## The role of an innovative disinfection system based on silver and hydrogen peroxide in infection prevention

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**Abstract:** Due to the current pandemic situation caused by SARS-CoV-2 the need of effective precautionary methods is increasing. Besides the transmission of this virus by aerosols induced to air, it is assumed that the transmission route of SARS-CoV-2 is mainly by contaminated surfaces. It has been demonstrated that viruses can contaminate dry surfaces and can be further transmitted to the host even after extended time. The amount of disinfection and hygiene systems has increased drastically over the recent year. Although, the conventional disinfection method via spraying and wiping is labour intensive and efficacy is dependent on the application. Aim of this study was to improve conventional disinfection methods. This new disinfection system based on hydrogen peroxide and silver nanoparticles displays a quick and effective alternative. The composition which was proposed in this study shows unique features in terms of application, health risk and effectivity. The novel application by vaporization helps to disinfect the environment and even the air to reduce virus spreading. New disinfection formulation shows efficacy on the surface proteins and genetic information of the virus. Integration of the effective disinfection method shown in this study in the current precaution measurements will help to reduce the spread of SARS-CoV-2.

### **1** Introduction

Disinfection and regularly hygiene methods are the most important factor in combating transmissible diseases. The current pandemic situation caused by SARS-CoV-2 has put those factors even more in perspective. Being a respiratory virus, SARS-CoV-2 can be transmitted via direct contact with infected people or via indirect contact through the saliva droplets of infected persons. When an infected person sneezes, coughs or speaks the virus can be transported through aerosols and persist on nearest surfaces [1].

Surfaces such as door handles, desks, cell phones or surfaces in public transportation can be contaminated and transmit the virus [2,3]. Thus, the transmission is untransparent

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and the virus spread followed by infections, is increased. Although, many studies on virus transmission on surfaces have been performed, the transmission time on hard surfaces can vary and the data on the survival of SARS-CoV-2 on surfaces is still missing. It has been shown that the virus can persist on dry surfaces up to 21 days [2–4] and moreover, viability in aerosols can last up to 3 hours [1].

This highlights the need of effective disinfection measurements. To combat this virus and other pathogens, the disinfection must be fast, effective and easy to apply. Current disinfection methods are mainly based on alcohol and can be applied via spray and swipe application. In this study, a novel disinfection method based on cold fogging of a suspension of silver nanoparticles and hydrogen peroxide was established.

#### 2 Mode of action of silver nanoparticles and hydrogen peroxide

Silver has been used as an antimicrobial agents since ancient times. The activity of silver is based on interaction with sulphate and phosphor groups of structures in microorganisms which are present in viruses and bacteria [5]. Due to the size of nanoparticles, the surface to volume ratio is highly increased. This leads to a higher and altered reactivity of the particles.

During the recent years, antiviral activity of silver nanoparticles against various viruses including SARS-CoV-2 has been described [6-10]. The silver nanoparticles can bind to the surface proteins of viral envelopes and thus, inhibit the binding of them to the receptors presented on host cells [11]. Moreover, silver nanoparticles can penetrate the viral envelope and bind to its genetic information (DNA or RNA) [12]. Due to this binding the viral replication cycle is impaired.

The addition of hydrogen peroxide leads to the increased release of silver ions and helps to stabilize the efficacy of the silver nanoparticles against viruses [13]. Moreover, hydrogen peroxide itself can bind to the viral shell and disturbs the integrity of it [14]. Even the application of silver nanoparticles in the combat of the pandemic caused by SARS-CoV-2 has been described so far [9,13].

The efficacy on bacteria depends as well on the binding to sulphate and phosphate groups [15]. Due to this, the silver nanoparticles can bind to the bacterial cell wall and disturb its integrity leading to apoptosis of the cell [16]. Moreover, the nanoparticles bind to proteins and enzymes of the bacteria [17]. This binding disturbs essential functions of the bacterial cell for example by binding to proteins of the respiratory system. Comparable to the activity on viral particles, the silver nanoparticle bind to the genetic information of the bacteria [18]. Thus, the bacterial cell replication is disturbed. Moreover, reactive oxygen species (ROS) are released inside the cell. Those ROS can disturb cellular functions and cell death is initiated [19].

Due to the multiple action sides of the silver nanoparticles, no resistance mechanism can be evolved. Hydrogen peroxide itself can produce ROS as well, but functions as an enhancer of the mechanisms induced by the silver particles [20].

Thus, the combination of silver nanoparticles and hydrogen peroxide is highly effective against bacteria and viruses. For both microorganisms, the combination lead to the toxic effects inactivating the microorganisms and preventing infection.

### 3 Application of cold fogging

Common disinfectants are applied via spraying and wiping afterwards. This method and its efficacy is highly dependent on the applicant. Therefore, personal conducting the disinfection must be extensively trained. But even after good incorporation, errors in disinfection can occur due to the differences of the materials. The disinfection method must

be adapted to each surface or subject, meaning the coating, the size, the form and the place in which it is presented. This makes the disinfection unique for each site of application.

In addition, spraying and wiping is quite labor extensive and the health of the workers might be impaired regarding the disinfectant applied. Application via cold fogging is a fast and easy disinfection method. By the technology of cold fogging, small aerosols of the disinfectant are produced and distributed in the environment. Those small water-based particles can reach every surface or object. The cold fogging method enables the disinfection of rooms, surfaces and the air. Due to the development of small particles the most hidden edges and grooves can be reached.

# 4 Combination of cold fogging and disinfectant based on silver nanoparticles and hydrogen peroxide

The novel disinfection based on silver nanoparticles and hydrogen peroxide presented in this study uses the advantages of cold fogging application and the composition of the disinfectant. The composition applied via fogging is easy to use and leaves no residues on the surfaces. Hydrogen peroxide itself is degraded to water and oxygen, whereas the silver nanoparticles can be integrated on the surfaces presented. Due to this fact, a long-term effect is produced on the disinfected surfaces.

Our studies have shown that this long-lasting depot can prevent surface contamination up to 8 hours after disinfection. If a viral particle is presented to the previously disinfected surface, the spread of infection is inhibited. Further, experiments performed on compatibility of the product and skin, eyes, lung cells, or mucosa have shown that there is no irritation on the different organs. This makes this disinfection method an easy-to-use tool. This disinfection method can be applied by every personal and requires almost no instruction. The cold fogging device must be applied correctly, is started and can be operated in a room without presence of the respective worker. The exposure time of 90 s per 35 m3 makes it a fast method to apply. This makes a load of disinfection material of 2 mL/ m3. This value was established during trials to gain a dense vapor without wetting the objects, and to get the disinfection efficacy as high as possible. Using this values, the disinfection method is a cheap way to disinfect multiple rooms.

# 5 Exemplarily application of the disinfection method and analysis of the residual microbes

In this study, an exemplarily proof-of-principle of the method should be evaluated. Besides laboratory experiments based on EU-norms, this study is a good approach to analyze close to reality settings.

Thus, a locker room of a volume of 30 m3 located in Remshalden, Germany, was disinfected using a Neburator® cold fogging device (IGEBA, Germany). A volume of 5 L of disinfectant composed out of a water-based suspension containing silver nanoparticles and hydrogen peroxide was filled in the tank of the device. The cold fogging device was placed near the door of the room to obtain an equal disinfection.

Before the disinfection process, surfaces and objects in the room were sampled for microbial analysis. Due to the extensive labor needed to detect viral counts, in this case the bacterial and fungal contamination was measured and analyzed. The cold fogging was conducted for 90 s and a reaction time for 30 min was set. Afterwards, the disinfection method was verified by sampling the same sites as before the disinfection. A schematic representation of the set-up of the fogging machine producing the vapor based on silver nanoparticles and hydrogen peroxide is shown in the following picture.



Fig. 1. Cold fogging of disinfection based on hydrogen peroxide and silver nanoparticles.

The samples were taken using Tryptic Soy Contact agar plates (Merck MilliPore) and sample sites were chosen randomly. Total aerobic count was determined after incubation at 32 °C for 3 days to detect bacterial contamination followed by incubation at 22.5 °C for 5 days to analyze growth of fungi and yeast. Microbial contamination was analyzed by counting colony forming units (cfu). Exemplarily sampling sites and respective microbial count before and after the disinfection are shown in Table 1.

Table 1. Sampling sites in a locker room exemplarily demonstrating the efficacy of the disinfection	ion
method by cold fogging using a product based on hydrogen peroxide and silver nanoparticles.	

Sampling site	Microbial count before disinfection [cfu/ 25 cm <sup>2</sup> ]	Microbial count after disinfection [cfu/ 25 cm <sup>2</sup> ]
Floor	Bacterial lawn,	4 cfu bacteria,
	fungal lawn	no fungi
Locker	23 cfu bacteria	0 cfu
Wall tiles	Bacterial lawn,	0 cfu
	Fungal lawn	0 clu
Water tap	65 cfu bacteria	0 cfu

As shown in the results above, almost all bacterial and fungal counts were diminished to zero after the disinfection. Although, pre-cleaning is recommended, in this room the disinfection was conducted on dirty surfaces. Nevertheless, the efficacy of the disinfection method could be proved. This example shows how effective the system works and how it can be verified easily.

The current study was performed to show the proof-of-principle method of the nebulization technique in combination with the disinfection product based on an example disinfection with subsequent bacterial count evaluation. Besides the presented results, individual studies have shown efficacy of the disinfection method against the surrogate virus of SARS-CoV-2, the bovine corona virus with activity up to 8 h after application (data not shown). This antiviral effect was described in many studies, showing that the inactivity and death of the microorganisms was due to the silver nanoparticles and hydrogen peroxide disinfection method and is not due a natural inactivation of the virus

particle on surfaces. Moreover, Dargahi et al. have shown that viral particle can persist up to 21 days on inanimate surfaces [4]. This result shows that the application of the presented cold fogging disinfection will reduce cross-contamination by having an activity time of 30 min.

### 6 Conclusion

Conventional disinfection methods have the drawback on time dependence and personal incorporation. The novel disinfection method presented in this study is based on silver nanoparticles and hydrogen peroxide. By the application using vaporization, the major disadvantages of conventional disinfection and hygiene systems can be solved. The combination of both chemicals is highly effective and can be measured by simple and direct sample taking. Application by vaporization enables the efficacy on all surfaces and objects in every room. Thus, the application is easy and fast to use. The long-term depot effect on surfaces is unique and helps to disrupt the infection chain of infectious diseases.

Although, bacteria and viruses differ in their structure, this study can be used to assume the efficacy as a combat against the SARS-CoV-2 pandemic. Current literature has indicated the activity of silver nanoparticles against SARS-CoV-2. The evaluation of the method based on cold fogging has shown that this application method is highly effective. Therefore, the method of cold fogging of disinfection based on silver and hydrogen peroxide can be used as a combat against the current situation of pandemic to apply easy and fast disinfection in every setting.

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