# Susceptibility of *Mycobacterium tuberculosis* to Non-Toxic Level Ozone

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## ABSTRACT

Decontamination of *Mycobacterium tuberculosis* (MTB) in the environment by specific methods, such as ultraviolet light and the toxic level ozone, has been effectively used. However, the efficacy of the non-toxic level ozone in the decontamination of MTB is not well documented. This study focused on the impact of non-toxic level (0.03-0.05 part per million) ozone in normal room air on MTB on a solid medium, M7H10. The non-toxic level ozone can reduce the number of MTB colonies as compared to the control. The number of MTB colonies was rapidly reduced, more than 95 percent, during the first two hours and gradually reduced during the remaining time of the experiment. The results suggest that the non-toxic level ozone, produced by specific devices, is promising for the decontamination of solid surfaces from MTB.

**Keywords:** Tuberculosis; Ozone; Decontamination; Susceptibility

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Since the emergence of AIDS in the early 1980s, tuberculosis has increasingly gained importance in clinical practice and has become more prevalent which necessitates the development of measures to control its transmission. *Mycobacterium tuberculosis* (MTB), a causative agent of tuberculosis, is primarily transmitted via aerosol droplets. To control its transmission, especially in high-risk areas such as hospitals or microbiology laboratories, decontamination of the germs in the environmental air and contaminated surfaces is important. Ultraviolet (UV) light has been effectively used to decontaminate air and solid surfaces from MTB.[1] However, UV light cannot be used during working hours with the presence of personnel because of its mutagenic effect. Furthermore, UV light cannot penetrate certain covered areas. Due to the very high oxidative capacity and germicidal effect of ozone, there are many industrial and medical applications. Ozone has been widely used as an effective tool to decontaminate water and food and for bio-cleansing contaminated spaces.[2-10] Currently, ozone is being used increasingly as an air disinfectant in many air conditioners and air purifiers. Ozone at the level under 0.064 part per million (ppm) is not toxic to humans.[11]

Previous studies have demonstrated bactericidal and bacteriostatic effects of ozone on MTB,[12-13] but none of them has verified the impact of non-toxic levels of ozone in normal atmosphere. This study was done to investigate the susceptibility of MTB on contaminated solid surfaces to non-toxic levels of ozone, 0.03-0.05* ppm.

* Maximum acceptable level of ozone, generated by any device that has been set by the United States Food and Drug Administration.[14]

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## MATERIALS AND METHODS

### Preparation of MTB

The H37Rv strain of MTB was prepared as a cell suspension with a density of 1 McFarland (McF No.1) standard from 3-week old colonies grown on the Lowenstein-Jensen medium. The suspension was diluted with distilled water 100 times and 10,000 times to $10^{-2}$ and $10^{-4}$ of McF No.1 standard, respectively. Then, 0.1 ml of the $10^{-2}$ and $10^{-4}$ diluted solutions, each was spread on M7H10 medium in disposable 9-cm Petridishes (Sterilin, Bibby Sterilin Ltd, Stone, Staff, UK), 8 and 20 plates, respectively.

### Production of ozone

Two ozone generators (Model AA020 and AB200, Active Science Co.,Ltd, Thailand) were used to generate ozone with an air concentration of 0.03-0.05 ppm in a laminar flow biological safety cabinet (BH2000 series class II cabinet, Clyde Apac, Australia). The ozone concentration was monitored by an ozone monitor (Ecozone™ Monitor Model E2-IX produced by Ecosensor, Inc, UK) and was adjusted manually.

### Susceptibility of MTB to ozone

Sixteen plates spread with 0.1 ml of a $10^{-4}$ cell suspension were placed in the biosafety cabinet while four other plates were sealed by plastic wrap and left outside the cabinet at room temperature. Four plates were brought out every two hours until the process was completed at 8 hour. The plates that were brought out were sealed and left outside the cabinet at room temperature as the 0-hour plates. After the 8-hour plates were brought out and sealed, all of the plates were then placed upside down in an incubator at $37^0$C for 20 days. The plates spread with

**TABLE 1.** The percentage survival of *Mycobacterium* colonies due to the effect of ozone at an air concentration of 0.03-0.05 ppm from the beginning dilution of the $10^{-4}$ McF No. 1 standard. The experiments were done twice. The results of each experiment are shown as experiment 1 and experiment 2.

| Round of experiment | Percent of survival colonies [CFU/plate] in each passing time[hour] | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Experiment 1 | 100 | 6 | 0 | 0 | 0 |
| Experiment 2 | 100 | 1.45 | 0.78 | 0.68 | 0.12 |
| Control | 100 | 50.97 | 45.93 | 16.27 | 1.83 |

0.1 ml of the $10^{-2}$ cell suspension were handled in the same way. Four plates were left outside while the other four plates were placed in the biosafety cabinet and then taken out after eight hours. All plates were then incubated at $37^{0}$C for 20 days as mentioned before. The entire experiment was repeated twice.

### Control for the susceptibility test

The air curtain of the biosafety cabinet caused the MTB media to become dry which may have caused a false reduction in the number of MTB colonies. Consequently, a control experiment was done using the procedure pre-viously described for the $10^{-4}$ diluted cell suspension but without exposure to ozone to eliminate the dryness factor which could affect the growth of mycobacteria.

### Counting the colonies

At 0 hour the colonies were counted by a dilution method while the others were counted directly on plates. Colonies were counted at 20 days after each incubation. The plate that showed no growth was further incubated for one week before being reported as having no growth.

### Statistical analysis

The arithmetic mean number of colonies was calculated from each four-plate set (quadruplets) in each experiment as colony forming units (cfu)/plate and the percent-reduction of MTB was calculated.

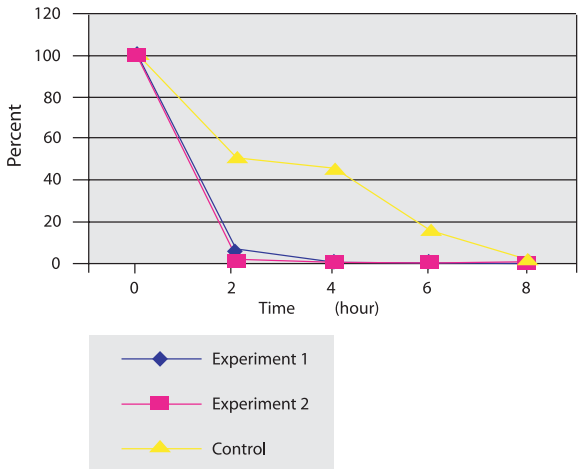



**Fig 1.** The percentage survival of *Mycobacterium* colonies due to the effect of ozone at an air concentration of 0.03-0.05 ppm from the beginning dilution the $10^{-4}$ McF No.1 standard. The experiments were done twice. The results of each experiment are shown as experiment 1 and experiment 2.

Table showing the colony counts of each quadruplets

**First experiment**

**$10^{-4}$ of MacFarland No.1**

| | Number of Mycobacterial colony | | | | | |
|---|---|---|---|---|---|---|
| Time (hour) | Plate 1 | Plate 2 | Plate 3 | Plate 4 | Mean | % Remain |
| 0 | 535 | 505 | 659 | 614 | 578.25 | 100 |
| 2 | 13 | 62 | 38 | 25 | 34.5 | 5.966278 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 |

**Second experiment**

**$10^{-4}$ of MacFarland No.1**

| | Number of Mycobacterial colony | | | | | |
|---|---|---|---|---|---|---|
| Time (hour) | Plate 1 | Plate 2 | Plate 3 | Plate 4 | Mean | % Remain |
| 0 | 1,151 | 1,116 | 1,241 | 1,496 | 1,251 | 100 |
| 2 | 14 | 7 | 20 | 32 | 18.25 | 1.458833 |
| 4 | 18 | 5 | 16 | 0 | 9.75 | 0.779376 |
| 6 | 10 | 17 | 4 | 3 | 8.5 | 0.679456 |
| 8 | 1 | 3 | 2 | 0 | 1.5 | 0.119904 |

## RESULTS

At the beginning, dilution of the $10^{-4}$ McF No. 1 standard, MTB, as measured by cfu, was rapidly reduced during the first two hours in experiments 1 and 2 to 94 and 98.55 percent, respectively (Fig.1). The MTB of the control experiment was gradually reduced (50.97 percent were reduced in the first two hours) during the course of the experiment.

As seen in the experiment of the $10^{-4}$ dilution, the experiment of the $10^{-2}$ dilution of 1 McF No. 1 standard also showed a reduction. (Table 1) at the 8th hour.

## DISCUSSION

We have shown that at the concentration of ozone at 0.03-0.05 ppm in air, which is non-toxic to humans, the reduction of mycobacterial cfu at the beginning dilution of the $10^{-4}$ McF No.1 standard was more than 90 percent in the first two hours and up to 100 percent during the following four to eight hours. A similar reduction was shown at the higher concentration of MTB. The experiments showed a high sensitivity of MTB on an open surface to ozone even at this low ozone level. Although we also found that MTB was reduced in the control experiment due to dryness of the medium, the reduction did not occur at the same speed as that in the plates exposed to ozone. However, we did not observe any difference in the survival percentage between the experiments and the control because the medium was too dry for MTB to grow readily. Further study may need to be conducted in a liquid medium to prevent the effect of dryness.

Because the air concentration of the ozone used in our experiment is non-toxic to humans, one can perform any activity in an environment containing this ozone concentration. Its germicidal effect on MTB was highest in the first two hours on solid surface and may also effectively decrease the MTB in air because of its greater surface area in contact with ozone. Moreover, ozone can spread to any place such as covered surfaces or spaces, which makes it more advantageous than UV light. This might enhance the reduction of MTB transmission among high-risk people such as laboratory technicians, doctors or others in close contact with MTB.

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## บทคัดย่อ

# ความไวของเชื้อวัณโรคต่อโอโซนในระดับที่ไม่เป็นอันตรายต่อมนุษย์


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ปัจจุบันมีการใช้แสงอัลตราไวโอเลตและโอโซนความเข้มข้นสูงทำลายเชื้อวัณโรคและเชื้อโรคต่างๆที่ปนเปื้อนในสิ่งแวดล้อมได้อย่างมีประสิทธิภาพ อย่างไรก็ดีไม่มีการศึกษาใดที่บ่งถึงความสามารถในการกำจัดเชื้อวัณโรคของก๊าซโอโซนในระดับที่ไม่เป็นอันตรายต่อมนุษย์ไว้อย่างชัดเจน การศึกษานี้ทำขึ้นเพื่อศึกษาผลกระทบของโอโซนในระดับที่ไม่เป็นอันตรายต่อมนุษย์ต่อเชื้อวัณโรคที่อยู่บนผิวอาหารเพาะเชื้อแบบแข็ง M7H10 ในบรรยายกาศปกติ พบว่าโอโซนในระดับที่ไม่เป็นอันตรายต่อมนุษย์สามารถลดจำนวนเชื้อวัณโรคได้เมื่อเทียบกับเชื้อวัณโรคที่ไม่ได้รับโอโซนถึงร้อยละ 95 ใน 2 ชั่วโมงแรก จากนั้นจำนวนเชื้อวัณโรคจะค่อย ๆ ลดลงอีกเล็กน้อยตลอดช่วงเวลาที่เหลือของการทดลอง จากผลการทดลองสรุปได้ว่าโอโซนในระดับที่ไม่เป็นอันตรายต่อมนุษย์สามารถใช้ลดจำนวนเชื้อวัณโรคที่ปนเปื้อนบนพื้นผิวของแข็งในบรรยากาศปกติได้