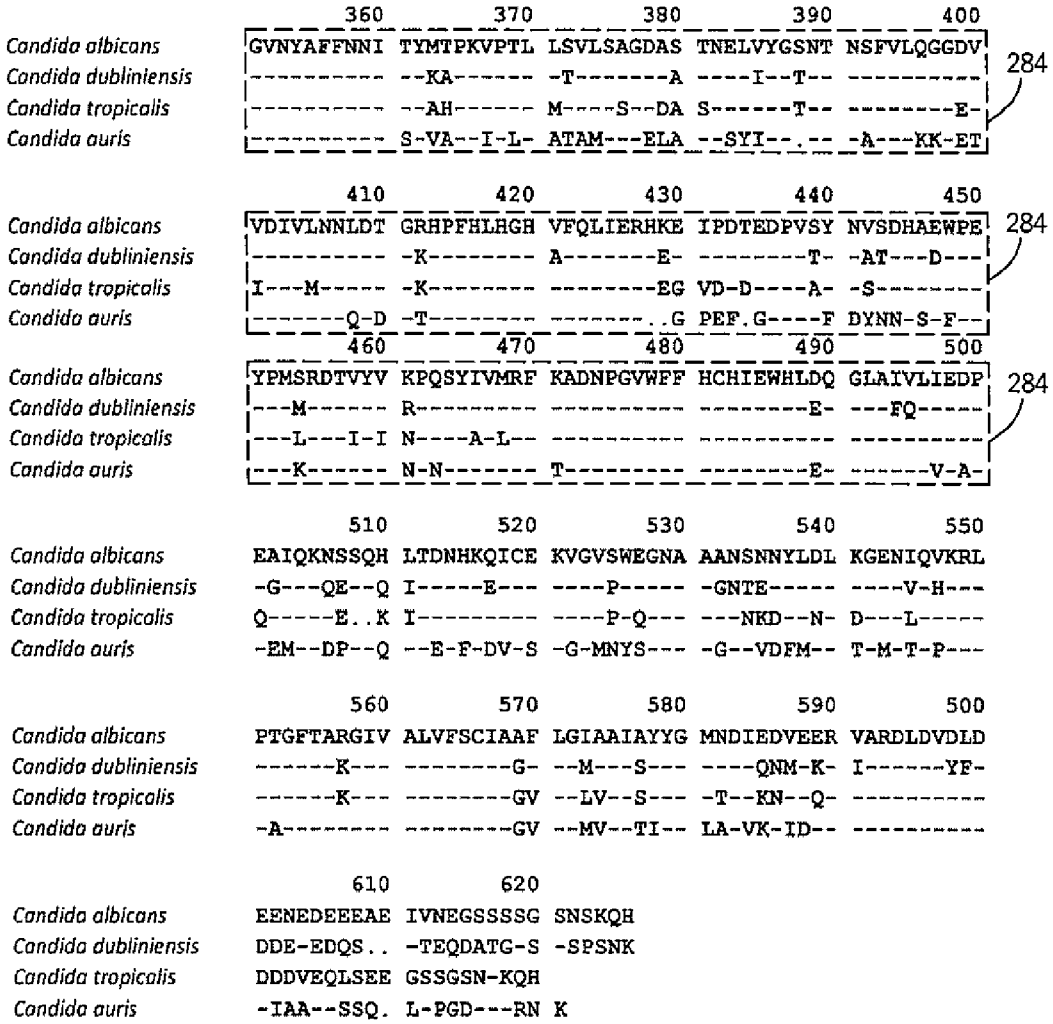


FIG. 19 Continued

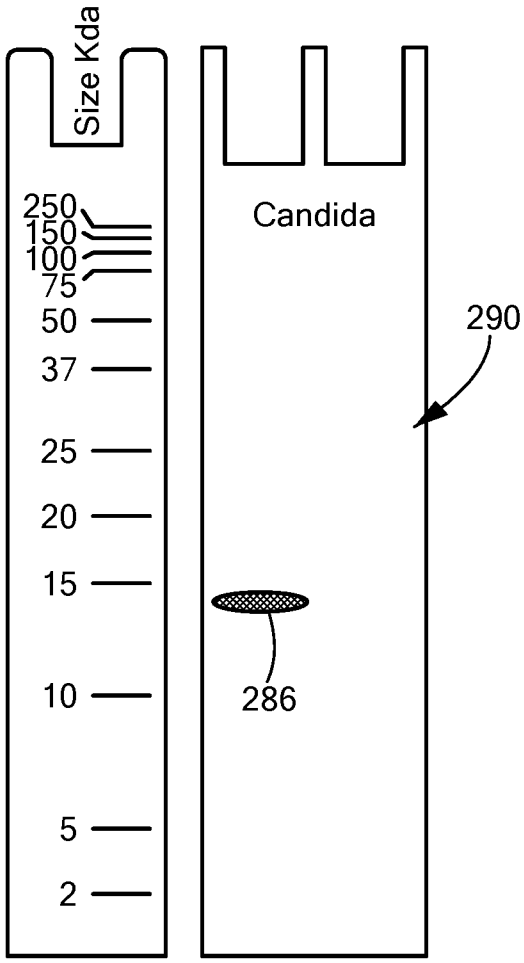


FIG. 20

Homology Diagram for α Gliadin Proteins or Prolamin in Common Grains

Gliadin protein is the immunogenic component of gluten and must be avoided by Celiac patients. Legend - standard single letter abbreviations are used for amino acids. [-] indicates homology. [.] indicates gap in sequence alignment. Amino Acid numbering aligned with example of Bread wheat αGliadin.

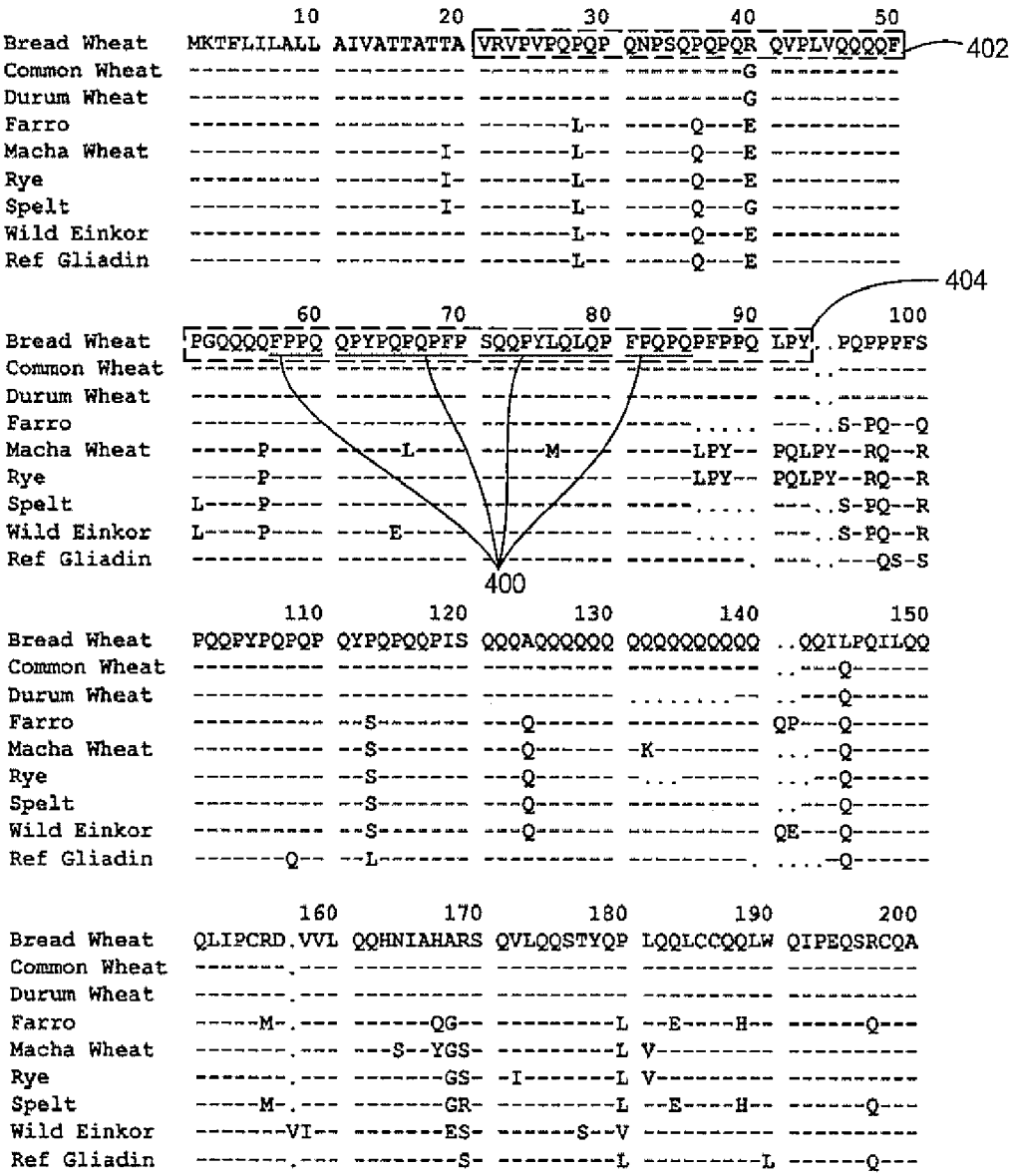


FIG. 21

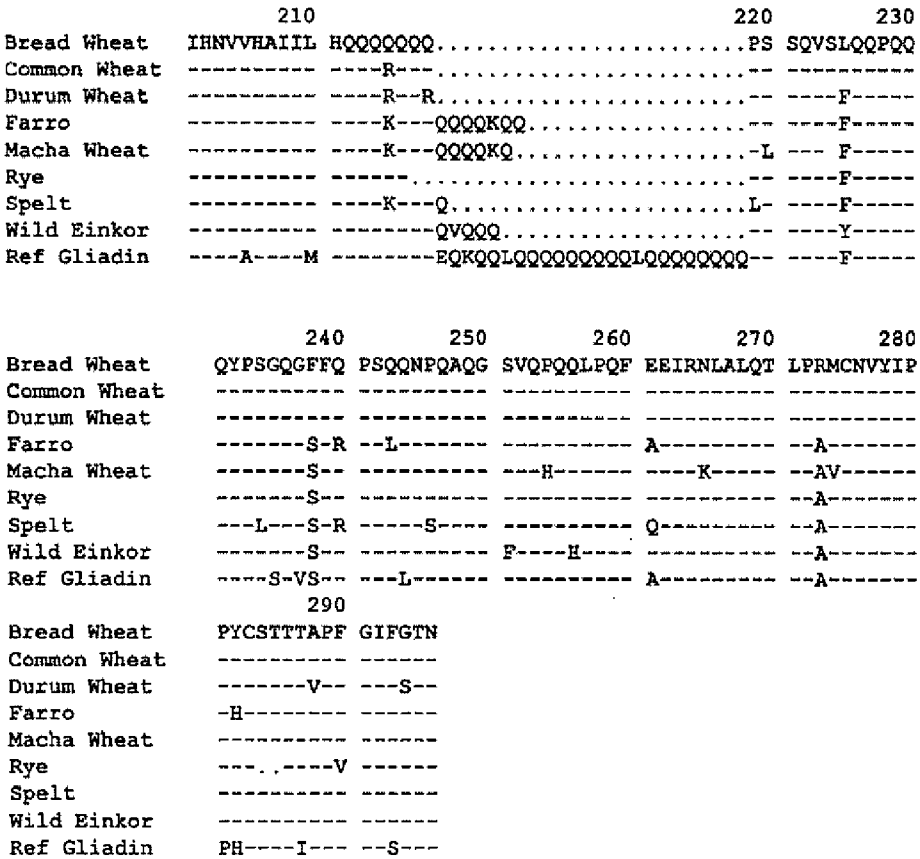


FIG. 21 Continued

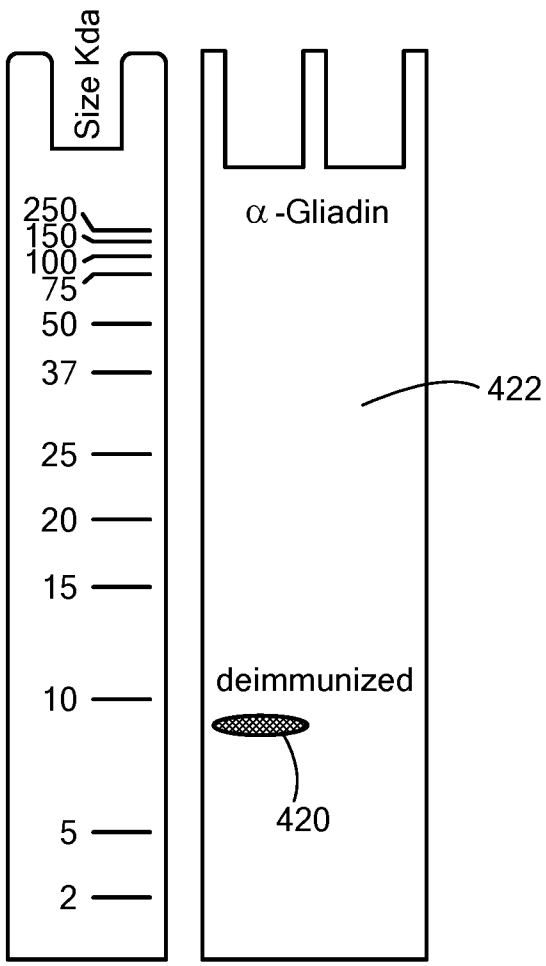

Size Kda
250
150
100
75
50
37
25
20
15
10
5
2
α -Gliadin
422
deimmunized
420

FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/24696

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61L 2/28, G01N 31/22 (2017.01)
CPC - G01N 31/22, G01N 31/226, G01K 1/02

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)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --------- Y	US 7,851,178 B1 (BELHUMEUR et al.) 14 December 2010 (14.12.2010) Abstract; Claims 4, 5; col 1, ln 29-35, col 2, ln 19-24, col 2, ln 28-37; col 3, ln 11-20, col 3, ln 22-23; Col 3, ln 35-43, col 3, ln 52-62	1-5, 22, 23, 25-27, 29-31 ---------- 24A, 24B
Y	WO 2014/056896 A2 (ALBERT LUDWIGS UNIVERSITAT FREIBURG et al.,) 17 April 2014 (17.04.2014) Abstract; Claim 2; Claim 6; ; p12, ln 17-18	24A, 24B
A	UNiProtKB A0A0B4WCZ5 (1 April 2015) http://www.uniprot.org/uniprot/A0A0B4WCZ5, entire document	6, 7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 August 2017	29 AUG 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/24696

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

--Please see continuation in first extra sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 22, 23, 24A, 24B, 25-27, 29-31 limited to SEQ ID NO 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/24696

Continuation of Box III Lack of Unity

Group I+: Claims 1-31, directed to a method for rapidly determining effective sterilization, deimmunization, and/or disinfection of equipment and/or supplies by a device, comprising determining the effectiveness of said procedure by determining if the defined surrogate protein having the predetermined sequence has been destroyed. The method for rapidly determining effective sterilization will be searched to the extent that the surrogate protein encompasses the polypeptide sequence of SEQ ID NO:1. It is believed that claims 1-7 and 22-31 encompass this first named invention, and thus these claims will be searched without fee to the extent that the defined surrogate protein encompass SEQ ID NO:1. Additional defined surrogate protein(s) will be searched upon the payment of additional fee(s). Applicants must specify the claims that encompass any additionally elected defined surrogate protein(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be that the defined surrogate protein encompasses the polypeptide sequence of SEQ ID NO: 2 (claims 1-5, 8-9 and 22-31).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the defined surrogate proteins recited therein. Each invention of Group I+ requires a defined surrogate protein having a predetermined amino acid sequence representative of an infectious agent, not required by any of the other inventions.

Common Technical Features

The inventions of Group I+ share the technical feature of a method for rapidly determining effective sterilization, deimmunization, and/or disinfection of equipment and/or supplies by a device, by providing a defined surrogate protein having a predetermined sequence representative of an infectious agent potentially contaminating the equipment and/or the supplies to be sterilized, deimmunized, and/or disinfected by the device; subjecting the defined surrogate protein to sterilization, deimmunization, or disinfection; and rapidly determining the effectiveness of said procedure by determining if the defined surrogate protein having the predetermined sequence has been destroyed, using a suitable assay.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 7,851,178 B1 to Belhumeur, et al. (hereinafter 'Belhumeur') (abstract "The present invention relates to a method of evaluating the efficiency of sterilization processes by measurement of degradation level of prion protein indicators. When exposed to sterilization conditions, prion indicators are degraded in a manner to proportionally indicate the level of degradation of prion proteins themselves on medical devices or other surfaces usable in surgery and health cares.", col 2, ln 31-32 "The indicator may be selected from the group consisting of Sup35p, Ure2p, Het-s protein, and combination thereof", col 3 ln 52-62 "degradation or alteration can also be estimated by Western Blot or dot blot using an antibody against the tagged protein to estimate the lack of or a modified detection signal being generated by any alteration of the target Sup35 protein. In addition, the indicator degradation could also be detected by color change of the solution, which would confirm sterilization. If needed, techniques such as circular dichroism, electron microscopy, fluorescent microscopy, FTIR, Congo Red binding or proteinase K digestion could also be used to detect the change in conformation of the sterilized protein").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Group I+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.