Experimental study on activities of the virus in low humidity indoor environments

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ABSTRACT

Japanese Building Maintenance Law defines that indoor humidity shall be maintained not lower than 40%. But it is sometime very difficult to comply with the law. Indoor air humidifies in office buildings mostly below 40% especially in wintertime. A request to deregulate the provision arises from HVAC industries. But a question concerning risk to deregulate the lower limit of humidity is arising. According to researches in 1960s, activity levels of influenza virus become higher when indoor relative humidity becomes lower than 40%. This fact is one of major basis of the provision but is rather old information. New *in vitro* study on this subject is conducted to update information on the effects of relative humidity on influenza virus. The results showed that higher the humidity became, the lower the virus infectivity became.

INDEX TERMS

Humidity; Virus activity; Japanese Building Maintenance Law; HVAC

INTRODUCTION

Indoor environments in Japanese commercial buildings were regulated by the Building Maintenance Law (BML) (Building Management Education Center, 1982), which has a provision of strict inspection system by regional health centres. The Law defines levels of carbon monoxide, carbon dioxide, SPM (Suspended Particulate Matter), temperature, relative humidity and airflow in indoors. Indoor air qualities in Japanese commercial buildings are protected by the Law, and Japanese workers never faced serious Sick Building Syndrome (SBS) affects, which was occurred intensely in Northern America and Europe in the 1980s. Among the six items mentioned above, indoor humidity levels in Japanese office buildings did not sometimes comply with the Law. Especially in winter, most of indoor humidity levels in Japanese office room become far lower than 40%. Therefore, people in HVAC (Heating Ventilating and Air-Conditioning) industry are complaining that the provision of humidity lower limit is too strict and unrealistic for building maintenance work. They sometime demand the Law to be deregulated so far as the lower limit of humidity is concerned. However, according to researches in the 1960s (Hemms *et al.*, 1960; Harper, 1963; Vlodavetz and Dmitrieva, 1966a,b; Balan, 1967), activity levels of influenza virus become higher when the indoor relative humidity becomes lower than 40%. This fact is one of the major bases of the provision but is rather old information. Revision of the data is needed. A new *in vitro* study on this subject is conducted to up-date information on the effects of relative humidity on influenza virus (Nakayama *et al.*, 1993; Komatsu *et al.*, 2002). The results showed that higher the humidity became, the lower the influenza virus infectivity became, and that the present provision of the Law is totally suitable.

EXPERIMENT I (Long-term exposure experiments on difference in infectivity of the virus in humidity levels of 30–40%)

Outline of the Experiment

Exposure chamber. The experimental apparatus consists of two airtight exposure chambers.

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Temperature and humidity conditions in one chamber were 26°C and 30% (does not comply with BML), while those in the other were 26°C and 40%, respectively (comply with BML). These humidity conditions were attained by equilibrium resulting from the co-localization with saline water solutions in each chamber. Humidity and temperature in the experimental laboratory were about 20% and 23° C, which were considerably dry conditions.

Virus tested (Komatsu *et al.*, 2002). Influenza virus A/Kitakyushu/157/93/H3N2 strain was used for the experiments. The cell used was MDCK (dog kidney), which has 430 passage histories. Both virus and cell were supplied by National Institute of Infectious Disease in Japan. MEM culture medium was used for the incubation of virus and cell. The medium for virus was compounded without adding stabilizer (such as sera, albumin, gelatine and so forth), so that test results reflect the effects of humidity on the virus as exactly as possible. The medium was poured in every 1 ml separately into a 10 ml vial and was freeze-dried by a freeze-dryer (Triomaster IIA-04) for 6 days. Water contain ratio of the test sample was about 1.4% when completion.

Exposure and sampling of virus tested (Harper, 1963). Virus tested was exposed to condition in each chamber and sampled at 24 and 72 h after the exposure, and also sampled before the exposure test.

Evaluation of infectivity of influenza virus. Influenza virus was set on MDCK culture cell, and then distributed on a plate. Infectivity was evaluated by Plaque method for exposure times of 0, 24 and 72 h, respectively.

Results

The results are shown in Table 1.

Table 1 Results of experiment I (long term exposure test on virus Infectivity, in PFU/ml)

Humidity and temperature	Exposure time (h)		
	0	24	72
Humidity: 30%, Temperature: 26°C (three samples)	$1.6 \times 10^{7.0}$	$1.3 \times 10^{2.0}$	$<1.0 \times 10^{2.0}$
Humidity: 40%, Temperature: 26°C (three samples)	$1.6 \times 10^{7.0}$	$<1.0 \times 10^{2.0}$	$<1.0 \times 10^{2.0}$

Attained humidity level. The humidity conditions actually attained in each chamber were 32 and 42% although we intended to set them to 30 and 40%. They are slightly higher than those obtained from a pretest.

Effects of humidity on the tested virus. Infectivity of influenza virus sampled before exposure (control condition) was $1.6 \times 10^{7.0}$ PFU/ml (PFL: Plaque Forming Unit). Infectivity of influenza virus sampled at 24 h after exposure in the condition of 30% was $1.3 \times 10^{2.0}$ PFU/ml, and that of 40% below the detection limit (less than $1.0 \times 10^{2.0}$ P1FU/ml). These results show that influenza virus was more inactivated in the condition of 40% humidity. However, difference in these results was not so clear. The results also show that the virus was almost inactivated after the 24 h of exposure, since a decrease ratio of PFU/ml values in both conditions were more than a factor of 10^5. Finally, in the case of 72 h of exposure, results obtained from two humidity conditions were below the detection limit and no significant difference between them was shown.

Discussion. Results of the experiment show that influenza virus infectivity completely disappeared after 24 h of exposure in the case of 40% humidity, which complies with BML provision, while about 30%, which does not comply with the Law, still remain a little. However, difference between them was not so clear. Exposure time of 24 h might be too long, and difference may be obtained from the test for shorter exposure term. Further experiments on the accuracy and reproducibility of the effects of humidity on the influenza virus for shorter exposure term is suggested.

EXPERIMENT II (Short-term experiment)

Outline of the Experiment

An additional experiment was conducted basically in the same manner as the long-term test mentioned previously. Exposure times were 0, 5 and 15 h, respectively.

Results
The results are shown in Table 2.

PFU values in 30% humidity condition were $2.6 \times 10^{6.0}$ (0 h exposure), $8.3 \times 10^{2.0}$ (5 h) and less than $1.8 \times 10^{1.0}$ (15 h). Similarly, those in 40% humidity were $2.6 \times 10^{6.0}$ (0 h), less than $1.0 \times 10^{1.0}$ (5 h) and also less than $1.0 \times 10^{1.0}$ (15 h), respectively.

Table 2 Results of experiment II (short-term exposure test on influenza virus infectivity, in PFU/ml)

Humidity and temperature	Exposure time (h)		
	0	5	15
Humidity: 30%, Temperature: 26°C (three samples)	$2.6 \times 10^{6.0}$	$8.3 \times 10^{2.0}$	$1.8 \times 10^{2.0}$
Humidity: 40%, Temperature: 26°C (three samples)	$2.6 \times 10^{6.0}$	$<1.0 \times 10^{2.0}$	$<1.0 \times 10^{2.0}$

Discussion
Results obtained from the 30% humidity condition test showed that infectivity was remaining while those from the 40% humidity test did not show infectivity. A difference was observed between the two tests.

EXPERIMENT III (Comparison of infectivities in various humidity conditions)
Purpose
Experiments in the conditions of wider humidity range (form 10 to 50%) were conducted since there was no significant difference in the results obtained from the humidity conditions between 30 and 40% in Experiment I., although test results of 40% were slightly higher than those of 30%.

Outline of the Experiment
Setting humidity conditions. Humidity conditions were adjusted by co-localizing the sample air with various water solutions in the test chamber as shown later. Using lithium chloride, 10% humidity was attained. Similarly, 30% was attained by magnesium chloride and silica gel, 40% by potassium carbonate and silica gel, and 50% by magnesium nitrate. Deep dishes containing saturated saline solution were placed in an air-tight chamber having a volume of 5.6 l. Silica gel was used to lower the humidity since 30 and 40% humidity conditions, which were actually realized in Experiments I and II, were apt to become higher than expected.

Outline of the Experiment
Influenza virus A/Hong Kong was used. Preparation began 3 days before the test and the tested virus was sampled after 3 and 5 h of exposure to air having a particular humidity condition. Incubation in the temperature condition of 34°C continued for 3 days after the sampling and counting.

Results
Figures 1 and 2 show the results obtained from 3- and 5-h exposure experiments.

Although infectivities of 5-h exposure test were reasonably lower than those from 3-h experiments, the overall trend of both cases was similar to each other. That is, the infectivity in both exposure times became lower when the humidity increased higher. For example, as for in the case of 5-h exposure, infectivity of 15% humidity was only a half of the control value while that of 50% decreased one millionth, and the infectivity of 30% was about 1/100 and was one order higher than that of 40%. This result is similar to that in experiments I and II.

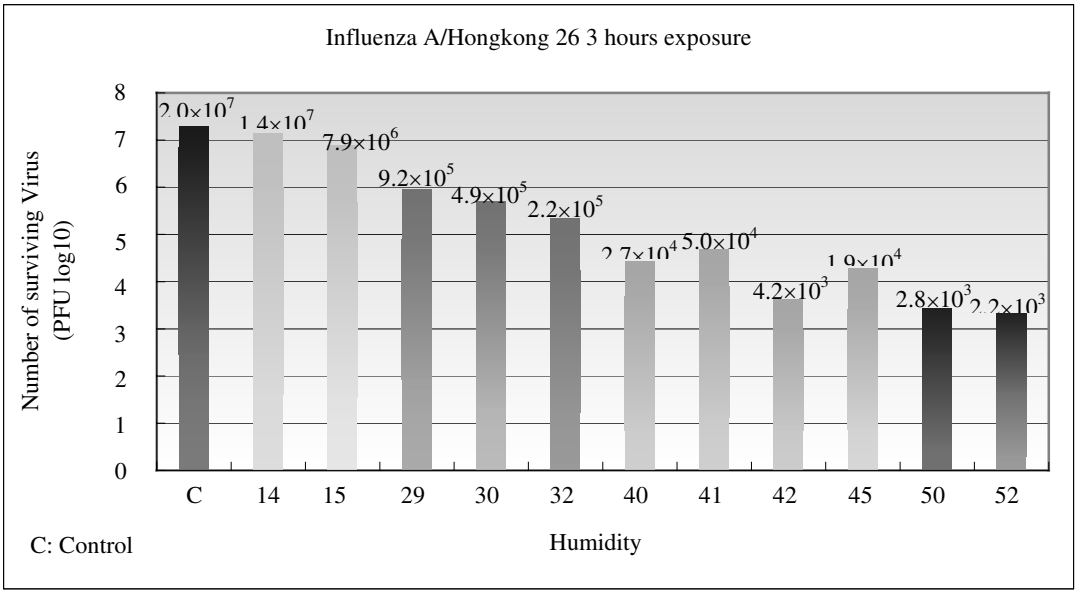

Figure 1 Effects of humidity on the virus infectivities after 3 h of exposure.

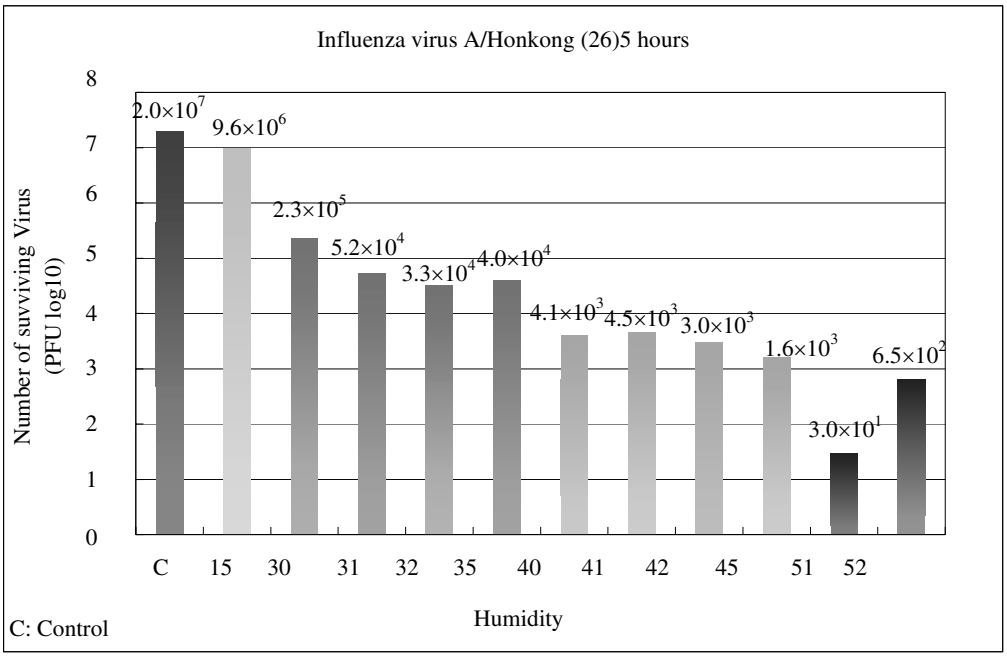

Figure 2 Effects of humidity on the virus infectivities after 5 h of exposure.

EXPERIMENT IV (Effects of humidity on HA activity or Hem agglutination activity of the Influenza A/Hong Kong)

Outline of the Experiment

A 0.5% solution of Goose Red Blood cell was used for the experiment. The test cell was mixed with influenza A/Hong Kong virus, which was cultivated in the conditions shown in Table 3, and HA activity of each condition was evaluated after that.

Table 3 Experimental conditions and results

Code	Humidity	Operation	HA activity
C-1	20%[a]	Thawing the frozen virus before the HA activity test	512
C-2	20%[a]	Storage for 5 h in sealed incubator with 26°C	512
550	50%	5-h exposure in a chamber with saturated solution of the magnesium nitrate	<2
540	40%	5-h exposure in a chamber with saturated solution of the potassium carbonate and silica gel	256
530	30%	5-h exposure in a chamber with saturated solution of the magnesium chloride and silica gel	256
515	15%	5-h exposure in a chamber with saturated solution of the lithium chloride	512

[a]Humidity in the laboratory.

Results

The results are shown in Table 3 and Figure 3.

The result of the HA activities of 15% humidity condition test was 512, and was the same value as controls, C-1 and C-2, while that of 50% humidity condition was less than.2. HA activity was almost lost. The activities in the conditions of 30% and 40% were 256, and were half of the control value.

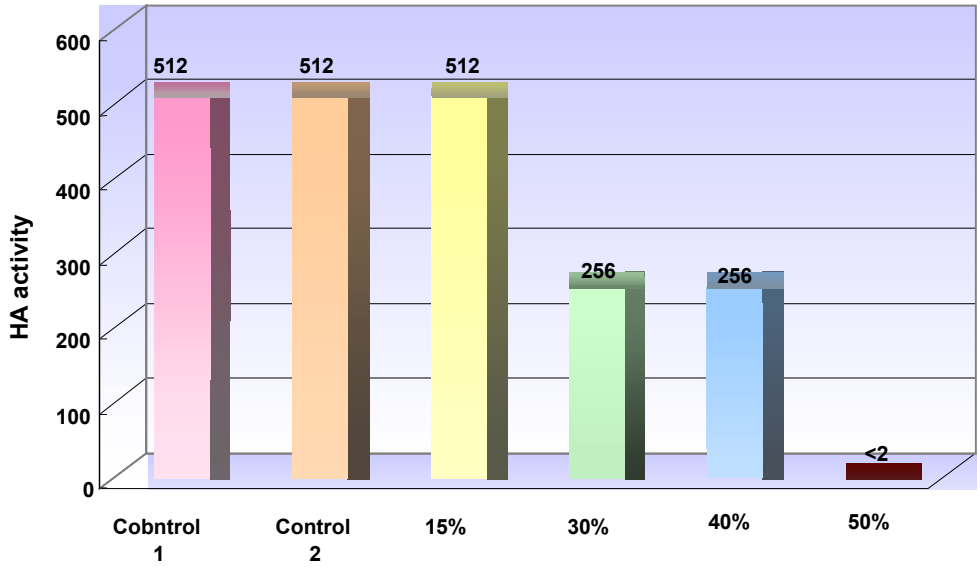

Figure 3 Result of virus HA activity experiment.

OVERALL DISCUSSION

1. Results of experiments I and II, which were conducted using influenza virus A/Kitakyuushu/157/93/ H3N2 strain, showed a difference in the results obtained from less than 24 h exposure tests for 30% and 40%, while those from 72 h exposure tests did not.
2. Results of experiment III, which was conducted using influenza virus A/Hong Kong, showed that results of infectivity obtained from 5 h exposure test in15% humidity condition was about half of those from control condition, while infectivity of 50% humidity condition was almost one millionth. As for the difference in infectivities between 30% and 40%, infectivity of 40% was one order lower than that of 30%.
3. HA (Hem agglutination) activity of influenza virus A/Hong Kong obtained from 5 h exposure test in 50% humidity condition was almost completely inactivated while that from 15% remained at the same level as the control condition. Those from 30% and 40% did not differ and were almost half of the control results.

4. Science tests of the present report were conducted only *in vitro*, an *in situ* study, which is conducted in a more practical condition, is needed as a further research subject.
5. Research on the higher limit of humidity is also important to revise the Law because higher the humidity it is easier for the microbes (such as fungi and bacteria) to propagate.

SUMMARY

1. Influenza virus were not inactivated in the humidity condition of less than 20% after several hours of exposure while they were almost completely inactivated in the humidity of 50%.
2. Difference in infectivities between 30% and 40%, which are borderline defined by Japanese Building Maintenance Law, was about order of ten, that is, infectivity in 30% was one order higher than that in 40%.
3. No difference in HA activity between 30% and 40% was found.
4. Preferable humidity in terms of inactivation of the influenza virus seemed 50%. However humidity condition of 40% showed considerable effect.
5. Therefore, present provision of humidity lower limit in BML is quite reasonable.
6. There is a necessity of further experiments on the accuracy and reproducibility of the effects of humidity on the influenza virus.
7. As the tests of the present report were conducted only *in vitro*, an *in situ* study, which is conducted in a more practical condition, is needed.

CONCLUSION

The present provision of humidity lower limit in BML is quite suitable. But there is a necessity of further experiments on the accuracy and reproducibility of the effects of humidity on the influenza virus.

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