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Technical note

Evaluation of disinfection efficiency in pet's hospital by using chlorine dioxide

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ABSTRACT

Microbial aerosols could cause various human and animal health problems and their control is becoming a significant scientific and technological topic for consideration. The main objectives of this study were to monitor bioaerosol levels of the pet's hospital and then to perform disinfection efficiency by applying chlorine dioxide. The air quality within these pet's hospitals should satisfy the guidelines specified by the Taiwan Environmental Protection Administration (TEPA). Accordingly, this study performed an experimental investigation into the efficiency of two different gaseous chlorine dioxide (0.3 mg m^{-3}) treatments in disinfecting a local pet's hospital, namely a single, one-off application and a multiple-daily application. In both cases, the ClO_2 was applied using strategically-placed aerosol devices. The air quality before and after disinfection was evaluated by measuring the bioaerosol levels of bacteria and fungi. The experimental results found that the average background levels of bacteria and fungi prior to ClO_2 disinfection were found to be 2014 ± 1350 and 1002 ± 669 CFU m^{-3}, respectively. A single ClO_2 application was found to total disinfected bacteria and fungi concentration levels by as much as 57.3 and 57.6%. By contrast, a multiple-daily ClO_2 application was found to total disinfected bacteria and fungi concentration levels by as much as 65.1 and 57.6%. Among the two disinfection methods, the multiple-daily ClO_2 application method was found to yield a higher disinfection efficiency for bacteria, i.e., 16.28 ± 0.92%. Thus, using a ClO_2 disinfectant to maintain the air quality is of great importance to reduce infectious diseases in the pet's hospital. Therefore, the results suggest that the air quality guidelines prescribed by the TEPA for pet's hospital and other animal facilities can best be achieved by applying chlorine dioxide at regular intervals. The ClO_2 aerosol devices can effectively restrain or disinfect airborne bacteria to improve the indoor air quality. Thus, it can be applied in pet's cosmetology institutions, hospitals, and other public areas, where bioaerosols are of great concern.

1. Introduction

Particles of biological origin account for approximately 24% of the total concentration of airborne particles [1]. Deterioration of indoor air quality due to the airborne bacterial consortia is a widespread environmental problem. The indoor environment can potentially cause greater risks to human occupants than the outside environment, because enclosed spaces can confine and accumulate aerosols to levels that cause health hazards. Additionally, average persons spend most of their time indoors. Microbial contamination of air has become of interest in the past two decades because of the correlation of sick building syndrome (SBS) with indoor air pollution [2]. In fact, the onset of SBS, which comprises a series of symptoms such as eye irritation, airways dryness, headache, sleepiness, and skin rash and itch, seems to be related to the presence of microbes or their components in indoor air [3,4]. Biocontamination has the same harmful effects as chemical pollutants on the health of individuals [5]. In the past few decades, the problem of microbial contamination of indoor air has become a subject of interest for many researchers; both for the possible effects on health and for the control measures to limit these effects [6,7]. Exposure to microbial pollutants is in fact related to many negative consequences, such as infectious diseases, toxic effects, allergies, and asthma [3].

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Bioaerosols may be suspended in the air, attached to indoor surfaces, or present in the dust accumulated within a building or any of its internal parts or operating systems (e.g., the inside walls, air-conditioning units, ducts, among others). Given favorable conditions, bioaerosols are able to grow and propagate rapidly through enclosed indoor environments, resulting in significant indoor air pollution [2]. Research has shown that long-term exposure to bioaerosols in indoor environments may lead to infectious disease, SBS, or organic dust toxic syndrome [8]. Furthermore, elevated levels of particulate air pollution are associated with decreased lung function, increased respiratory problems, and enhanced rates of chronic obstructive pulmonary disease, cardiovascular disease and lung cancer [9]. As a result, exposure to bioaerosols in public spaces has emerged as a matter of growing concern in recent years [1,10,11]. In general, the concentration and size distribution of indoor bioaerosols depend on a wide range of biotic and abiotic factors. For example, previous studies have shown that the moisture content of building materials, the relative humidity and temperature of the local environment, the air exchange rate, the presence of human activities, and the number of people and pets all significantly affect the concentration level of indoor bioaerosols [12–14]. In non-industrial indoor environments, airborne bacteria are generated mainly by the presence of humans and related activities such as talking, sneezing, coughing, walking, washing, toilet flushing, and so forth [15]. Thus, while indoor environments are supposed to be protective, they can in fact become contaminated with particles which present different and sometimes more serious risks than those in outdoor environments if their concentration levels exceed recommended safety limits.

To safeguard public health, the National Institute of Occupational Safety and Health in America and the American Conference of Governmental Industrial Health (ACGIH) have ruled that the total number of bioaerosol particles in indoor environments should not exceed 1000 CFU m^{-3}, while the total culturable count for bacteria should be no higher than 500 CFU m^{-3} [16]. Furthermore, the Taiwan Environmental Protection Administration (TEPA) Taiwan has stated that for indoor public spaces, the bacteria concentration should be no higher than 1500 CFU m^{-3}, while the concentration of fungi should not exceed 1000 CFU m^{-3} [17].

Taiwan lies in a subtropical zone, and is usually warm and humid throughout the entire year. As a result, the local climate is highly conductive to the growth of bioaerosols [18]. According to the results of one long-term monitoring study, the concentration of biological contamination in Taiwan is much higher than the value of 1000 CFU m^{-3} recommended by the WHO [19]. Thus, to satisfy the TEPA guidelines for the air quality in indoor environments, effective disinfection treatments are required.

Pet shops and pet hospitals are two potential workplaces associated with the possibility of being exposed to bioaerosols, since pets are known as a potential source for indoor bioaerosols [14,20,21]. As a result, stringent disinfection protocols are required to ensure the health and general well-being of the cafeteria's consumers. The gaseous chlorine dioxide (ClO_2) is one of several techniques used for the remediation of structures impacted by microbial growth [6]. ClO_2 can destroy all manner of microorganisms, including bacteria, spores, fungi, viruses and even protozoans [22,23]. ClO_2 dissolves readily in water, forming a stable state of small particles. Under room temperature conditions, the ClO_2 content within the water evaporates and propagates naturally through the local environment, providing a disinfection function. Being in gaseous form, the ClO_2 molecules have the ability to penetrate into building cavities, wall cavities and other hard-to-access areas, and therefore provide an extremely thorough disinfection function [24]. Moreover, ClO_2 also exhibits a good degree of fungicidal activity when applied in solution form [25,26]. This study has been carried out on the ClO_2 disinfection of airborne bacteria in polluted indoor air due to its great potential to protect public health.

In a study performed by the US EPA, it was shown that ClO_2 results in no physiologically relevant alterations in human health provided that it is present only in low concentrations [27]. Accordingly, the present study with two different ClO_2 ultrasonic aerosol procedures were performed, namely single and multiple ClO_2 application. The air quality in the pet's hospital before and after ClO_2 disinfection was evaluated in terms of the bioaerosol levels of bacteria and fungi. The air quality results were then analyzed in order to determine the relative disinfection efficiencies of the two different methods.

2. Materials and methods

The study was conducted in the pet's hospital in Taiwan. Prior to disinfection, air samples were collected and analyzed in order to determine the background concentration levels of bacteria and fungi. ClO_2 disinfection was then carried out using two different application procedures. On each sampling day, air samples were collected over a 5-h period in order to evaluate the reduction in the bacteria and fungi concentration levels. The ClO_2 disinfection process for each mode was executed triple. The details of the experimental procedure are described in the sections below.

2.1. Study area and sampling time

Fig. 1 presents the floor plan of the pet's hospital considered in the present study. Sampling was conducted in four different areas of pet's hospital. The air samples were collected in accordance with the NIEA (National Institute of Environmental Analysis) guidelines specified by the TEPA. On each sampling day, indoor air samples for determining the biological and nonbiological contaminants in the pet's hospital were collected between the hours of 10:00 am and

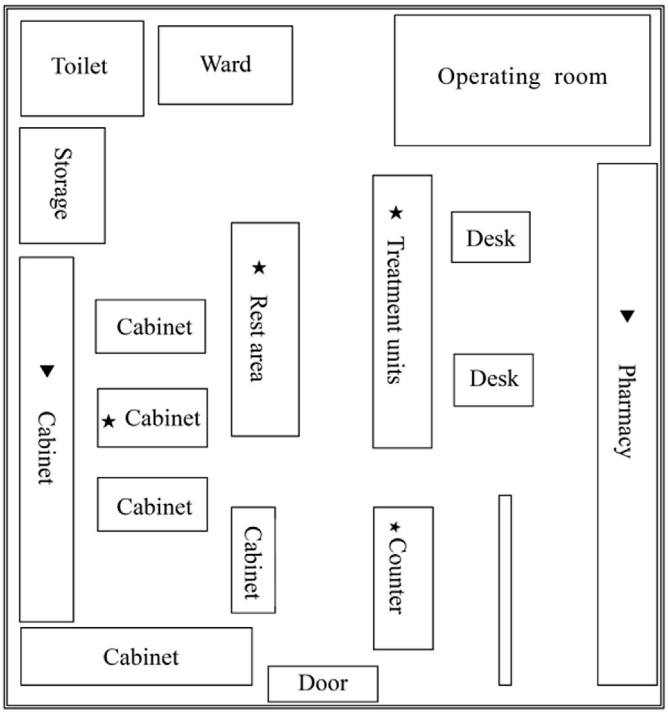

Fig. 1. Floor plan of pet's hospital (★ = sample collection location; ▲ = location of ultrasonic aerosol devices).

3:00 pm. The samples were then analyzed in order to determine the concentrations of the various biological (i.e., bacteria and fungi) components. To ensure the reliability of the analytic results, each sample was analyzed in triplicate. To investigate the effects of environmental factors on the ClO_2 disinfection efficiency, the relative humidity and temperature were measured each time a sample was collected using a TES-1364 Humidity Temperature Meter (TES, Taiwan). Finally, the airflow velocity and CO_2 concentration within the pet's hospital were measured using a Q-TRAK IAQ meter (Model 7565, TSI, USA).

2.2. Disinfection methods

According to the Occupational Safety and Health Administration of the USA and the ACGIH, the 8-h time-weighted average of ClO_2 in the workplace should not exceed 0.3 mg m^{-3} [28]. As stated above, the pet's hospital used in the present study had a volume of 300 m^3. Thus, to satisfy the 8-h TWA limit of 0.3 mg m^{-3}, fumigation was performed using a 360 mL ClO_2 solution (250 mg L^{-1}). The ClO_2 solution was applied using ultrasonic aerosol devices placed at three different locations within the pet's hospital, with each device containing 120 mL ClO_2 solution (250 mg L^{-1}). In general, the free radical of ClO_2 exists in gaseous form at temperatures of 11 °C or more [26]. In the present study, the average temperature within the pet's hospital was always higher than 11 °C, and thus, the ClO_2 solution evaporated upon application, resulting in the gradual release and propagation of ClO_2 molecules into the pet's hospital environment.

One each sampling day, sampling and disinfection were performed at 10:00 and 11:00 am, respectively. Air samples were then collected on an hourly basis until 3:00 pm. Two different disinfection modes were considered, namely a single application mode (SAM), and a multiple application mode (MAM). In the SAM mode, the ClO_2 solution was applied at 11:00 am and was not replenished as it nebulized. In the MAM mode, the ClO_2 solution was also applied at 11:00 am and replenished every 2 h. Thus, the overall ClO_2 dosage of SAM and that of MAM were different. In every case, the disinfection/sampling process was limited to a single day only.

2.3. Air sample collection

On each sampling day, air samples were collected prior to the disinfection process in order to determine the background concentration levels of bacteria and fungi. In both stages of the experiment (i.e., the initial stage with no ClO_2 disinfection and the second stage with a periodic, one-off application of ClO_2), air samples with a volume of 1000 L were collected in accordance with the relevant Taiwan NIEA guidelines (i.e., NIEA E301.12C for bacteria and E401.12C for fungus [29]). The samples were collected using a MAS-100 Eco Microbial Air Sampler (Merck, Germany, 100 L min^{-1}) containing petri dishes with Tryptic Soy Agar (TSA) plates and Malt Extract Agar (MEA) plates. Following a 10-min collection period, the petri dishes were removed from the sampler in order to cultivate the bioaerosols. For the bacteria bioaerosols, the TSA plates were incubated at a temperature of 30 ± 1 °C for 48 ± 2 h. Meanwhile, for the fungi bioaerosols, the MEA plates were incubated at 25 ± 1 °C for 4 ± 1 d. The bacteria and fungi levels were then evaluated by counting the number of colonies formed on the respective agar surfaces.

2.4. Statistical analysis

Significant differences among the disinfection efficiencies of the two ClO_2 application procedures, were evaluated by means of the Duncan analysis of variance test (ANOVA, $\alpha = 0.05$; Version 12, SPSS, USA, 2003).

3. Results and discussion

As described in the previous section, the relative humidity, temperature, airflow velocity, CO_2 level, residual bacteria, and residual fungi in the pet's hospital were recorded each time an air sample was collected. Table 1 shows the average bacteria and fungus concentration levels of the pet's hospital before and after ClO_2 treatment, respectively. Note that the annotation "before" refers to the samples collected in the first stage of the study (i.e., no ClO_2 disinfection), while the annotation "after" refers to the samples collected in the second stage of the study (i.e., periodic, single and multiple application of ClO_2). These results were presented both for the time period immediately before the disinfection process was carried out and at the time of sampling. Table 1 summarizes the experimental measurement results (mean ± standard deviation (SD)) for the environmental parameters and the bacteria and fungi concentration levels before and after the disinfection process, respectively. It can be seen that before disinfection, the average temperature was 27.2 ± 1.0 °C, while the relative humidity was 63 ± 8%, the airflow velocity was 5.8 ± 3.2 m min^{-1}, while the CO_2 level was 925 ± 499 ppm. As shown in Table 1, the average CO_2 level in the earth's atmosphere is around 300 ppm. CO_2 levels of more than 800 ppm may cause symptoms such as headaches and nausea in some individuals. Moreover, prolonged exposure to CO_2 levels of more than 1000 ppm may cause permanent damage to the respiratory system or brain [30]. Therefore, it is important to keep the indoor pet's hospital properly ventilated. A one-way ANOVA test was performed to test for significant differences among the average temperature, relative humidity, airflow velocity and carbon dioxide level in the two different application methods. Moreover, a correlation analysis was performed to investigate the relationship among the temperature, relative humidity, airflow velocity and carbon dioxide. The results of the ANOVA test showed that there were no significant differences among the temperature, relative humidity, airflow velocity and carbon dioxide level in the SAM and MAM application methods ($p > 0.05$). Moreover, no significant relationship was observed among the environmental conditions for the two different methods.

At temperatures of 11 °C or more, the free radical of ClO_2 is found in gaseous form [26]. Since the average temperature within of pet's hospital was always higher than 11 °C, the ClO_2 solution gradually evaporated, resulting in the release and propagation of chlorine dioxide molecules into the environment. ClO_2 has strong oxidizability, and therefore exists virtually entirely in a molecular state following application. As a consequence, it readily penetrates and destroys the cell membranes of bacteria. The loss of the cell membrane suppresses respiration in the bacterium body and renders the phosphotransferase mechanism inactive. As a consequence, the bacterium dies [30,31]. Figs. 2 and 3 show the bacteria and fungi concentration levels in the pet's hospital with both SAM (Figs. 2a and 3a) and MAM (Figs. 2b and 3b), where 0 h represents

Table 1
Experimental data before and after disinfection (ClO_2 treatment) (mean ± SD).

Items	Before ClO_2 treatment	After ClO_2 treatment	
		SAM	MAM
Total number of samples	72	144	144
Temperature (°C)	27.2 ± 1.0	24.2 ± 0.7	27.1 ± 0.6
Relative humidity (%)	63 ± 8	63 ± 5	60 ± 4
Airflow velocity (m min^{-1})	5.8 ± 3.2	3.7 ± 3.3	5.8 ± 1.8
Carbon dioxide (ppm)	925 ± 499	583 ± 124	966 ± 164

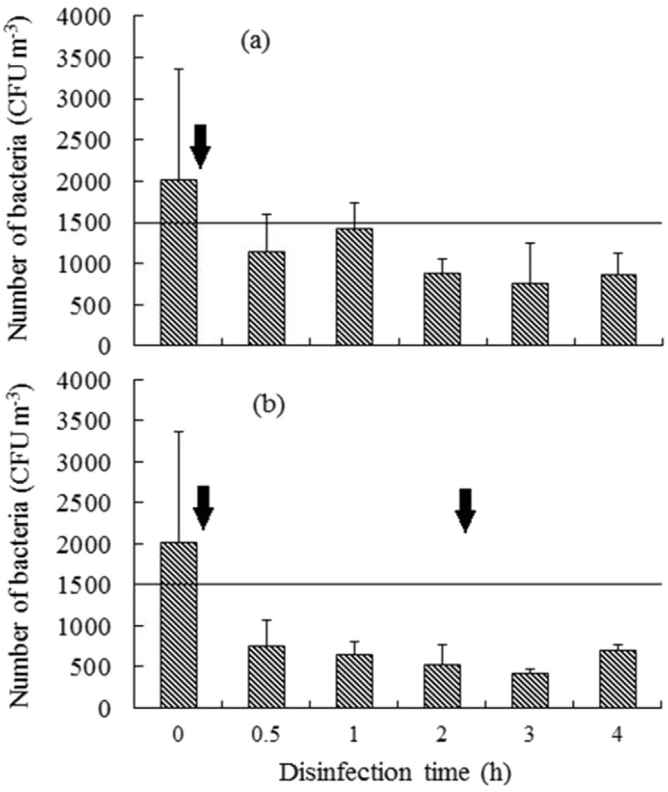

Fig. 2. Impact of two disinfection modes on indoor bacteria bioaerosol concentration (*downwards arrow* addition of ClO_2), where (a) represents single application and (b) represents multiple applications.

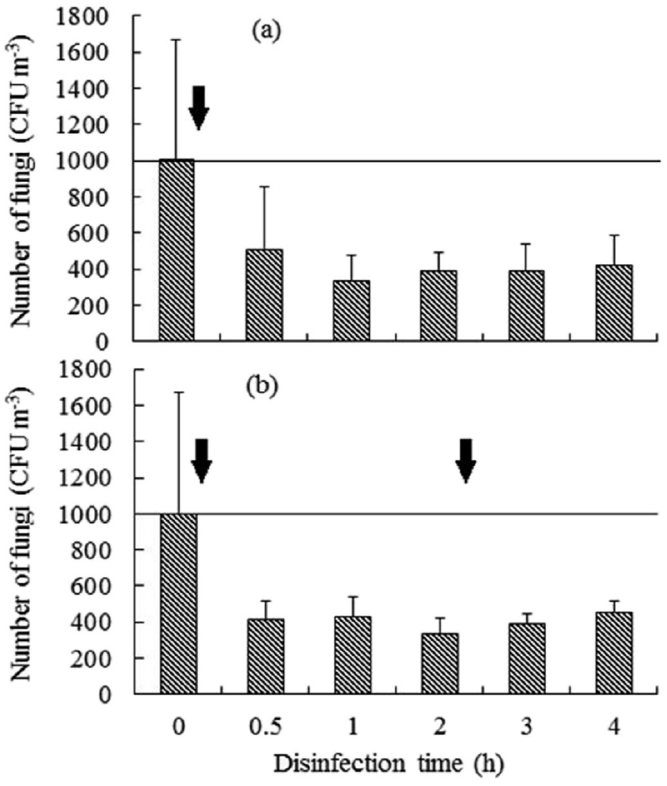

Fig. 3. Impact of two disinfection modes on indoor fungi bioaerosol concentration (*downwards arrow* addition of ClO_2), where (a) represents single application and (b) represents multiple applications.

the time before the disinfection process. The results presented in Figs. 2b and 3b show that the multiple application treatment reduces both the bacteria and the fungi concentrations were lower than the maximum permissible concentration levels prescribed by the TEPA. Figs. 2a and 3a show that the SAM application method reduces the residual bacteria and fungi concentration levels to a value compatible with the TEPA guidelines. Fig. 2a and b also show the variation in the bacteria concentration level. It is seen that the bacteria concentration level is generally lower than the maximum permissible level (1500 CFU m^{-3}) in both disinfections. In addition, it is seen that the level of bacteria following disinfection in SAM is higher than MAM. Therefore, it is seen that the multiple-daily ClO_2 treatment has an apparent effect in improving the air quality. As shown in Fig. 2, it is seen that for the residual bacteria concentration the hourly, SAM the SD of the bacteria concentration level is greater than MAM the SD of the bacteria concentration. In addition, given MAM disinfection, the residual bacteria and fungus concentration following disinfection is lower than that SAM disinfection. The concentration of airborne bacteria and fungi in indoor settings is determined primarily by the presence of humans and pets [32]. In other words, it is inferred that the MAM method provides an improved disinfection performance.

Fig. 3 shows the fungus concentration levels in the pet's hospital for both SAM (Fig. 3a) and MAM (Fig. 3b) application methods, indicating that the ClO_2 treatment reduces the fungus concentration level. Therefore, it is seen that for the residual fungi concentration the hourly, SAM the mean ± SD of the fungi concentration level is greater than MAM the mean ± SD of the fungi concentration. The mean ± SD with multiple disinfection was significantly lower, indicating disinfection being effective. Comparing the results presented in Fig. 3 for the ClO_2 application methods, it is seen that the multiple application method yields the higher disinfection efficiency.

The bacteria and fungi disinfection efficiencies of the two disinfection methods are summarized in Table 2. The disinfection per hour was determined to get the average disinfection for comparing the average disinfection efficiency of the two methods in this study. As shown, the SAM and MAM disinfection methods result in 14.32 ± 3.22% and 16.28 ± 0.92% bacterium disinfections per hour, respectively, and 14.41 ± 3.96% and 14.15 ± 0.71% fungi disinfections. According to the results of the Duncan ANOVA test, the residual bacteria level following SAM disinfection is significantly higher than that following the MAM treatment ($p < 0.05$). However, there is no significant difference among the two different treatment methods in the residual fungi level. The results presented in Figs. 2b and 3b show that the multiple application treatment reduces both the residual bacteria and the fungi concentration levels to a value compatible with the TEPA.

As shown in Table 2, the SAM and MAM disinfection methods result in 57.3 and 65.1% total bacterium disinfections per day, respectively, and 57.6 and 57.6% fungi disinfections. The results presented in Fig. 2b show that the MAM disinfection method also

Table 2
Disinfection efficiencies of two disinfection methods for bacteria and fungi bioaerosols

Items	SAM	MAM
Disinfected bacteria per hour (%)	14.32 ± 3.22	16.28 ± 0.92
Disinfected fungi per hour (%)	14.41 ± 3.96	14.15 ± 0.71
Residual bacteria (CFU m^{-3})	806.5 ± 259.5[a]	702.5 ± 74.2[b]
Total disinfected bacteria (%)	57.3	65.1
Residual fungi (CFU m^{-3})	424.0 ± 159.0	435.0 ± 28.3
Total disinfected fungi (%)	57.6	57.6

[a–b]Within the same column, entries annotated with different superscripts exhibit a statistical difference according to Duncan ANOVA test ($p < 0.05$).

reduces the residual bacteria concentration levels to value compatible with the TEPA. The results of the present study suggest that when the bacteria and fungi concentrations exceed the suggested value, disinfection method can reduce bioaerosol levels. By comparing the two disinfection methods, multiple disinfection (i.e., daily) was more effective.

4. Conclusions

This study has performed an experimental investigation into the effectiveness of ClO_2 as a disinfection agent for pet's hospital in Taiwan. Two different ClO_2 application methods have been considered, namely a single treatment, and multiple treatments at 2 h intervals. In every case, disinfection was performed using 0.3 mg m^{-3} of ClO_2, and the ClO_2 was allowed to ultrasonic aerosol procedures and propagate through the air. Conclusions obtained from this study include the following:

1. The experimental results have shown that the application of ClO_2 results in a moderate reduction in the average levels of bacteria and fungus within the pet's hospital. In the SAM method, the ClO_2 is applied only once a day. However, in the MAM method, the ClO_2 needs to be replenished on a 2-hourly basis. The results suggest that from both disinfection efficiency perspective and a cost and convenience perspective, the MAM treatment protocol represents the most appropriate means of satisfying the TEPA guidelines for the indoor air quality in pet's hospital.
2. The ClO_2 aerosol devices can effectively restrain or disinfect airborne bacteria to improve the indoor air quality. Thus, it can be applied in workplaces, hospitals, and other public areas, where bioaerosols are of great concern.

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