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Effectiveness and Safety of Ultrasonic Fogging Method with Hypochlorite Solution for Controlling Microorganisms

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Introduction

In 2020, the COVID-19 pandemic has posed immeasurable impacts on people's lives and economic activities. In early March, the balance between supply and demand of alcohol for hand disinfection began to tighten. In early April, the Ministry of Health, Labor and Welfare received an offer to supply high-concentration ethanol products from some brewers, and issued a statement "High-concentration ethanol products can be used as substitutes for the ethanol for hand disinfection only if it is unavoidable at medical institutions etc.". In addition, some organizations, companies, and local governments that own equipment for producing electrolyzed water distributed "hypochlorous acid water" to people out of good will.

Workers engaged in the food industry know well that "hypochlorous acid water" is used as an antimicrobial food additive and disinfectant, regulated in terms of available chlorine concentration and pH, which is regulated in terms of available chlorine concentration and pH, but is subject to restrictions if distributed in containers. However, many general citizens did not know the details, so took it home and used in response to a direction that "it is safe because it's a food additive." In addition, solutions with different pH, concentration, and manufacturing method were also distributed as "hypochlorous acid water."

In response, the Ministry of Health, Labor and Welfare, the Ministry of Economy, Trade and Industry, and the Ministry of Education, Culture, Sports, Science and Technology issued documents from March to June, with occasional revisions, to indicate the liquid properties and manufacturing method of hypochlorous acid water, verification of effectiveness of (weakly acidic) hypochlorous acid water against novel coronavirus, and pros and cons of its spatial fogging. In addition, contents of media reports led to confusion and anxiety in citizens. Of particular concern is the notices (office communications) from ministries and agencies without showing any scientific evidence that inhaling sprayed droplets of hypochlorous acid water is harmful, its effect is uncertain, and spraying or fogging is not recommended.

Here I use a generic term of "hypochlorite solution" for dilute solutions containing hypochlorous acid with available chlorine concentration of 10-100 mg/L regardless of the method of its production to describe basic concepts of microbial control by the ultrasonic fogging method in indoor spaces, and then discuss its safety and effectiveness in microbial control based on basic scientific data.

"Hypochlorous acid," the main active factor of chlorine disinfection

Hypochlorous acid is a disinfectant that has been used since the mid-1800s and is known as an active component of chlorine disinfection. Hypochlorous acid began to be used for hand disinfection by obstetricians and gynecologists under the name of chlorine water, which led to the confirmation of its effects for the first time[1)]. The beginning of hypochlorous acid was "being effective for hand disinfection." Currently, hypochlorous acid is commonly used as an industrial product under the name of sodium hypochlorite (NaOCl), and is used widely as the main component of household bleach, baby goods disinfectants, and bathroom mold remover.

A typical example of chlorine disinfection is tap water disinfection. It has disinfecting and microbiostatic effects on various microorganisms while it is harmless to human health, as far as residual chlorine concentration in tap water is properly controlled at 0.1-1.0 mg/L. Hypochlorous acid supports hygienic water that we can drink directly by twisting the faucet. About 3% of tap water is disinfected with chlorine gas and 97% with sodium hypochlorite. In addition, hypochlorous acid is also used to disinfect pools with residual chlorine concentration of 0.4-l.0 mg/L, thereby preventing infection due to human-derived soils and microorganisms.

Currently, the hypochlorite solutions used for cleaning and disinfecting equipment, instruments, appliances, foodstuffs, and so on in the food industry include dilute sodium hypochlorite solution, hypochlorous acid water (strongly, weakly, or slightly acidic) and alkaline electrolytic sodium hypochlorite, as well as weakly acidic hypochlorite solutions that were prepared by mechanically mixing acidic solutions or carbon dioxide gas with sodium hypochlorite solutions. The main cleaning or sterilizing component in these solutions is hypochlorite (HOCl), with greatly different efficacies of cleaning and disinfection that vary with the degrees of HOCl dissociation ($HOCl \leftrightarrows OCl^- + H^+$) depending on the pH of solutions. This means that weakly acidic solutions with larger proportion of HOCl have stronger sterilization capability, while alkaline solutions with larger proportion of OCl^- have stronger cleaning power, if we assume that the hypochlorite solutions have the same available chlorine concentration[2)]. Moreover, HOCl can easily volatilize (move from liquid phase to gas phase) by stirring, bubbling, spraying, or air-forced vaporization of the solution, since it is volatile. On the other hand, OCl^- is non-volatile and stays in the solution[3,4)]. It is necessary to fully understand these characteristics to determine the liquid properties (pH, concentration) of hypochlorite solution for ultrasonic fogging.

Basic activities for food hygiene

Thorough implementation of basic activities for food hygiene is required for effective spatial spraying of hypochlorite solution.

At manufacturing sites for general industrial products, the basic activities for an increase in the efficiency of manufacturing process are arrangement and sweeping of the work space, elimination of unnecessary items, maintenance of dirt-less conditions, and education of the manufacturing staffs. Here, clean conditions mentioned are the situation where arrangement and order are kept. These activities can be applicable to manufacturing sites in various industries, and naturally they are also effective in food factories. However, it should be noted that dirt-less conditions mentioned above refer to "visually clean" states. Microbial control measures require the cleanliness in terms of macroscopically invisible soils and microorganisms. Being unable to achieve even visually clean states is far from successful microbial control.

Microbial control technology can be roughly divided into four categories: cleaning, disinfection, microbiostasis, and separation. Cleaning is a technique that uses water as a medium to remove soils and microorganisms on equipment and devices away from the system. Disinfection is a technique that kills microorganisms. Microbiostasis is a technique to suppress the growth of microorganisms by applying the conditions that are disadvantageous for the growth of microorganisms. Separation is a technique to prevent the contact of products with harmful substances by isolating them from environmental factors. Fully using any one of these control techniques is not sufficient to achieve microbial control. The idea to control microorganisms by effectively combining the four techniques in a manner suitable for each site is necessary. These concepts were often called hurdle theory.

Where are the microorganisms to be controlled?

Spatial ultrasonic fogging with hypochlorite solution aims to control harmful microorganisms present in indoor spaces. So where are the microbes? In any spaces, the numbers of microorganisms present on "solid surfaces" are much higher than those of "airborne microorganisms". Microorganisms are most abundant on "floor surfaces" among various solid surfaces followed by the surfaces we touch by hands, which applies to both food factories and living spaces. Airborne microorganisms can be controlled by exchanging air with ventilation or supplying clean air through HEPA filters; however, countermeasures against adherent microorganisms that cannot be eliminated by simply exchanging air remain challenging. The true purpose of spatial fogging is to control surface adherent microorganisms without wetting the surface and without manual manipulation.

Controlling microorganisms does not mean sterilizing products or equipment. It means reducing the number of microorganisms to the point that they do not impair the quality of products or human health.

Two forms of hypochlorous acid in spatial fogging

The ultrasonic fogging method atomizes hypochlorite solution into fine droplets by using an ultrasonic vibrator to spray them into spaces. The fine droplets blown out from the fogger soon begin to fall downward due to gravity, and become finer within the space with the volatilization of water and hypochlorous acid. Then, they fall onto floor surfaces or float in rooms as invisible droplets. The hypochlorous acid volatilized from the fine droplets diffuses in rooms as "gaseous hypochlorous acid". Thus, the hypochlorous acid blown out

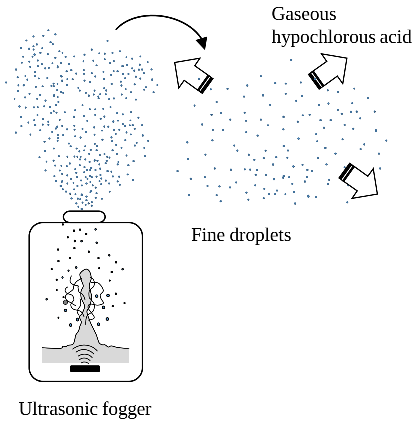

Fig. 1. Two forms of hypochlorous acid blown out from the fogger.

from the fogger exists and acts in two forms, the molecules present in fine droplets and the gaseous molecules that diffuse into the air in a room (Fig. 1).

The fine droplets (liquid phase) contain obviously higher concentrations of hypochlorous acid than those in space (gaseous phase). As such, greater disinfection can be achieved with direct contact of fine droplets, although their contact sites are limited. Although spatial density of gaseous hypochlorous acid is low, it diffuses uniformly, which allows it to come into contact with any surface in rooms. In addition, its concentration can be easily controlled.

Safety criteria and basic data

Safe utilization of the actions of hypochlorous acid in fine droplets and gaseous hypochlorous acid requires appropriate control and management of their concentrations.

Hypochlorous acid in fine droplets

Currently, no criterion for working environment is defined for liquid fine droplets. Therefore, companies dealing with hypochlorite solutions individually conduct various safety tests, such as inhalation toxicity, oral administration, skin irritation eye irritation tests, and so on, using experimental animals to confirm the safety of fogged fine dropltets[5-8].

For example, no notable changes were observed in body weight and general conditions in both male and female rats in a 90-day subchronic inhalation toxicity test, nor in hematological and lung histopathological examinations, which have been reported in academic literatures[6, 8].

Gaseous hypochlorous acid

Safety criteria for gaseous chemical substance includes the environmental criteria defined by the Industrial Safety and Health Act (Japan) and the acceptable concentration by the Japan Society for Occupational Health[9]; they are established to be 0.5 ppm (= 500 ppb) for chlorine gas (Cl_2). These concentrations correspond to those that do not pose health problems after the exposure for 8 h a day and 40 h a week. This value is premised on working environments; more specifically, it is a criterion in the field where large amounts of hypochlorite solution are used in food factories and the like. There is no criterion applicable to living environments; long-term exposure to chlorine gas with a concentration of 500 ppb is unlikely.

Currently, no working environment criterion for hypochlorous has been defined for two reasons. One of the reasons is the lack of standard gas for hypochlorous acid. The other is that chlorine gas, when reacted with water in the living body, becomes quickly converted to hypochlorous acid (eq. 1), which in turn affects the living body, making it reasonable to estimate the effects of hypochlorous acid on the living body from those of chlorine gas. In addition, the reaction in eq. 1 produces hydrochloric acid (HCl), strong acid, as a by-product, suggesting that chlorine gas has greater effects on living tissues.

$$Cl_2 + H_2O \rightarrow HOCl + HCl \qquad (1)$$

As is described in the next section, even ultrasonic fogging of dilute hypochlorite solution (10-100 ppm) does not result in the concentration higher than the criterion of 500 ppb with an equilibrium reached at much lower concentration.

Unique odor

People feel so-called "chlorine odor" in the environments of facilities where hypochlorite solutions are used. The identity of this odorous component is gaseous hypochlorous acid volatilized from the solutions. In my sense, approximately 10 ppb gaseous hypochlorous acid can be perceived by olfactory senses. It means that hypochlorous acid is much safer than odorless chemicals because the odor eliminates the worry about long-term exposure without noticing its presence at high concentrations (> 500 ppb).

Indoor concentrations resulting from ultrasonic fogging

Hypochlorite concentrations in fine droplets

The concentrations of hypochlorite ($HOCl + OCl^-$) in fine droplets decrease as the distance from the fogger increases, since the hypochlorous acid volatilizes from the fine droplets

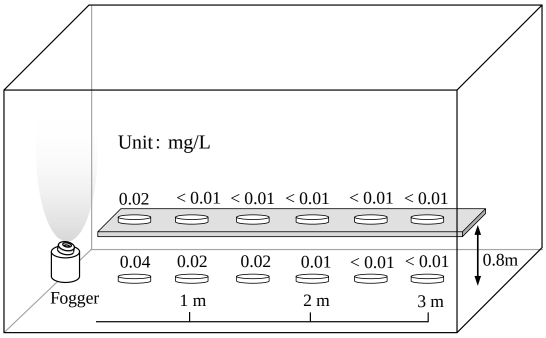

Fig. 2. Measurement of hypochlorite concentrations at different locations in a meeting room (90 m^3) due to fine droplets of hypochlorite solution generated by ultrasonic fogging.
-Meeting room: empty, closed, no air agitation.
-Hypochlorite solution: pH 5.8, 50 mg/L, 1-h fogging at 300 mL/h.

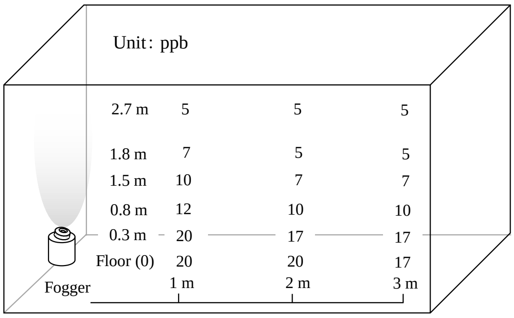

Fig 3. Measurement of the distribution of hypochlorite concentrations at different locations in a meeting room (90 m^3) due to gaseous hypochlorite generated by ultrasonic fogging.
-Meeting room: empty, closed, no air agitation
-Hypochlorite solution: pH 5.8, 50 mg/L, 1-h fogging at 300 mL/h

as described above. A straightforward method of determining the amount of hypochlorite reached is collecting fine droplets in a container of a defined size to measure available chlorine concentration, while another method is capturing hypochlorite into the solutions containing fluorescent probe reagents to measure the fluorescence intensities of the solutions[10-12].

Figure 2 shows the concentrations (mg/L) of available chlorine (HOCl + OCl$^-$) collected in pure water (10 mL) in petri dishes after hypochlorite solutions (pH 5.8, 50 mg/L) were fogged in an empty meeting room (90 m^2) for 1 h (300 mL/h) using an ultrasonic fogger (1.6 MHz). A spray port was placed 1 m above the floor, and petri dishes were placed on the floor at intervals of 0.5 m from the fogger and on a desk of 0.8 m high. Available chlorine concentrations were higher as the positions were closer to the fogger, and they were higher on the floor than on the desk. Previous studies reported that the amounts of hypochlorite reached the sites of measurements were inversely proportional to the distances[11]. The highest available chlorine concentration on the floor was 0.04 mg/L at a distance 0.5 m away from the fogger, which is less than the criterion for tap water. At the distance of 2 m or more, available chlorine concentrations were below detection limit (<0.01 mg/L). On the desk of 0.8 m high, the concentration was only 0.02 mg/L at a distance 0.5 m away from the fogger, and below the detection limit at distances of 1 m or more. These results indicate that the amounts of hypochlorite reached were higher on the floor surface because fine droplets tend to fall downward, and that the amounts reached were lower at the higher position (0.8 m). Thus, the hypochlorite in fine droplets we receive on our faces in sitting and standing positions (positions of inhalation) was extremely low concentrations and amounts when certain distances were kept from the fogger.

Concentrations of gaseous hypochlorous acid

Chlorine gas detectors equipped with controlled potential electrolysis sensors can be used for simple measurements of the concentrations of gaseous hypochlorite[13].

Figure 3 shows the distribution of gaseous hypochlorous acid concentrations (ppb [v/v]) in a room after hypochlorite solutions (pH 5.8, 50 mg/L) were fogged with an ultrasonic fogger in an empty meeting room (90 m^3) for 1 h (300 mL/h) in a condition similar to that in Fig. 2. The positions for measurement were 1, 2, and 3 m away from the fogger at various heights above the floor. The concentrations of gaseous hypochlorite were higher at the positions closer to the floor and became lower toward the ceiling. This is because volatilized gaseous hypochlorous acid molecules are adsorbed and absorbed by the fine droplets, and then volatilizes again during the fall of fine droplets toward floor surfaces. These results let me speculate that repeating this eventually would result in the formation of a gradient of hypochlorous acid concentration from the floor surface toward the ceiling. In addition, the concentration of gaseous hypochlorous acid is almost uniform regardless of distance, although it tends to be slightly higher near the fogger. From the results of this experiment, it is found that the concentrations of gaseous hypochlorous acid on our faces in sitting and standing positions (positions of inhalation) are 1/50-1/100 of the criterion concentration (500 ppb).

Concentrations of gaseous hypochlorous acid assuming excessive fogging

Figure 4 shows the changes of gaseous hypochlorous acid concentration over time in a small chamber (1 m^3), which mimicked a meeting room, after spraying a hypochlorite solution (pH 5.8, 50 mg/L) with an ultrasonic fogger (2.4

MHz) for 1 h (150 mL/h). Relative humidity reached nearly 100% after 20 minutes of operation, after which condensation began to appear on the floor, sides and ceiling of the chamber, indicating that excessively fogging state was reproduced.

The concentration of gaseous hypochlorous acid increased over time during the operation, eventually reaching an equilibrium. In this experiment, the equilibrium value was 120 ppb, which was about 1/4 of the criterion. This is because of gradual decrease of the water and hypochlorous acid levels that volatilized from the fine droplets due to vapor-liquid equilibrium resulting from the increase in relative humidity and the concentration of gaseous hypochlorous acid. Consequently, the fine droplets fell and were adsorbed to the wall surfaces of chamber while maintaining their droplet sizes at the spray port, resulting in condensation. Thus, excessive fogging does not necessarily cause continuous increase in the concentration of gaseous hypochlorous acid.

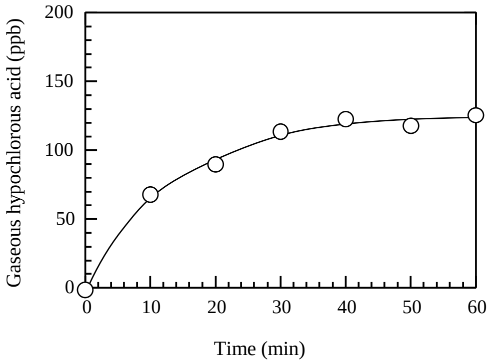

Fig. 4. Time course of gaseous hypochlorous acid concentrations in a small chamber (1 m^3) during ultrasonic fogging with hypochlorite solution.
-Chamber: empty, closed, no air agitation
-Hypochlorite solution: pH 5.8, 50 mg/L, 1-h fogging at 150 mL/h

Disinfection/inactivation effects

Hypochlorite present in fine droplets

Figure 5 shows the changes in the survival ratio of *Escherichia coli* when ultrasonic fogged fine droplets of hypochlorite solution (2-4 mg/L) adjusted to pH 6.0 and 10.2 were brought into direct contact with *E. coli* cells on the membrane filter at a distance of 0.3 m from the fogger[10]. Horizontal axis representing apparent concentration-time (*CT*) product values (mg·min/m^3) calculated from available chlorine concentrations, the amounts fogged, and air flow volume, while vertical axis representing logarithmic values of survival ratio (log N/N_0). The survival ratios decreased linearly at all pH after 20 minutes of contact (first-order reaction) with 4-log decrease. These results indicate that disinfecting effect depend on *CT* values, and that fogged fine droplets of hypochlorite solution of 2-4 mg/L, that are slightly higher than the criterion for tap water, are also effective for disinfection.

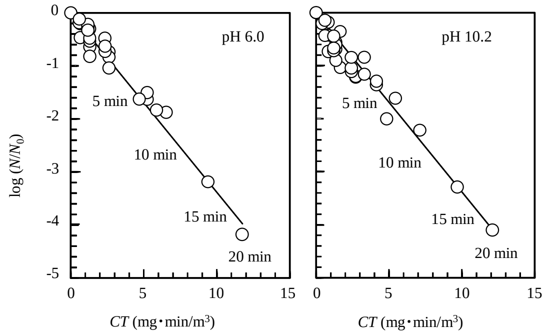

Fig 5. Inactivation of *E. coli* on membrane filters by ultrasonic fogging with pH-controlled hypochlorite solutions[10].
-Hypochlorite solution: 2-4 mg/L
-Fogging conditions: 3.0 ml/min at a flow rate of 0.01 m^3/min

In general, the disinfection effects of hypochlorite solutions depend on HOCl concentration; therefore, weakly acidic solutions (pH 6.0) exert greater disinfection effects. However, since the concentrations of HOCl decrease significantly before reaching targets to disinfect due to its high volatility, disinfecting effects of ultrasonic fogging sometimes do not differ so much between weakly acidic and weakly alkaline solutions as observed in liquid phases.

Table 1 shows the changes in infectivity titers of influenza A virus when rayon non-woven fabrics applied with the viruses were brought into direct contact with ultrasonic fogged fine droplets of hypochlorite solution (50 mg/L) adjusted to pH 6.0 and 10.2 for 10-30 minutes at a distance 0.4 m away from the fogger[12]. The infectivity titers were calculated from the numbers of plaques formed after infection to canine liver cells (MDCK cells). Initial virus infectivity titer was 6.38-log$_{10}$PFU/0.1 ml. When distilled water was used for fogging

Table 1. Inactivation of influenza A (H1N1) virus existing on nonwoven rayon fabric by ultrasonic fogging with pH-controlled hypochlorite solutions[12].

Solution	pH	FAC conc. (mg/l)	Virus titer (log$_{10}$ PFU/0.1 ml)		
			10 min	20 min	30 min
Distilled water	6	−	5.21	4.69	4.25
Hupochlorite solnution	6	50	< 1	< 1	< 1
	10	50	< 1	< 1	< 1

Initial virus titer was 6.38 log$_{10}$ PFU/0.1 ml.
Fogging conditions: 2.0 mL/min at flow rate of 0.05 m^3/min

for 10-30 minutes, logarithmic infectivity titers were reduced to 1.18-2.13. When hypochlorite solution was used for fogging, no infectivity titer was detected after fogging with solutions of pH 6.0 and 10, with the inactivating effects in reduced logarithmic infectivity titers of 4 or more obtained after the exposure of only 10 minutes or less.

The influenza virus is a single-stranded RNA virus with an envelope consisting of lipid bilayer membrane. The virus has three types of protein protrusions: hemagglutinin (HA), neuraminidase (NA), and ion channel (M2), on the surface of its envelope. The decrease in infectivity titer is due to damages to the envelope, viral RNA, and related enzymes, as well as the inhibition of the activities of HA and NA14). HOCl permeates the viral envelope and inactivates influenza virus quickly15,16). In addition, the inactivating activity of hypochlorite solutions is known to include damaging viral RNA on the basis of the results of PCR17, 18). Inactivation of the adsorption activity of HA at the initial stage of virus infection or that of the NA activity at the final stage should decrease virus infectivity titers. Hypochlorite ions (OCl^-) have strong cleansing and proteolytic effects2), thereby damaging influenza viral HA and inhibiting hemagglutination reactions (HA titer)16). Thus, OCl^- also contributes significantly to reduced infectivity titer (inactivation) of influenza virus.

Gaseous hypochlorous acid

In the experiment described herein, bacteria and viruses were brought into contact with gaseous hypochlorous acid released from hypochlorite solutions by forced-air vaporizing method in order to clearly distinguish its effects from those of hypochlorite in fine droplets.

Figure 6 shows the change in logarithmic count of viable cells of *Vibrio parahaemolyticus* (6×10^4 CFU/plate) on a wet agar plate when exposed to gaseous hypochlorous acid (25-50 ppb) for 60 minutes in a small chamber (0.3 m^3, 22°C, 72%RH) 19). The counts of viable cells decreased over time during the exposure, reaching below detection limit (<10 CFU/plate) after 30 minutes. In addition, re-plotting logarithmic counts of viable cells with *CT* values on horizontal axis showed that a 20-minute survival curve (*CT* value: approximately 150 ppb・min) can be approximated by linear fitting (data not shown), indicating that the disinfection effects of gaseous hypochlorous acid depend on *CT* values. These results indicate that gaseous hypochlorite exhibits good sterilization effects even at low concentrations.

Figure 7 shows the decrease in logarithmic infectivity titers when influenza A virus (10^5-10^6 TCID 50/mL) on petri dishes were placed on a table at a distance of 1.5 m from a forced-air vaporizing apparatus (1.2 m in height) in a 25 m^3-closed room (20°C, 50% RH) and exposed to gaseous hypochlorous acid (10-15 ppb)20). In this experimental conditions, only slight spontaneous decay of influenza virus was observed within 180 minutes (control). The exposure to gaseous hypochlorous acid resulted in a decrease of 2.4-log in 60 minutes and 4.2-log or more in 120 minutes (which is below the lower limit of detection), as compared with the control.

In forced-air vaporizing system, the amounts of hypochlorite released depend on the undissociated hypochlorous acid (HOCl) concentration of hypochlorite solutions3,4). In addition, the amount of gaseous hypochlorous acid released per unit time increases depending on the volumes of air flow4). This is because the driving force in mass transfer from liquid phase to gaseous phase is the difference between the HOCl concentrations of solutions (bulk) and gas-liquid interface. Another contributing factor is the decrease in mass transfer resistance from liquid phase to gaseous phase resulting

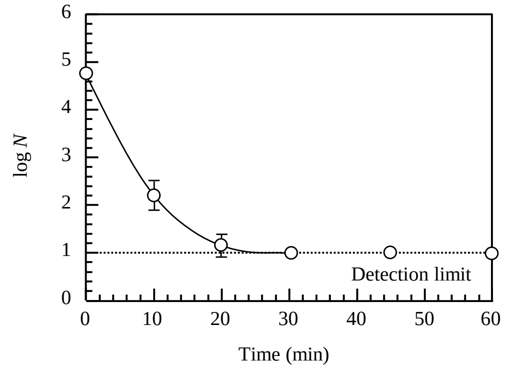

Fig. 6. Disinfection effect of gaseous hypochlorious acid against *V. parahaemolyticus* on wet agar plates19)
-Gaseous hypochlorous acid: 25-50 ppb

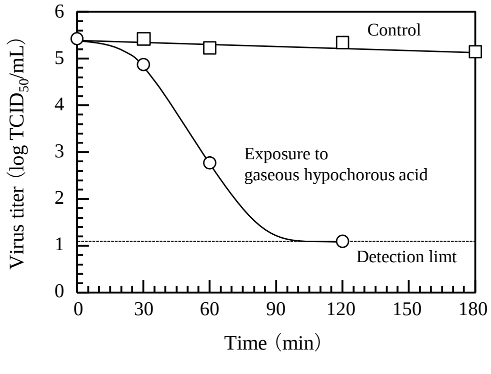

Fig. 7. Inactivating effect of gaseous hypochlorous acid against influenza A virus on dry solid surface20)
-Gaseous hypochlorous acid: 10-15 ppb

from thinner thickness of the gas-phase boundary film due to the increase in the volumes of air flow. Thus, the amount of hypochlorous acid released can be controlled by the pH and concentration of hypochlorous acid of solutions, as well as the volume of air flow.

Furthermore, the disinfection and inactivating effects of gaseous hypochlorous acid are known to increase with the increase of relative humidity21), indicating that the adjustment of relative humidity is an important factor in the control of microorganisms by gaseous hypochlorous acid in indoor spaces.

Conclusion

It has so far been considered that the transmission of novel coronavirus mainly occurs through droplets and direct contact. However, with the progress of re-examination, WHO issued a view that aerosol infection (airborne infection) cannot be completely excluded on the basis of some studies suggesting its possibility in poorly ventilated and enclosed spaces. *Mycobacterium tuberculosis* and measles virus are known to transmit through aerosol. Are the conventional measures, e.g., ventilation, hand washing, alcohol disinfection, wearing masks, and the avoidance of three types of close contact, sufficient as preventive measures against these infectious agents? Is there any other microbial control technology that can be combined based on the hurdle theory?

"Chlorine disinfection of tap water" is a world-class technology of Japan. So is the technology using hypochlorite solution. Educational and nursing care facilities from nursery schools to universities, as well as some hospitals have been using ultrasonic fogging of hypochlorite solutions. Very few accidents with clear causal relationships with hypochlorite solution have been reported. I believe that "ultrasonic fogging with hypochlorite solution" described herein coupled with conventional protective measures is an effective approach for microbial control. National and local governments should urgently compile scientific data on their safety and effectiveness as well as the research going on at home and abroad, and take measures against the second and third wave of novel coronavirus.

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