Ascertaining the 24 Hours Residual Inactivation Efficacy of the Chemical Composition of Legionella-X Viral Off (Fortified with Silver Nanoparticles) Against Bacteria and Enveloped Viruses After Impregnation of Surgical Masks Using JIS L 1902:2015 Test Method

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Abstract

Due to the SARS, MERS, Covid-19 and Influenza-A Epidemics, the medical staff and frontline staff of the public sectors are exposed to the all types of viruses. Facial masks are used as the first line of defense to shield the frontline staff against said viruses. Researchers from the Magna Think Tank Group feel that all facial masks and gowns can be further enhanced if they are impregnated with Legionella-X Viral Off Hospital Grade Disinfectant fortified with Aqueous Colloidal Silver Nanoparticles to decimate any bacteria and viruses that landed on facial masks. This article covers the mechanism and the Test Method of JIS L 1902: 2015 [1,2,3] used to ascertain the antibacterial and antimicrobial inactivation residual efficacies of Legionella-X Hospital Grade Disinfectant fortified with Colloidal Silver Nanoparticles at 24 hours after impregnation into surgical masks and gowns made from both hydrophilic and hydrophobic materials.

Keywords: Legionella-X Viral Off**,** Aqueous Colloidal Silver Nanoparticles, JIS L 1902: 2015, Disposal Surgical Masks and Sleeved Gowns, antibacterial and antimicrobial residual efficacies.

Introduction

During the SARS [4,5,6], MERS [7,8,9], Covid-19 [10,11,12,13,14, 15,16,17,18,19,20],and Influenza-A [21,22,23.24,25] Epidemics , the medical staff and frontline staff of the public sectors are exposed to the all types of viruses [26,27,28,29,30]. Facial masks are used as the first line of defense to shield the frontline staff against said viruses [31]. Researchers from the Magna Think Tank Group feel that the safety measures all facial masks and gowns can be further enhanced if they are impregnated with Legionella-X Viral Off Hospital Grade Disinfectant fortified with Aqueous Colloidal Silver Nanoparticles to decimate any bacteria and viruses that landed on facial masks and surgical gowns.

Colloidal silver nanoparticles are an ancient remedy that was once used to treat bacterial, viral and fungal infections [41]. The current emergence of resistant viral strains and the side effects linked with prolonged use of anti-viral drug calls for an alternative source to decimate viruses [42]. Silver Nanoparticles have mainly been studied for their antimicrobial potential against bacteria but have also proven to be active against several types of viruses including human immunodeficiency virus, hepatitis B virus, herpes simplex virus, respiratory syncytial virus, and monkey pox virus [36]. The use of silver Nanoparticles provides an interesting opportunity for novel antiviral therapies. Since metals may attack a broad range of targets in the virus there is a lower possibility to develop resistance as compared to conventional antivirals [43].

Nanoparticles are determined as particles with dimension less than 100 nm. Their singular physical (e.g., plasmonic resonance, fluorescent enhancement) and chemical (e.g., catalytic activity enhancement) properties derive from the high quantity of surface atoms and the high area/volume relation, in fact, as their diameter decreases, the available surface area of the particle itself increases dramatically and as a consequence there is an increase over the original properties of their bulk materials[44].

In consideration that biological interactions are commonly multivalent, the interplay between microbes and host cells usually associates multiplex copies of receptors and ligands that bind in a harmonize manner, culminating in enhanced particularities, adaptabilities and strengths of such interactions that allow the microbial agent to take possess of the cell under attack. The attachment and entry of viruses into host cells represent a terrific example of such multivalent interactions between viral surface components and cell membrane receptors [32]. Interfering with these recognition events,

and thereby blocking viral entry into the cells, is one of the most promising strategies being pursued in the development of new antiviral drugs and preventive topical microbicides [33,34,35].

Emerging and re-emerging viruses are to be considered a continuing threat to human health because of their amazing ability to adapt to their current host, to switch to a new host and to evolve strategies to escape antiviral measures [36].

Viruses can emanate because of adaptability in the host, the environment, or the vector, and new pathogenic viruses can arise in humans from existing human viruses or from animal viruses. Several viral diseases that emanate in the last few decades have now become rooted in human populations globally. Such examples are SARS coronavirus, Chikungunya virus, West Nile virus, Hendra virus, Monkey pox virus, Hantavirus, Nipah virus, and the threat of pandemic influenza viruses, most recently of Covid-19.

Most viruses are, indeed, provided by an extraordinary genetic adaptability, which has enabled them to escape antiviral inhibition and in certain cases to regain advantage over the host by mutagenesis that create new viral strains with acquired resistance to most of the antiviral compounds available [36].

In view of the above, the authors feel that it is imperative to nib the problems at its buds by preventing cross contamination via inactivation of the viruses and bacteria while using said personal protective equipment.

A potent synergistically chemical formulation was hence conceptualized to be used for impregnation of surgical mask and gowns.

The functions of each raw material mentioned in the said chemical composition are herein described below.

The Legionella-X Viral Off [37] chemical composition comprising of aqueous colloidal silver nanoparticle, Isopropanol Alcohol, alkyl didecyl dimethyl ammonium chloride, water and peppermint essential oil [37], is used for impregnation of the surgical masks and gowns.

Colloidal Silver Nano Particles- The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications. Silver Nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China, and they are said to show good antimicrobial action [38].

The aqueous colloidal silver nanoparticle is used as co-disinfectant [39] and to enhance the excellent residual effect of antimicrobial properties of the disinfectant impregnated into the disposal surgical mask and sleeved gowns.

The aqueous colloidal silver nanoparticle's antimicrobial effect has been demonstrated in numerous applications against different types of microorganisms[45]. The mechanism behind the bactericidal efficacy of the aqueous silver Nanoparticles is through its binding to disulphide or sulfhydryl groups in cell wall proteins [46]. Silver nanoparticle also binds to DNA. Through these binding events, metabolic processes are disrupted, leading to cell death. The size of the colloidal silver nanoparticle ranges from 2-150nm. The optimal size of spherical silver Nanoparticles (AgNPs) for off-resonance surface-enhanced Raman scattering (SERS) was found to be ~50 nm based on the equivalent Ag content in AgNP colloids. Hence, the preferred size of the colloidal size nano-size is 50 nm [40][47].

Silver Nanoparticles have demonstrated an excellent antibacterial property, it is used as a potent disinfectant [40].

According to the aspect of this journal, it is the object of the authors to provide the Chemical Composition for impregnation via spraying into disposal surgical masks and surgical sleeved gowns to inactivate bacteria and enveloped viruses such as Coronavirus, SARS, MERS, Influenza A and Influenza B. The said chemical composition comprising of aqueous colloidal silver Nanoparticles, Isopropanol Alcohol/ Ethanol, Chlorhexidine gluconate/Quaternary Ammonium Compound (alkyl dimethyl benzyl ammonium chloride, alkyl didecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride) and Peppermint/ Spearmint Essential oils and water [37].

The chemical composition and its functional properties are herein described:

Isopropanol Alcohol- In embodiment Isopropanol is added to the chemical composition for inactivating enveloped viruses via denaturation of the capsid protein and killing bacteria by destroying peptidoglycan/ lipopolysaccharide cell wall of the bacteria. It also enhances the hydrophilic properties of peppermint/spearmint essential oils used in the chemical formulation

Twin Chain Quaternary Ammonium Compound - The said twin quaternary ammonium compound causes disruption of intermolecular interactions and dissociation of lipid bilayers. Alkyl Didecyl Dimethyl Ammonium Chloride is a broad spectrum bactericidal and fungicidal and is used as disinfectant cleaner for linen. It is widely used in hospitals, hotels and other industries. a Peppermint/Spearmint Essential Oils and water.

Water- In another further embodiment, water is used as a catalyst and has a major role in denaturing the capsid proteins of the enveloped viruses and cell membranes of bacteria. It also serves a carrier of all the above-mentioned ingredients of the said chemical composition.

Peppermint Oil- In a further embodiment, Peppermint/Spearmint essential oils is used to enhance the antimicrobial properties of the said chemical composition. Peppermint essential oil is the preferred essential oil because of its antimicrobial effects against both Gram-positive and Gram-negative bacteria. Peppermint essential oil is also found to possess antiviral and fungicidal activities.

In another embodiment of said chemical composition, Isopropanol/Ethanol is used to increase the hydrophilic properties of the peppermint/spearmint essential oils and to facilitate the ease of said chemical composition to be impregnated into disposal surgical masks and sleeved gowns made from hydrophobic materials. It is also used for the denaturation of the capsid protein of the enveloped viruses such as Coronavirus, SRAS, MERS, Influenza A, Influenza B and for destroying the peptidoglycan/ lipopolysaccharide cell wall of the bacteria. The preferred alcohol is Isopropanol Alcohol because it less dehydrating on living tissue and so is a better solution for disinfecting skin than ethanol. It is also effective against bacteria spores.

In yet another embodiment of the said chemical composition, chlorhexidine gluconate /Quaternary Ammonium Compound (alkyl dimethyl benzyl ammonium chloride, alkyl didecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride) is used as a co-disinfectant for the said chemical composition. Chlorhexidine gluconate is the preferred choice due to its a broad-spectrum biocide property and its effectiveness against Gram-positive bacteria, Gram-negative bacteria and fungi. It has excellent for both bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria) making it an excellent choice for the said chemical composition used for the impregnation disposal surgical masks and sleeved surgical gowns.

In yet another embodiment of the said chemical composition Peppermint/ Spearmint essential oil is used to further enhanced the antimicrobial effect of the said invention. The preferred essential oil is peppermint, when used in a mixture of polar solvent such as Isopropanol Alcohol it is highly effective against Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus aureus than Pseudomonas aureus and Serratia marcescens. Peppermint essential oil is also found to possess antiviral and fungicidal activities.

In yet another further embodiment, water is primarily used to as a catalyst and has a major role in denaturing the proteins of cell membranes of viruses and bacteria. Also, the function of water slows down evaporation of said chemical composition and increases the

surface contact time with the membrane and as a carrier for the all the above-mentioned ingredients of the said chemical composition. Preferred water is municipal fresh water due to its ease of access.

The dry inactivation residual efficacy against enveloped viruses and bacteria of Legionella-X Viral Off [37] a hospital grade disinfectant was further enhanced by fortifying it with Colloidal Silver Nanoparticles for the purpose of increasing its inactivation residual efficacy after impregnation of surgical masks, gowns and clothing.

The JIS L 1902:2015 Textiles-Determination of Antibacterial Activity and Efficacy of textile Product was used to ascertain the dry inactivation residual efficacy of Legionella-X Viral Off at 24 hours.[3]

The Scope of the Test for JIS 1902:2015 is briefly herein described. [3]

This Standard specifies quantitative and qualitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens and antibacterial efficacy.

This Standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, bedclothes, home furnishings and miscellaneous goods, regardless of the type of antibacterial agent used (organic, inorganic, natural or man-made) or the method of application (built-in, after-treatment or grafting).

Based on the intended application and on the environment in which the textile product is to be used and also on the surface properties of the textile properties, the user can select the most suitable of the following four determination methods on determination of antibacterial activity.

a) Absorption method - An evaluation method in which test bacterial suspension is inoculated directly onto the specimens.

b) Transfer method- An evaluation method in which test bacteria are placed on an agar plate and transferred onto the specimens.

c) Printing method- An evaluation method in which test bacteria are placed on a filter and printed onto specimens.

d) Halo method -A qualitative method to evaluate by the existence of halo.
The colony plate count method and the ATP (ATP = Adenosine Tri-phosphate) lumi-nescence method are also specified for measuring the enumeration of bacteria.

e) Terms and definitions For the purpose of this Standard, the following term and definition apply.

f) Control specimen-Specimen which is the same textile product as the textile product to be tested, but without antibacterial treatment, used to validate the condition of test effectiveness.
If control specimen is not available, a 100 % cotton standard adjacent fabric (cotton No. 3-1) specified in **JIS L 0803** is used as the control specimen, after 10 cycles of washing for 10 min at a temperature of 60 °C without detergents or any brighteners, rinsing twice for 5 min and air-drying.

g) Antibacterial agent (Legionella-X Viral Off) Product designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria.

h) Antibacterial finish. Treatment designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

i) Antibacterial Activity. Activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria.

j) Antibacterial efficacy-Efficacy of antibacterial activity shown by antibacterial finish. It is evaluated by the existence of halo and antibacterial activity value.

k) Plate count method-Method in which the number of bacteria present after incubation is calculated by counting the number of colonies according to a ten-time dilution method.
The results are expressed in "CFU (Colony Forming Unit)".

l) Luminescence method

Method in which the amount of adenosine triphosphate (hereafter referred to as "ATP") contained in bacterial cells is measured.
The results are expressed in "moles of ATP".

m) Neutralizer

Chemical agents used to inactivate, neutralize or quench the antibacterial properties of antibacterial agents

n) Halo

The part where the growth of test bacteria produced around the sample is suppressed when the antibacterial processed sample is placed and incubated on the culture medium containing the test bacteria.

4 Safety precaution

The test methods specified herein require the use of bacteria. These tests should be carried out by person with training and experience in the use of microbiological techniques. Safety precautions shall be checked against and comply with regulations.

The test procedure and results are herein described below:

The Japanese Industrial Standard (JIS) issued a testing method to determine the antibacterial activity (expressed as an Index), of antimicrobial-treated (compounded) materials or articles.

The antibacterial activity is calculated dividing the number of bacteria present after 24 hours of cultivation onto a testing treated article/product (C) into the number of bacteria present after 24 hours of cultivation onto the corresponding untreated (without antimicrobial agent) article/product (B).

The following formula is established for the calculation of the value of antimicrobial activity:

R= log (B/C)

where,

B: average of the number of viable cells of bacteria on the untreated test piece after 24 h

C: average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h

The value of antimicrobial activity obtained by the testing method of this Standard shall not be less than 2.0 for the antimicrobial efficacy of antimicrobial products.

An antibacterial index of >2.0 (≥99% killing ratio) of a treated article with antimicrobial agent might be considered as “Antibacterial Article”.

Antibacterial efficacy

From the testing result, the antibacterial efficacy of the test specimen of Legionella-X Viral Off (Fortified with Silver Nanoparticles) is as shown in Table F.1.

Table F.1 Antibacterial efficacy based on JIS L 1902:2015

Antibacterial value A	Antibacterial efficacy
2.0 < A < 3.0	Effect
3.0 < A	Full effect
>5.8	Legionella-X Viral Off

Test Result of Legionella-X Viral Off fortified with Silver Nanoparticles

Tested by PSB TUV using JIS L 1902-2015 as follows:

TEST REPORT: 7191232782-CHM20-01-RC
11 MAR 2020

PSB Singapore

RESULTS

Test microorganism (Bacterial cells inoculated per test piece)	Average of the number of viable cells of test microorganism per test piece		Antibacterial Activity Value **(Criteria: Not Less than 2.0)**
Klebsiella pneumoniae (ATCC 4352)	0 hour	24 hours	
Control (Blank)	37 000	13 000 000	-
Legionella-X Viral Off Hospital Grade Disinfectant for Inactivating Bacteria and Enveloped Viruses on Impregnated Disposal Masks made from both Hydrophobic and Hydrophilic Materials	Less than 20	Less than 20	More than 5.82

Test microorganism (Bacterial cells inoculated per test piece)	Average of the number of viable cells of test microorganism per test piece		Antibacterial Activity Value **(Criteria: Not Less than 2.0)**
Staphylococcus aureus (ATCC 6538P)	0 hour	24 hours	
Control (Blank)	29 000	13 000 000	-
Legionella-X Viral Off Hospital Grade Disinfectant for Inactivating Bacteria and Enveloped Viruses on Impregnated Disposal Masks made from both Hydrophobic and Hydrophilic Materials	Less than 20	Less than 20	More than 5.82

Conclusion

Based on the above test result by TUV/PSB, Legionella-X Viral Off fortified with Silver nanoparticles have a dry residual inactivation efficacy of 99.99984% against bacterial and virus making it an excellent disinfectant for impregnating surgical mask, gown, and clothing.

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[3] JIS L 1902 – Antimicrobial Test – Fabrics JIS L 1902 is an antimicrobial product test for testing antibacterial activity and efficacy on textiles**.** https://www.situbiosciences.com/product/jis-l-1902-antimicrobial-test-fabrics/

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His research activities include the generation of functionalized and non-functionalized natural compounds with mineral nanoparticles for medical purposes.

He has participated and published many articles on Nano Medicine in International Scientific Journals, in addition to collaborating with the engineering institute of the Autonomous University of Baja California in projects involving areas of health.

He is the founder and director of Ixchel Medical Center and is the Medical Adviser of the Magna Think Tank Group.