

# Inactivation of Enveloped Viruses (Coronaviruses, H5N1), with Legionella-X Range of Chemical Disinfectants

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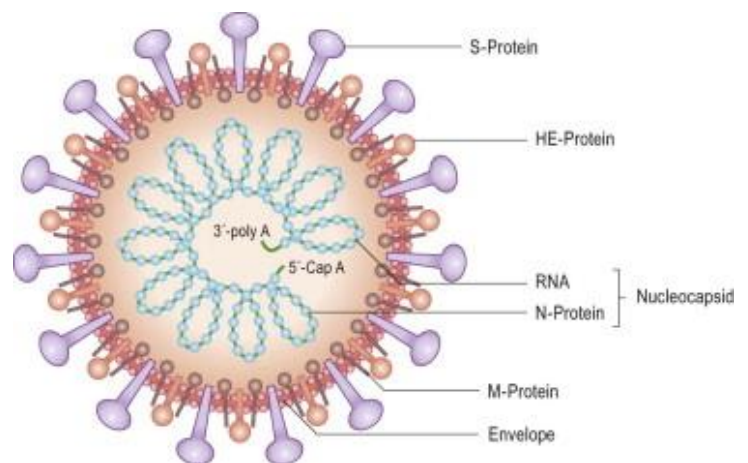
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**Abstract**— In November 2002 the Severe acute respiratory syndrome induced by coronavirus (SARS-CoV) was first identified in China. It caused a global outbreak with 8,098 probable cases including 774 deaths [1]. World Health Organization (WHO) review comprehensive protocol for cleaning and disinfection of hospitals and other settings after the occupation of people with Severe acute respiratory syndrome [2]. In view of the above, the Legionella-X range of disinfectants [3] was developed to combat the Enveloped Viruses such as Coronavirus and Influenza Viruses. Legionella-X disinfectant contains synergistic chemical composition to inactivate enveloped viruses by denaturation of the protein of Coronaviruses and Influenza Viruses (H5N1). This article covers the synergistic chemical used and the mechanism involved inactivating the above-said viruses. Complete formulation with blending procedures and the inactivating efficacy of an Enveloped Virus using H5N1 are also furnished in this article.

**Keywords**- Enveloped Virus, Coronavirus, H5N1 Virus, Legionella-X Disinfectants, Quaternary Ammonium Compound, MERS, SARS

**INTRODUCTION**- Coronaviruses (CoVs) are enveloped positive-sense RNA viruses, associated with the subfamily Coronavirinae and are

characterized by club-like spikes that protrude from their surface (see picture 1).



Picture 1

It has an exceptional large RNA genome, and an uncommon replication strategy [4]. Coronaviruses (CoVs) are the largest group of viruses belonging to the Nidovirales order, which includes Coronaviridae, Arteriviridae, and Roniviridae families [4]. The Coronavirinae comprise one of two subfamilies in the Coronaviridae family, with the other being the Torovirinae.

They are a comprehensive classification of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute

Respiratory Syndrome (SARS-CoV). Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known coronaviruses are circulating in animals that have not yet infected humans [5].

The Coronavirinae are further subdivided into four groups, the alpha, beta, gamma and delta coronaviruses. The viruses were initially sorted into these groups based on serology but are now divided by phylogenetic clustering.

Alpha, Beta, Gamma and Delta are the four main sub-grouping of Coronaviruses. There are four main sub-groupings of coronaviruses, known as alpha, beta, gamma, and delta [6].

Two strains of human coronavirus, 229E and OC43 [7] are known to cause close to 25% of colds that exhibit symptoms similar to those caused by the rhinoviruses (e.g. runny nose, sneezing, and cough). However, recent zoonotic strains of coronavirus characterized by species-jumping from animals to humans have gained notoriety and become of particular concern over the past decade.

The SARS-CoV (Severe Acute Respiratory Syndrome coronavirus) outbreak of 2002-2003 originated in bats and spread indirectly to humans via intermediate animals (e.g. civet cats) [8] From the earliest reported cases in southern China, the virus eventually spread to 28 countries over the course of eight months; thousands are believed to have been infected and 774 deaths were reported .

SARS-CoV is thought to be transmitted most readily by respiratory droplets (droplet spread) produced when an infected person coughs or sneezes.

The **virus** also can spread when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eye(s)

More recently, the MERS-CoV (Middle East Respiratory Syndrome Coronavirus) outbreak originating in Saudi Arabia in April of 2012 has made headlines due to its high mortality rate of 45% and rapid spread to 9 countries (6); clusters of cases have

continued to be reported in the Middle East through the end of 2013 [9].

The transverse species from bats to become endemic in humans, coronaviruses 229E and OC43 are proliferate from human-to-human person by way of contaminated aerosols. However, the potentiality for transmission from tainted fomites remains of concern as proven by the continued activity of strain 229E more than three hours after drying onto porous and non-porous materials, including aluminum and sterile sponges; strain OC43 remained infectious up to one hour after drying on the same surfaces [10]. The SARS-CoV virus is infectious and its incursion rate is approximated to range from 10%-60%. Predominantly, some victims are considered great spreaders with the capability to spread the disease to large number patients (usually more than 4), with some reports documenting transmission of the virus to more than 100 contacts. Despite of the fact that steroids and ribavirin have been used empirically for therapy, no efficacy data from controlled studies exist to prove that either drug affects outcome favorably.

The zoonotic SARS coronavirus strain desiderated both respiratory and intestinal replication routes for human hosts. A contemplative study was carried out on 138 patients infected with SARS-CoV found that almost 40% of patients developed diarrhea [14] and that SARS-CoV genomic material was detectable in the stool of patients for more than 10 weeks after onset of the initial illness.

Environmental transmission of coronaviruses via fomites and liquids can be curtailed given the proper application of disinfection protocols [10].

In view of the above, a complete range of Legionella-X high-level disinfectants capable of killing most pathogens, including all types of viruses, vegetative bacteria, mycobacteria, and bacterial spores, were developed to meet each unique application of inactivating Enveloped Viruses, bacteria using synergistic chemical composition of Quaternary Ammonium Compound, surfactants, alcohol, essential oils and a synergistic chemical composition of Chlorhexidine Gluconate, surfactant, essential oil and water [15,16].

## How does SARS transmit?

Most coronaviruses spread the same way other cold-causing viruses do, through infected people coughing and sneezing, by touching an infected person's hands or face, or by touching things such as doorknobs that infected people have touched

SARS is transmitted principally by close human-to-human contact. In the context of SARS, close contact means having cared for or lived with someone with SARS or having direct contact with respiratory secretions or body fluids of a patient with SARS. (Examples of close contact include kissing or hugging, sharing eating or drinking utensils, talking to someone within 3 feet, and touching someone directly [12].

Close contact does not include activities like walking by a person or sitting across a waiting room or office for a brief time.) The virus that causes SARS is transmitted by the spread of respiratory droplets produced when an infected person coughs or sneezes [12]

When a person coughs or sneezes, small amounts of fluid are propelled for about 3 feet through the air and land on the mouth, nose or eyes of persons who are nearby. The virus also can spread when a person touches a surface or object contaminated with these infectious droplets and then touches his or her mouth, nose, or eyes. It is possible that the SARS virus might spread more broadly through the air (airborne spread) or by other ways that are not now known [12].

## Combating Coronavirus and H5N1 Virus through Disinfection.

Human coronaviruses were first identified in the mid-1960s. The seven coronaviruses that can infect people are: Common human coronaviruses 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS) SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS) 2019 Novel Coronavirus (2019-nCoV) [19].

The H5N1 highly pathogenic influenza viruses' subtype have infected more than 600 people since 1997, resulting in the deaths of approximately 60% of those infected. [20] The influenza A viruses circulating in avian species rarely infect humans. However, since 1997, highly pathogenic avian influenza viruses of the H5N1 subtype have infected more than 600 people. Infection of humans with these viruses typically leads to severe respiratory disease that often progresses to multiorgan failure; approximately 60% of confirmed cases of highly pathogenic H5N1 influenza infection have resulted in death. The first fatal infections of humans with highly pathogenic avian H5N1 influenza viruses were reported in Hong Kong in 1997 [25].

Since their emergence in the late 1990s, highly pathogenic avian H5N1 influenza viruses have undergone multiple reassortment events with avian influenza A viruses of different subtypes, including H6N1, H9N2 and H5N1 [26,27,28,29,30,31]. Hence, the currently circulating highly pathogenic H5N1 viruses represent a diverse group of viruses. Moreover, the viral surface glycoprotein HA (the major viral antigen), has evolved through point mutations, leading to several genetically and antigenically distinct clades and subclades.

The major clades circulating during the past years include clades circulating in Egypt, Israel, the Gaza strip and the West Bank, circulating in China, Bangladesh India and circulating in Indonesia. [8,9,10]. Although genetically and antigenically diverse, highly pathogenic avian H5N1 viruses share the ability to cause high mortality in poultry and infect humans. Recently, the HA gene of highly pathogenic avian H5N1 influenza viruses of clade 2.3.4.4 has reassorted with the neuraminidase (NA) and other viral genes originating from different avian influenza viruses, giving rise to novel viruses of the H5N2, H5N6 and H5N8 subtypes.

Many studies have assessed the virulence and pathogenicity of highly pathogenic avian H5N1 influenza viruses in different cell types and animal models including chickens, ducks, mice, guinea pigs, ferrets, pigs and nonhuman primates (reviewed in [11,12]). Mice are typically used to assess the virulence and immunogenicity of influenza viruses because they are inexpensive and multiple immunological reagents are available.

However, mice are not a natural host of influenza viruses and typically do not transmit viruses. Ferrets infected with influenza viruses show signs of respiratory infection like those observed in humans, and influenza viruses can transmit among ferrets via respiratory droplets.

For decades it was assumed that infectious diseases were spread primarily by the airborne route or through direct patient contact, and the surrounding environment played little or no role in disease transmission. Up until 1987 the Centers for Disease Control and the American Hospital Association focused on patient diagnosis due to the belief that nosocomial infections were not related to microbial contamination of surfaces (21). Over the years of studies have changed the perspective on viral transmission to include a more complex multifactorial model of disease spread. There is now growing evidence that contaminated fomites or surfaces play a key role in the spread of viral infections (22, 23, 24).

Disinfection define as a process that eliminates innumerable or all pathogenic microorganisms, excluding bacterial spores, on inanimate objects. In health-care settings, objects usually are disinfected by liquid chemicals.

The length of survival of Coronaviruses ranges from 24 to 72 hours on fomites and in stool samples; Up to 72–96 hours on dry inanimate surfaces [17]. Hence chemical disinfectants need to be employed to disinfect all fomites to prevent infection.

Chemical disinfectants are basically classified into 3 classes according to their mechanism of action—denaturants, reactants, and oxidants [18]. Denaturants, such as quaternary ammonium compounds, phenolics, and alcohols, execute by disarraying protein and lipid structures, making these products especially potent against lipophilic enveloped viruses [18].

This article covers only denaturant disinfectant. It relates to the composition of a fast-acting disinfectant against the highly pathogenic Enveloped Virus including H5N1 and Coronaviruses by synergistically blending a major portion alcohol with minor portion of twin-chain quaternary ammonium compound, essential oil, non-ionic surfactant, and solvent. The use of

synergistic combination of twin-chain quaternary ammonium compound, ethyl alcohol or methyl alcohol, non-ionic surfactant, essential oil and water as a fast-acting disinfectant to combat the current high lethality and virulence H5N1 and Coronavirus Virus have been an area of considerable research and investigation of the said authors.

### **The Mechanism of the Inactivation of the Viruses is herein described.**

The Legionella-X range of disinfectants is highly effective against enveloped viruses enveloped in a lipid coat. The detergent used in Legionella-X disinfectant interrupts the interactions between the molecules in the virus's lipid coating. Most enveloped viruses cannot exist without their lipid coating so are destroyed when exposed to these detergents. Other viruses may not be destroyed but they are unable to reproduce rendering them non-infective. The detergent used in the formulation creates an environment in which the aggregation reaction between the lipid coat and the detergent happen more rapidly. The detergent typically used is nonyl phenol non-ionic surfactant. The denature alcohol used in formulation facilitates the denaturation of the haemagglutinin (HA) proteins (see figure 2). There is a 100% viral death achieved using this chemical formulation.

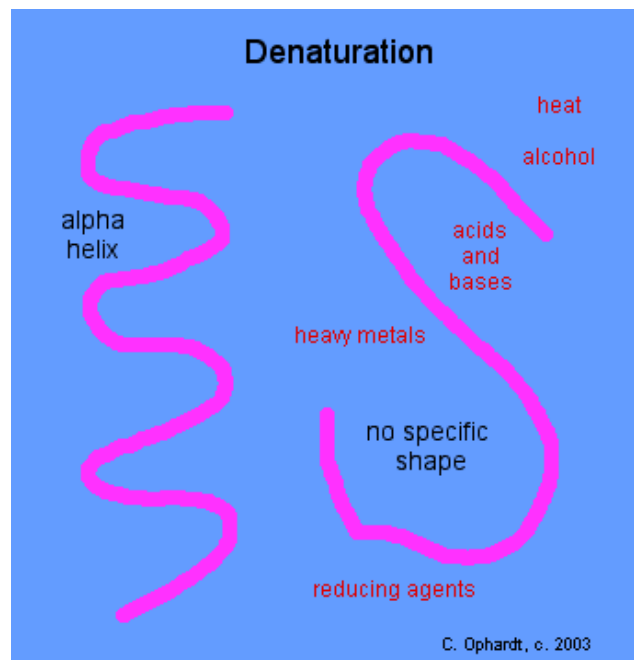


Figure 2

## **The Synergistically Chemical Composition of Legionella-X is herein described**

The composition of a fast-acting disinfectant against the highly pathogenic Enveloped Coronavirus and H5N1 is a synergistic mixture of a major portion of alcohol with minor portion of twin-chain quaternary ammonium compound, essential oil, non-ionic surfactant, and solvent. The use of synergistic combination of twin-chain quaternary ammonium compound, ethyl alcohol or methyl alcohol, non-ionic surfactant, essential oil and water as a fast-acting disinfectant to combat the current high lethality and virulence Enveloped Coronavirus and H5N1 Virus has been an area of considerable research and investigation of the said authors.

The chemical composition that effectively inactivate Enveloped Coronavirus and H5N1 virus, consists of synergistic combination of twin-chain quaternary ammonium, alcohol, non-ionic surfactant, essential oil, and water. It includes the use of anti-viral agent using Quaternary Ammonium Compound[32,33,34]-Didecyl Methyl Ammonium Chloride, Ethyl Alcohol [35] as a synergistic anti-viral and antimicrobial agent, Alcohol Ethoxylate [36] as the cleaning and emulsifying agent, peppermint or spearmint [37,38,39] to further enhance the synergistic effect of the anti-viral properties, water [40]to enhance the solubility of above said chemicals and to create an exothermic reaction [40]with alcohol [40] to further enhance the solubility essential oil in said composition.

Avian influenza is an infection caused by avian (bird) influenza (flu) viruses. These influenza viruses occur naturally among birds. Wild birds worldwide carry the viruses in their intestines, but usually do not get sick from them. However, avian influenza is very contagious among birds and can make some domesticated birds, including chickens, ducks, and turkeys, very sick and kill them [41,42]

Infected birds shed influenza virus in their saliva, nasal [43] secretions, and feces. Susceptible birds become infected when they have contact with contaminated secretions or excretions or with surfaces that are contaminated with secretions or excretions from infected birds. Domesticated birds may become infected with avian influenza virus through direct contact with infected waterfowl or other infected

poultry, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus.

Usually, “avian influenza virus” refers to influenza A viruses found chiefly in birds, but infections with these viruses can occur in humans. The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans.

However, confirmed cases of human infection from several subtypes of avian influenza infection have been reported since 1997. [44] Most cases of avian influenza infection in humans have resulted from contact with infected poultry (e.g., domesticated chicken, ducks, and turkeys) or surfaces contaminated with secretion/excretions from infected birds.

During an outbreak of avian influenza among poultry, there is a possible risk to people who have contact with infected birds or surfaces that have been contaminated with secretions or excretions from infected birds.

Of the human cases associated with the ongoing H5N1 outbreaks in poultry and wild birds in Asia and parts of Europe, the Near East and Africa, more than half of those people reported infected with the virus have died. Most cases have occurred in previously healthy children and young adults and have resulted from direct or close contact with H5N1-infected poultry or H5N1-contaminated surfaces. In general, H5N1 remains a very rare disease in people. The H5N1 virus does not infect humans easily, and if a person is infected, it is very difficult for the virus to spread to another person.

Nonetheless, because all influenza viruses have the ability to change, scientists are concerned that H5N1 virus one day could be able to infect humans and spread easily from one person to another. Because these viruses do not commonly infect humans, there is little or no immune protection against them in the human population. If H5N1 virus were to gain the capacity to spread easily from person to person, an influenza pandemic (worldwide outbreak of disease) could begin.

No one can predict when a pandemic might occur. However, experts from around the world are watching the H5N1 situation in Asia and Europe very closely and are preparing for the possibility that the virus may

begin to spread more easily and widely from person to person.

Due to the high lethality and virulence of H5N1 Virus, its endemic presence, its increasingly large host reservoir, and its significant ongoing mutations, the H5N1 virus is the world's largest current pandemic threat. The world is teetering on the edge of a pandemic that could kill a large fraction of the human population.

In view of the above, the authors have researched and invented a highly effective disinfectant to protect those who are exposed to the pathogenic Coronaviruses and H5N1 virus through direct or direct contact with the said viruses.

The authors have discovered the chemical composition of a high efficacy disinfectant against Enveloped Coronavirus and H5N1 Virus by synergistically blending a major portion of alcohol and a minor portion of twin-chain quaternary ammonium compound, Spearmint and Peppermint essential oil and alcohol ethoxylate surfactant with water.

In an embodiment the ethyl alcohol ( $C_2H_5OH$ ) or methyl alcohol is used as a co-disinfectant.

In another embodiment of the invention Didecyl Dimethyl Ammonium Chloride is used as the main disinfecting agent against H5N1 Virus and Coronavirus.

In yet another embodiment of the invention alcohol ethoxylate surfactant is used as cleaning agent.

In yet another embodiment of the invention the peppermint essential oil is used a synergistic co-disinfectant.

In yet another embodiment of the invention water is used as co-carrier and to increase the solubility properties of the synergistically blended chemical raw materials. It is also used to create an exothermic reaction with alcohol during manufacturing process to enhance the solubility of the essential oil in the said chemical composition.

The foregoing embodiments are susceptible to considerable variation in its practice, limited to the specific exemplifications set forth hereinabove. Rather, the foregoing embodiments are not within the spirit and scope of the appended claims, including the equivalents thereof available as matter of law.

The authors' intent is to dedicate all disclosed embodiments to the public to save lives should a pandemic strikes the globe.

### **Detailed Description of Blending Procedure is herein described.**

1) A chemical composition of a fast-acting disinfectant and cleaner against highly pathogenic H5N1 Virus comprising of all the steps of a to f sequentially:

a. First mixing alcohol and solvent in the mixing tank to create an exothermic reaction for the purpose of enhancing the solubility of oil based chemical product in said mixture.

b. Second adding quaternary ammonium compound in said mixture to increase the anti-viral and antimicrobial properties of said mixture at a temperature range of 32°C to 35°C.

c. Third adding spearmint and peppermint essential oil to said mixture to further enhance the synergistic effect of the anti-viral and antimicrobial properties of said mixture at a temperature range of 32°C to 35°C.

d. Fourth adding alcohol ethoxylate surfactant to increase the cleaning properties of said mixture at a temperature range of 32°C to 35°C.

2) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus mentioned in 1a, wherein the alcohol used is Ethyl Alcohol or Methyl Alcohol is added to the mixing tank containing solvent at temperature range of 20°C to 25°C, and stir said mixture for 5 to 10 minutes to allow exothermic reaction to take place till the temperature of the said mixture reaches 32°C to 35°C before adding the quaternary ammonium compound, essential oil and non-ionic surfactant is Alcohol Ethoxylate is added to the said chemical composition.

3) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as in mentioned point 2, wherein the alcohol used is Ethyl Alcohol  $C_2H_5OH$  to enhance the synergistic effect of the anti-viral and antimicrobial properties.

4) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in 1b, wherein the quaternary ammonium compound is twin-chain quaternary ammonium compound is added to the above said chemical composition at 32°C to 35°C to increase its anti-viral properties of the chemical composition.

5) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 4, wherein the twin-chain quaternary ammonium compound used is Didecyl Dimethyl Ammonium Chloride due to its high viral efficacy against H5N1 virus.

6) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in 1c, wherein the Essential Oil is Peppermint or spearmint is added to the said chemical composition at 32°C to 35°C and allowed to mix for 10 to 15 minutes to further enhance the synergistic effect of the anti-viral and antimicrobial properties of said composition.

7) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in 1d, wherein the non-ionic surfactant is Alcohol Ethoxylated at a temperature range of 32°C to 35°C to increase the cleaning and emulsifying properties of said chemical composition.

8) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in 1a, wherein the solvent used is water due to its excellent solvency for said chemical composition and to create an exothermic reaction with alcohol to enhance the solubility of the essential oil.

9) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 8, wherein the water content ranges from 10% to 80% wt/wt.

10) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 3 wherein the alcohol ethyl alcohol is used due to its excellent compatibility with said chemical composition.

11) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 10, wherein the composition content of ethyl alcohol ranges from 10% to 80% wt/wt.

12) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned point 5 wherein the composition content of Didecyl Dimethyl Ammonium Compound ranges from 0.5% to 5% wt/wt.

13) A chemical composition of a fast acting-disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 6 wherein the essential oil used is peppermint to enhance the anti-viral and antimicrobial properties of the said composition.

14) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus H5N1 Virus as mentioned in point 13

wherein the peppermint used is *Mentha Peperitia* due to its excellent

15) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 13 wherein the content composition of the peppermint ranges from 0.1% to 5% wt/wt.

16) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 1 wherein the composition prepared comprising:

- a) 10-80 wt % Ethyl Alcohol
- b) 0.5- 5 wt % of Quaternary Ammonium Compound
- c) 0.1- 3 wt % of Essential Oil
- d) 0.5- 3 wt % Alcohol Ethoxylate
- e) 10- 50 wt % Solvent

17) A chemical of a fast-acting disinfectant and cleaner against pathogenic Coronavirus and H5N1 Virus as mentioned in point 16 wherein the composition prepared comprising:

- a) 70% of Ethyl Alcohol
- b) 1.5% of Quaternary Ammonium Compound
- c) 4% of Essential Oil
- d) 2.5% Alcohol Ethoxylate
- e) 22% of water

In accordance with this journal article, its' objective is to provide the test procedures to ascertain the disinfectant efficacy of said disinfectant against Enveloped Virus such as Avian Influenza H5N1 Virus.

A sample of Legionella-X disinfectant was sent to Division of Pathology, Department of Clinic, Reproduction Pathology, Pathology Faculty Veterinary Medicine Bogor Agriculture University, Republic of Indonesia for testing its efficacy against H5N1 Virus. [27,28,29,30,31,32]

**The method and test procedures are herein described.**

The purpose of the test is to ascertain the efficacy of Legionella-X disinfectant against Enveloped Avian Influenza H5N1 virus.



## 1.1 Observation Parameter

The test parameter is based on the observation of the percentage of death virus after the introduction of said invention/disinfectant.

## 2.0 Test Method [27,28,29,30,31,32]

Using Isolated AI H5N1 virus from Tasikmalaya 2005, collected from Microbiology Department of Faculty of Veterinary, Bogor Institute of Agriculture (IPB). The test was done in the unit of the Integrated Services of Medicine of Microbiology, Faculty of Veterinary IPB. Five live egg embryos Specific Pathogen Free (SPF) were used as medium for the test.

The haemagglutination-inhibition (HAI) assay is a traditional method for assessing immune responses to influenza virus haemagglutinin (HA) and for identifying influenza virus field isolates. The HA protein on the surface of influenza virus agglutinates erythrocytes.

## 2.1 Test Procedure [44,45,46,47, 48,49]

Three components were used; AI H5N1 Virus, Live Egg Embryos and said disinfectant 100% concentration. The preparation of the disinfectant solution was done by diluting 1 part of said disinfectant to 1part distilled water by weight. Subsequently, 2 ml of AI H5N1 Virus ( $10^9$  EID<sub>50</sub>) was introduced in to the said disinfectant solution and then the mixture was incubated for 15 minutes at 37°C.

0.2 ml of said mixture was then injected into 11 days old live embryo via allantois and kept in the incubator at 37°C, observation was then carried out daily till the death of embryo. The liquid of the allantois was taken out for rapid test, HA/HI using AI standard serum.

## 3.0 Results

Based on observation all the embryos died two days after the introduction of the mixture of said disinfectant and H5NI Virus. The liquid from the allantois of the death embryo was then taken for rapid test HA/HI using AI standard serum.

The results as tabulated below:

**Table1. The Efficacy Result of said invention/disinfectant against AI H5NI Virus**

Concentration	Dilution	Percentage of Inactive Virus AI H5NI (C
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100	1:1	100
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Based on Table 1, the said invention/disinfectant has the ability to inactivate 100% of AI H5NI virus.

## 4.0 Conclusion

Based on the said efficacy test, the said invention/disinfectant is highly effective against Avian Influenza H5N1 Virus.

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