Ascertaining the 60 Days Residual Inactivation Efficacy of Legionella-X Viral Shield Self-Disinfecting Coating Using JIS Z 2801:2010/A1:2012 Test Method

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Abstract

The Coronavirus is primarily a respiratory illness, and it typically spreads via airborne droplets from an infected person's coughs or sneezes [1,2,3,4,5,6,7]. Viral particles can survive for a time on surfaces, but the coronavirus life span on surfaces depends on variables like temperature and humidity [8,9,10,11]. Early research has demonstrated that the virus's survival depends on the type of surface it lands on. The Coronavirus virus can survive anywhere between three hours and seven days, depending on the material [12,13,14,15,16,17]. A healthy person can then unknowingly touch those inanimate surfaces, people's hands can transport the viral particles to different surfaces, even the face, where it can enter your body through your eyes, mouth or nose [18,19,20,]. In view of the above, Magna and its Think Tank Group felt that it was essential not only to disinfect all inanimate public surfaces but also to provide all inanimate surfaces with a coating shield against any viruses or bacteria that landed on it. This article covers the residual inactivation efficacy of Legionella-X Viral Shield and its 60 days anti-bactericidal residual inactivation efficacy using JIS Z 2801:2010/A1:2012 Test Method [21,22,23].

Keywords:

Legionella-X Viral Shield, JIS Z 2801:2010/A1:20012, Residual Inactivation Efficacy, Convid-19 Virus

Introduction

Based on scientific research the COVID-19 Virus is known to be spread mainly by inhaling droplets released when an infected person coughs or sneezes, these droplets can also land on surfaces [1,2,3,4,5,6,7]. A healthy person can then unknowingly touch those surfaces and oftentimes, people's hands can transport the viral particles to different surfaces, even the face, where it can enter your body through your eyes, mouth, or nose [18,19,20]. Hence, health officials keep reminding us not to touch our faces and to wash our hands as frequently as possible.

Recent research has validated that the virus's survival depends on the type of surface it lands on. The live virus can survive anywhere between three hours and seven days, depending on the material [12,13,14,15,16,17]. The study followed earlier research that also tested the coronavirus life span on household surfaces. The prior study, published March 17 in the New England Journal of Medicine, suggested the virus could live up to four hours on copper, up to a day on cardboard, and up to three days on plastic and stainless steel [24,25]. The researchers compared the new coronavirus' life span on surfaces to that of the SARS coronavirus. They found that both coronaviruses lived the longest on stainless steel and polypropylene, a type of plastic used in everything from toys to car parts. Both viruses lasted up to three days on plastic, and the new coronavirus lasted up to three days on steel. On cardboard, however, the new coronavirus lasted 24 hours or three times the eight hours that SARS did.

Another study, published March 1 in the Journal of Hospital Infection, looked at the life spans of other coronaviruses found in humans on various surfaces. The SARS coronavirus, at a temperature of 68 degrees Fahrenheit (20 degrees Celsius), lasted for two days on steel, four days on wood and glass, and five days on metal, plastic, and ceramics. (The researchers also found that one strain of SARS lasted up to nine days on a plastic surface at room temperature.) SARS survived for two to eight hours on aluminum and less than eight hours on latex.

According to Rachel Graham, an epidemiologist at the University of North Carolina, smooth, nonporous surfaces like doorknobs and tabletops are better at carrying viruses in general. Porous surfaces like money, hair, and cloth fabric do not allow viruses to survive as long because the small spaces or holes in them can trap the virus and prevent its transfer. According to research the Covid-19 Virus typically lasts on common surfaces such as Glass – 5 days, Wood – 4 days, Copper-4 hours, Aluminium-2-8 hours, Plastic & stainless-steel – 3 days, Cardboard – 24 hours, ceramics examples dishes, pottery and mugs 5 days [26,27,28,29,30,31,32]. See predicter of decay of the Corvid-19 virus in Chart 1 below.

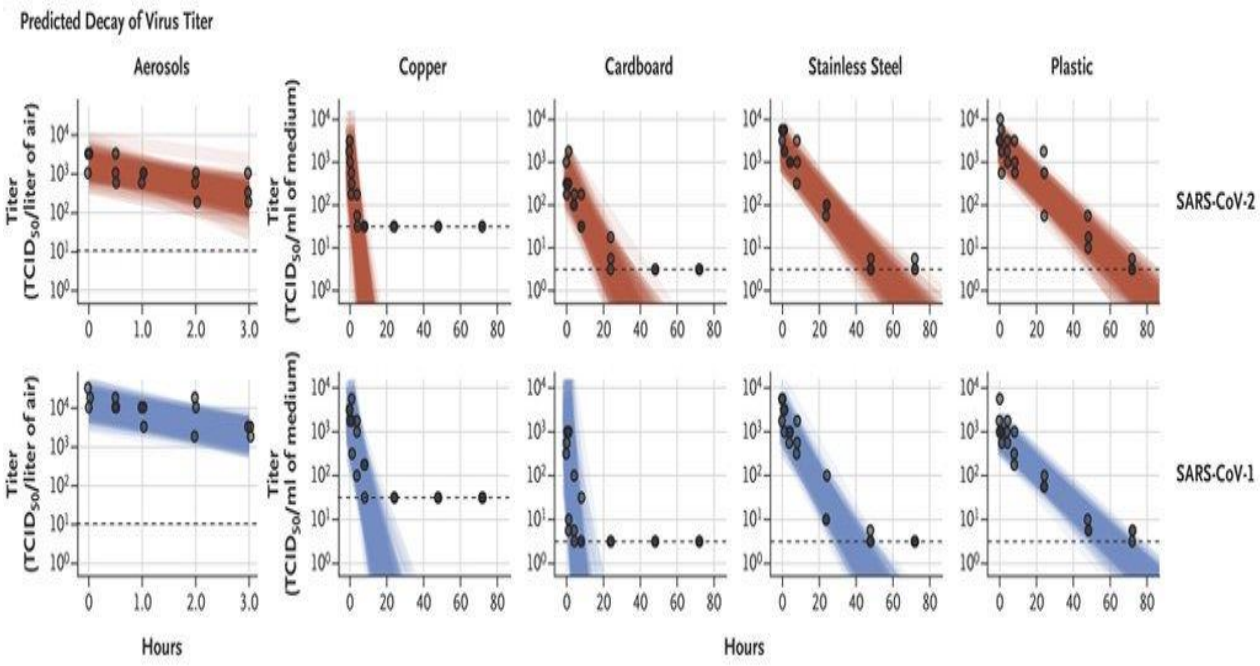

Chart 1

The study followed earlier research that also measured the coronavirus lifespan on a range of household surfaces. The prior study, published March 17 in the New England Journal of Medicine, suggested the virus could live up to four hours on copper and up to a day on cardboard. The researchers found that the virus lasted up to three days on plastic and stainless steel — a shorter time than the results in the Lancet study. See chart 2 below.

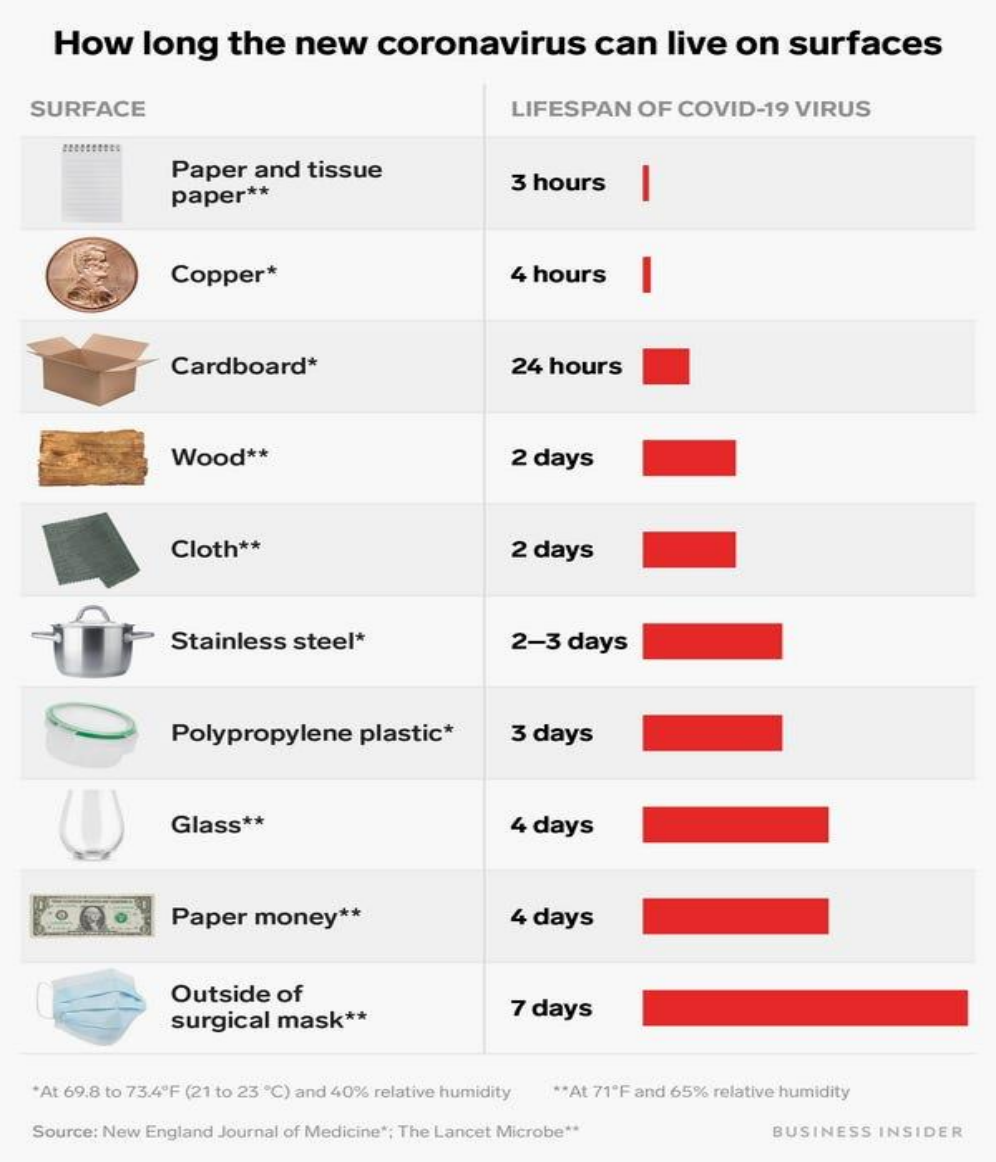

Chart 2

The CDC guidelines on how to protect the public at large include: Clean and disinfect surfaces that many people come in contact with. These include tables, doorknobs, light switches, countertops, handles, desks, phones, keyboards, toilets, faucets, and all high-contact surfaces in public. Wash your hands often with soap and water for at least 20 seconds immediately when you return home from a public place such as the bank or grocery store.

The lingering infectious period of Covid-19 on hard surfaces promoted Magna and its Think Tank Group to research and develop a shield-coating for all inanimate surfaces after they have been disinfected.

In view of the above Legionella-X Viral Shield with high residual inactivation, efficacy was developed to combat Covid-19 Virus. The said product was developed to protect all inanimate surfaces including, plastics, metals, wood, leather, fabrics, etc. up to 60 days against the Covid-19 Virus.

Legionella-X Viral Shield is a water-based broad-spectrum self-disinfecting coating containing synergistically blend of twin-chain quaternary ammonium compound, colloidal silver and copper nanoparticles, and another proprietary compound.

Legionella-X Viral Shield Self-Disinfecting Coating inactivates viruses by denaturation and reactive oxygen specimen (ROS) [33]. It employs said dual mechanisms to inactivate both viruses and bacteria.

Its residual inactivation efficacy of 60 days was validated using JIS Z 2801:2010/A1:2012 Antibacterial Activity. The said test calls for antibacterial activity and efficacy of antibacterial products on plastic products, metal products, ceramics, excluding textiles and the photocatalyst products. The said standard is corresponding to ISO 22196:2007 for the measurement of antibacterial activity on plastic surfaces [34,35,36,37,38]. The details of JIS Z 2801:2010/A1:2012 is herein described below.

Testing method

Bacteria to be used for the test

The species of bacteria to be used for the test shall be as follows, and the test shall be carried out on the respective bacteria.

a) Staphylococcus aureus
b) Escherichia coil

Examples of bacterial strain to be used for the test are shown in table 1. If the bacterial strain is contributed by the agency of culture collection other than that shown in table 1, it shall be obtained from member agencies of World Federation for Culture Collections (WFCC) or Japan Society for Culture Collections (JSCC), and it shall be the bacterial strain of the same series as that shown in table 1.

Table 1 Bacterial strain used for the test

Type of bacteria	Preservation number of bacterial strain	Agency of culture collection
Staphylococcus aureus	ATCC 6538P FDA 209P NBRC 12732 CIP 53.156 DSM 346 NCIB 8625	American Type Culture Collection Food and Drug Administration Bioresource Information Center, Department of Bio-technology of National Institute of Technology and Evaluation Collection des Bacteries de l'Institu t Pasteu r Deutsche Sammiung von Mikroorgs.nismen und Zellkulturen Gmbh National Collection of Industrial and Marine Bacteria Ltd.
Escherichia coli	ATCC 8739 NBRC 3972 CIP 53.126 DSM 1570 NCTB 8545	American Type Culture Collection Bioresource In formation Center, Department of Bio-technology of Nations I Institute of Technology and Evaluation Collection des Bacteries del'insti tut Pasteur Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh National Collection of Industrial and Marine Bacteria Ltd

Preparation of test inoculum

One platinum loop of bacteria of the test bacteria preincubated in a) shall be dispersed evenly in a small amount of 1/500 NB, and the bacteria concentration shall be estimated with direct microscopic observation or other appropriate methods. This inoculum shall be diluted with 1/500 NB appropriately and adjusted so that the bacteria concentration becomes 2.5 x 10.^{5} to 10 x 10^5 cells/ml, and this shall be used as the test inoculum. if the test inoculum is not used immediately, it shall be cooled on ice (0°C) and shall be used within 2 h after storage.

The inoculation with test inoculum shall be as follows.

1) Each test piece shall be placed in a sterilized petri dish making the test surface up. The test surface shall be the surface of the product on which antibacterial treatment is performed. Even when the antibacterial treatment is processed to depth, the cross-section shall not be used as the test surface.

2) Exactly 0.4 ml of test inoculum of shall be taken with a measuring pipette and instilled onto each test piece in the petri dish. The volume of inoculated inoculum on the test piece whose size is other than the standard size shall. be proportionally divided by the ratio of the area of covering film. Even if the test piece is of the standard size when the volume of inoculum based on the provision is inoculated on the test piece of very good wettability such as ceramics, tile, enamel, and glass, the film may move at a small slant and the inoculum may escape from the edge of the film. In this case, the volume of inoculated inoculum may be reduced up to 1/4 of the specified volume. However, even when the volume of inoculated inoculum is reduced, the bacteria concentration inoculated on the test piece shall he 6.2 X 10^3 to 2.5 x 10^4cells/cm^2.

3) The instilled test inoculum shall be covered with a film; the film shall be gently pressed so that the test inoculum spreads across the film while paying attention so that it does spillover from the edge or film, and the lid of the petri dish shall be placed (see figure 1 and 2). The standard size of the film shall be the square of 40 mm ± 2 mm. If the test piece is not that of the standard size, the size shall be adjusted so that that the film can be placed within 2.5 mm to 5 mm from four sides of the test piece, but the size of the film shall not. be reduced to less than 400 mm^2. Further, if it. It is difficult to adhere to the film closely since the shape of the test piece is not flat if the test inoculum spreads over the test piece without covering the film since the test piece is hydrophilic or water-absorbent or the like, the process of covering the film may be omitted. When the covering process of film is omitted, the standard size of the test piece shall be the square of 40 mm ± 2 mm (within. 10 mm in thickness).

   On the inoculation with test inoculum if it is difficult to prevent the leakage beyond. the edges of the film on the case where the surface of the sample is very hydrophilic and the like, the volume of inoculum may be reduced tip to 0.1 ml. In this case, the concentration of bacteria cells in inoculum shall be increased to provide the same number of bacterial cells as that of inoculated inoculum of normal volume.

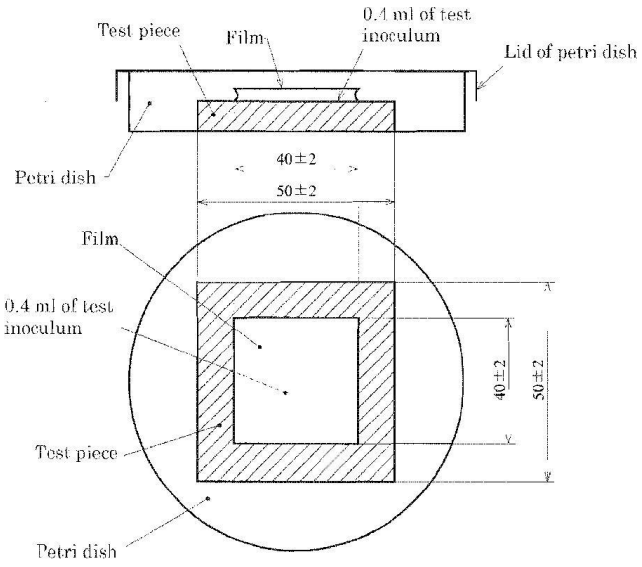

Figure 1

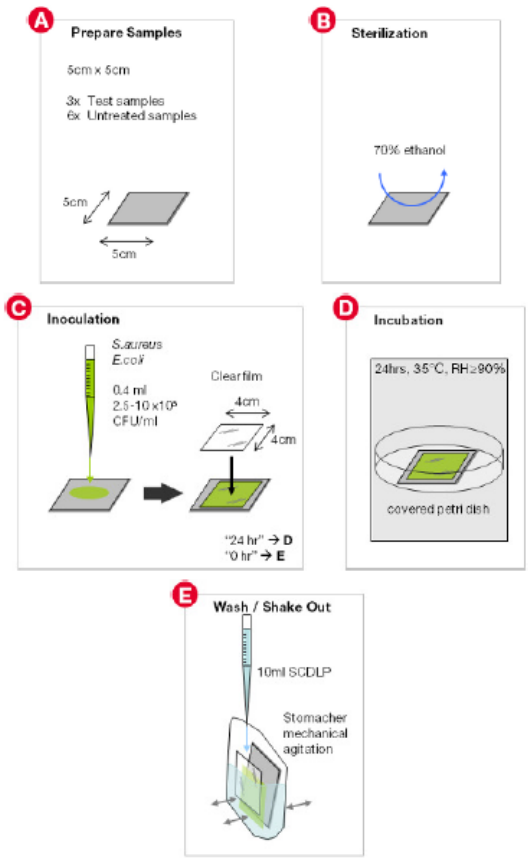

Figure 2

Incubation of inoculated test piece with test inoculum. The petri dish containing the inoculated test piece with the test inoculum (three untreated test pieces and three antibacterial test pieces) shall be incubated at a temperature of 35 °C ± 1°C and relative humidity of 90 % or more for 24 h ± 1h.

NOTE: The antibacterial effectiveness of a product is evaluated from the antibacterial activity obtained from the test at the incubation temperature specified here; however, the test at the temperature established. considering the actual use of the antibacterial product (such as room temperature) may be carried out together if agreed upon all parties concerned with delivery.

The wash-out of test bacteria inoculated shall be as follows.

1) **Test piece immediately after inoculation with test inoculum**. For three untreated test pieces immediately after inoculation with test inoculum, the covering film and the test piece shall be placed on another petri dish respectively with caution so that the inoculum does not spill. By adding 10 ml of SCDLP broth, the inoculum on the untreated test piece shall be washed out, with a measuring pipette at least four times, and this wash-out inoculum shall be recovered completely. The washings shall be immediately proceeded to the measurement of the number of viable bacteria.

2) **Test piece after incubation**. For the test piece after the incubation of the test bacteria shall be washed out. The washings shall be immediately proceeded to the measurement of the number of viable bacteria.

3) **For the wash-out of test bacteria** the method such that the covering film and the test piece are placed in a sterilized stomacher pouch by using sterilized tweezers with caution so that the inoculum does not spill, 10ml of SCDLP broth is added with a measuring pipette and the test piece and the covering film are kneaded sufficiently with hands or an extractor (such as stomacher) for the microbial test is applicable. Or if other methods show a recovery rate equivalent to or superior to the method. above, such methods may be used. If it is difficult to wash out the test bacteria with 10

ml of SCDLP broth because of the size and characteristics of the test piece, the volume may be increased.

Measurement of the number of viable bacteria by agar plate culture method

Exactly 1 ml of the wash-out of test bacteria inoculated shall be taken with a measuring pipette and added in a test tube containing 9.0 ml of phosphate-buffered physiological saline of and sufficiently mixed. Then, 1 ml shall be taken from this test tube with a new measuring pipette and add in another test tube containing 9.0 ml of phosphate-buffered physiological saline, and sufficiently mixed. These procedures shall be repeated to prepare 10-fold serial dilutions. 1 ml each of the washings and each dilution shall be dispensed into two sterilized Petri dishes. To each petri dish, 15 ml to 20 ml of the plate count agar warmed at 46°C to 48°C shall be added and sufficiently mixed. By placing the lids, the Petri dishes shall be left as they are at room temperature. After solidifying the culture medium, the Petri dishes shall be turned over, and incubated in the incubator at a temperature of 35°C ±1°C for 40h to 48h. After incubation, the number of colonies in a serially diluted petri dish in which 30 to 300 colonies appear shall be measured, as a rule. If the number of colonies is less than 30 in the agar plate dispensed with 1 ml of the washings, the number of colonies shall be measured for this plate. If there are not any colony formations in an agar plate, then "< 1" shall be recorded. Further, if the number of colonies is not inversely proportional to the dilution ratio since it is considered that the formation of colonies is inhibited by the effects of the antibacterial agent, the number of viable bacteria shall be determined using a method which forms colonies without being affected by the antibacterial agent with the use of an inactivating agent or dilution.

Calculation of the number of viable bacteria

The number of viable bacteria shall be obtained by counts of colonies measured according to equation (1).

$$N = \frac{C \times D \times V}{A} \quad \text{..... (1)}$$

where,

N: number of viable bacteria (per 1 cm^2 of test piece)

C: count of colonies (average count, of colonies of two Petri dishes adopted)

D: dilution factor (that of dilution. dispensed into Petri dishes adopted)

V: volume of SCDLP broth used for wash-out (ml)

A: surface area of covering film (cm^2)

In the case where the covering film is omitted. *A* shall be the surface area (cm^2) of the antibacterial test piece or the untreated test piece.

The number of viable bacteria shall be expressed with two significant figures y. rounding off the third significant figure. When the count. of colonies, *C is* "*<].*" *C* is taken as "1", and the number of viable bacteria shall he calculated corresponding to *V*, *A, D* at that time. For example. when *V* is 10 ml, *A* is 16 cm^2, and *D* is 1, it shall be expressed as "<0.63".

Test results

The test: results shall be as follows.

a) Determination of conditions of test validation When the following three test conditions are all satisfied, the test, shall be determined to be valid. Unless all the conciliators are satisfied, the test shall be determined to be not valid, and a retest has to be carried out.

1) The following equation (2) is established for the logarithmic value of the number of viable bacteria immediately after inoculation on the untreated test piece.

$$\frac{L_{max} \_ L_{min}}{L_{mean}} \leq 0.2 \quad \text{..... (2)}$$

where,

L_{max}: maximum logarithm number of viable bacteria

L_{min}: minimum logarithm number of viable bacteria

L_{mean}: average of logarithm numbers of viable bacteria of three test pieces

2) The average of the number of viable bacteria immediately after inoculation on the untreated test piece shall be within the range of 6.2×10^3 to 2.5×10^4cells/cm^2.

3) The number of viable bacteria on the untreated test piece after 2-4 h shall be not, less than 62 cells/cm^2 for all three test pieces. When a film is used for the untreated test piece: however, the number of viable cells of

bacteria after 24h shall be not less than 6.2 x 10^2 cells/cm^2 for all three test pieces.

b) Calculation of antibacterial activity When the test has been determined to be valid, the antibacterial activity shall be obtained according to equation (3). The value shall be recorded to the first decimal place by rounding the second decimal place down. When the number of viable bacteria is "<0.63", it shall be taken as "0.63" and the average of logarithm numbers shall be calculated.

$$R=(U_t - U_0) - (A_t - U_0) = U_t - A_t \quad \text{.....(3)}$$

where,
R: antibacterial activity
U_0: average of logarithm numbers of viable bacteria immediately after inoculation on untreated test pieces
U_t: average of logarithm nu m hers of viable bacteria after inoculation on untreated test pieces after 24h
A_t: average of logarithm. numbers of viable bacteria after inoculation on antibacterial test piece after 24 h

Record of test results

The following matters shall be listed. in the test results of antibacterial products such as plastic products.

a) Number or title of this Standard
b) Commencement date of the test
c) Type, size, shape, and thickness of antibacterial-treated test piece and untreated test piece
d) Type, size, shape, and thickness of the film

Conclusion

Based on the said JIS Z 2801:2010/A1:2012 Test Method, the residual inactivation efficacy of Legionella-X Viral Shield against Positive-gram stained and Negative-Stained is 99.9998% and 99.99995% respectively.

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About the Authors

Nelson Cheng Ph.D. (Honorary Doctorate) is the founder and chairman of Magna Group, consisting of Magna International; Magna F.E. Chemical Pte., Ltd.; Magna Chemical Canada, Ltd.; Magna Australia Pvt., Ltd.; and Lupromax International Pte., Ltd.

He graduated as a marine engineer under the United Nations Development Program Scholarship and received his Doctor Honoris Causa from the University of Baja California (UABC).

He is recognized as Singapore's leading inventor and the Singaporean with the highest number of patents from the Intellectual Property Office of Singapore. He owns several World-Wide Patents including VCI Mineral Stone Paper. He has invented more than 500 chemical, lubricant and anti-corrosion products with more than 230 products assigned with NATO Stock Numbers and marketed in more than 30 countries.

He is the inventor of several technologies for corrosion protection including, Vappro VCI (Vapour Corrosion Inhibitors) and Vappro CRI (Concrete Rebar Inhibitor), Molecular Reaction Surface Technology (MRST), Colloidal corrosion inhibitors (CCI) and Heat Activated Technology (HAT).

He has written more than 120 Research Papers and Technical Journals, published in National Association of Corrosion Engineers (NACE), International Journal of Emerging Technology and Advanced Engineering (IJETAE), International Journal of Current Trends in Engineering & Technology (IJCTET), Cambridge University Press, Acedemia.edu, ResearchGate, Intech Open and co-authored several anti-corrosion books.

Nelson has received and accorded several accolades including the 2015 Winner of the Asia Packaging Award, Top 10 Most Inspiring Entrepreneur 2015, Winner of the Global

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Patrick Moe is the senior technical manager of Magna International Pte. Ltd. He has a BSc in Industrial Chemistry, Grad. Dip and MSc in Environmental Engineering.

His key responsibilities at Magna International as follows: assisting the CEO in research and development of new products, finding out customers' needs and develop customized new products, helping in synthesizing new compounds by making appropriate modifications of known methods, recommending and implementing methods to increase the quality of products and service, management of hazardous raw materials.

He has co-authored more than 55 Technical Journal on Corrosion and Lubricants. He is a member of the National Association Corrosion Engineers (NACE) and the World Corrosion Association (WCA).

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He has authored different peer-reviewed articles and a book chapter. Moreover, Dr. Beltrán has directed several researchers, granted from different government institutions. He has also served as a reviewer of different high impact journals such as Materials Science and Engineering C, Nanomedicine: Nanotechnology, Biology and Medicine, and Biotechnology and Biotechnological Equipment. His research interests are focused on Biomaterials, Tissue Engineering, Cellular and Molecular Biology, and Corrosion of Materials.

Dr. Ernesto Alonso Valdez Salas

Dr. Ernesto Alonso Valdez Salas is a passionate and renounced physician based in Mexicali Baja California. He has a medical degree and a master's degree in surgery with a specialization in gastroenterology at the Universidad Autonoma de Guadalajara, he received his of Doctor of Sciences from Universidad Autonoma de Baja California.

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 NanoMedicine 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.