VALIDATION OF ELECTRONIC AIR FILTER FOR FILTRATING *MYCOBACTERIUM TUBERCULOSIS* AND VIRUS FROM CONTAMINATED AIR

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Aerosol transmission of *M. tuberculosis* (MTB) and viruses that cause respiratory infections has been increasing. Air filtration is one option for reducing pathogens in indoor air. However, few research studies have validated the efficiency of electronic air filters for filtering bacteria and viruses. This study aimed to evaluate the filtrating efficiency (FE) of an electronic air filter for filtering MTB and viruses in contaminated air, compared with a high efficiency particulate air (HEPA) filter, and no filter. An enclosed chamber was constructed, in the middle of which an air filter could be placed for testing. 5×10^8 each of MTB H37Ra and T7 virus were sprayed into one side of the chamber using a nebulizer. On the other side, an impinger air-sampler was used to collect the sprayed samples. Then, MTB and virus samples were respectively detected by nested PCR and PCR; the culture method was used as the gold standard. All of the sprayed MTB and T7 virus could be detected by nested PCR and PCR with a sensitivity of 10 *f*g for MTB H37Ra and 1 *p*g for T7 virus. However, since most MTB fail to culture, and PCR was not quantifiable, additional FE tests of both filters were performed with other bacteria--*S. aureus* (10^5 cfu sprayed) and *E. coli* (10^4 cfu sprayed). Based on the culture results, the FE of both filters could be calculated. It was concluded that both types of air filter could filter out the sprayed T7 virus (particle size 0.04 μm), *S. aureus* (particle size 1 μm), and *E. coli* (particle size 2 μm) with FE > 99.0 %.

Keywords: validation, electronic air filter, filtrating**,** *M. tuberculosis*, T7 virus, contaminated air.

The aerosol transmissions of *M. tuberculosis* and virus that cause respiratory infection have been increasing. Airborne transmission occurs when bacteria or viruses adhere on dust particles or on small respiratory droplets that may become aerosolized. Airborne bacteria and viruses transmit when people coughing, sneezing, talking, laughing, singing or exhaling. The airborne transmission of pathogenic microorganisms include *Mycobacterium tuberculosis*, causing of Tuberculosis (TB) (CDC, 1994), Smallpox, Chickenpox, Influenza (Bean *et al*., 1983), Measles, German measles, Mumps (Committee on Infectious Diseases and Committee on Hospital Care, 1998), and Severe acute respiratory syndrome (SARS) (Drosten *et al*., 2003). TB, an infectious lung disease, is currently pandemic and continues to be a major public health problem, particularly in developing countries. Over one-third of the world's population has been exposed to the TB bacterium, and new infections occur at a rate of one per second (http://www.who.int/ mediacentre/factsheet/fs104/ en/index.htn).

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The experiment for detecting *M. tuberculosis* and viruses from contaminated air has been considered. *M. tuberculosis* H37Ra, an attenuated tubercle, has been used as the surrogate microorganisms representing for *M. tuberculosi*s (Brosch *et al*., 1999). While bacteriophage T7 a double stranded DNA virus, has been used as the surrogate of mammalian virus from contaminated air (Tseng and Li, 2005). The molecular method such as plain Polymerase Chain Reaction (PCR) and nested PCR was first demonstrated as a mean to detect bacteria and viruses in air sample (Alvarez *et al*, 1994; Sawyer *et al*., 1994).

The AGI-30 impinger is user friendly, inexpensive, reliable, portable, easily sterilized, and has high biological sampling efficiency. The typical sampling duration is approximately 20 mins and the volume of collection medium about 20 ml (Maier *et al*., 2000).

A novel electronic air filter developed by Alpine company, Thailand, is a non Ionizing electronic air filter system. This product has been awarded from many agencies both national and international (http://www.alpinefilter.com/). The company has claimed that, this electronic air filter can trap the particulate of dangerous microorganisms like *M. tuberculosis* and

viruses from the air, however no study has been performed to validate this product.

This study aimed to validate the efficiency of a novel electronic air filter, a non ionizing electronic air filter system developed by Alpine company, on filtrating *M. tuberculosis* and T7 virus from indoor air. The result of this study would be useful for the consumer in the decision to purchase the appropriate air filter system for protecting their health.

MATERIALS AND METHODS

1. Experimental chamber model for electronic air filter validation

An experimental chamber model was set for electronic air filter validation, by using *M. tuberculosis* H37Ra and T7 virus as surrogate microorganisms for *M. tuberculosis* and virus, respectively. The performance of electronic air filter in filtering the microorganisms was compared with that of commonly used of HEPA filter. All steps of operation were performed in closed system, in that the model was placed in biosafety cabinet type II (Molten Maxisafe 2000 Model 1-2, Denmark) (Figure 1). The chamber model was constructed using 1 cm thick transparency plastic board, to make chamber box with 28 cm widths, 36 cm lengths and 36 cm heights. The air inlet was flowed from one end of the experimental chamber, pass through the tested filter at the center, and the air outlet was collected from place at the other end. The input air was sprayed using nebulizer (model Aerofamily, Italy), and flowed through the filter. The air outlet at the other end of the chamber contained the built in electric fan, which could suck the input air pass through the filter to the other side of chamber to the air outlet. The air outlet was connected with modified air impinger which further connected with vaccuum air pump.

2. Preparation of inoculums of *M. tuberculosis* H37Ra

M. tuberculosis H37Ra (non human pathogenic strain) was used as the surrogate for human pathogenic *M. tuberculosis* strain. A loopful colony of *M. tuberculosis* H37Ra was grown in 15 ml Middlebrook 7H9 medium at 37 ºC for 7 days, and further adjusted the cell number to 10^8 cells/ml.

3. Preparation of inoculums of T7 bacteriophage

3.1 Amplification and preparation bacteriophage T7 lysate

Bacteriophage T7 was amplified by infection of high titer phage lysis to the *E. coli* BL21 culture. First 50 ml of Lubria-Bertani (LB) broth in 250 ml erlenmeyer flask was inoculated with single colony of *E. coli* BL21 and then shaked at 250 rpm over night at 37 ºC. After that, this overnight culture was diluted in 50 ml LB broth (1:100 dilution) and shaked at 37 ºC until Optical Density at 600 nm (OD600) reach 0.6-0.8. When *E. coli* grew into log phase, the culture was infected with 5 μl of high titer phage lysate and shake at 37 ºC until lysis. The T7 lysis was recognized by "clearing" of the culture, strings of cell, and then swirl to mix. The infected culture was centrifuged at 7,000 rpm for 15 mins. The supernatant was transferred to a new 50-ml centrifuged tube. Amount of phage in the supernatant was checked using phage titration method.

3.2 Titration of T7 phage lysate

The 100 fold serial dilution was performed in 1,000 μl volume. The T7 phage dilution was prepared by this mean to obtain all dilutions covered 10^{-2}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10}. One hundred microlitre of each dilution was added to 300 μl overnight culture of *E. coli* BL21. This BL21-T7 mixture was incubated at room temperature for 15 mins. After that, this infected *E. coli* was added to 3 ml LB-Top agar in 10 ml test tube and mixed by vortexing. The mixture was poured onto prewarmed (37 °C) LB agar plate. After the agar harden, the plate was turned and incubated at 37 °C for 3 hrs to observe the clear plaque. The numbers of plaque in each plate were counted and further calculated using the following formulae; Number of plaque × dilution series × 100 = pfu/ml.

4. Air sampling and detection

Five milliliters each of *M. tuberculosis* H37Ra (10^8 cells/ ml) inoculum, and inoculums of T7 phage (10^8 pfu/ ml), were respectively sprayed using nebulizer (model Aerofamily, Italy), for 30 mins. The electric fan at the outlet end of the chamber sucked the sprayed air pass through the filter to the other side of chamber to the outlet. This outlet was connected with modified air impinger that further connected with vacuum air pump (with flow rate at 40 L/min). The collecting air sample was trapped in distilled water in the impinger (20 ml for *M. tuberculosis*

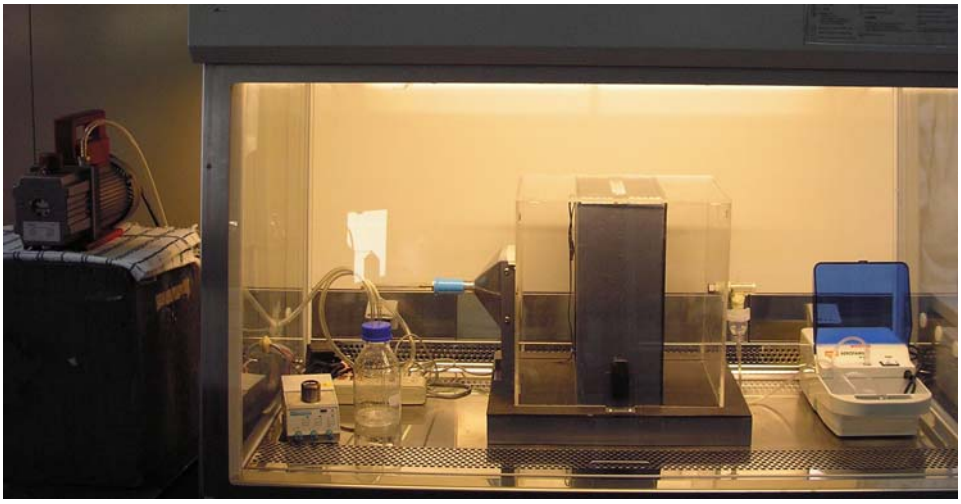

Figure 1. A laboratory setting for electronic air filter validation

H37Ra, and 35 ml for T7 bacteriophage (virus)). The collected bioaerosol samples trapped in distilled water of the impinger were further tested.

5. *Mycobacterium tuberculosis* H37Ra detection

5.1 DNA preparation

DNA of *M. tuberculosis* H37Ra was prepared from 20 ml of bioaerosol collected water in impinger air sample. The 20 ml of sample was filtered through 0.2 μm membrane filter (Isopore™, Ireland), using 25 ml syringe, then the cell pellet was rinsed with 800 μl of PBS, then 400μl was used for spread plate on Middlebrook 7H10 agar, and the other 400 μl was used for DNA extraction. Four-hundred microlitre of cell suspension was heated at 80 °C for 20 mins, to kill the cells. Fifty microlitres of lytic enzyme was added, and then incubated at 37°C overnight. After that, 75 μl of Proteinase K solution (5 μl Proteinase K and 70 μl of 10% SDS) was added and heat the sample at 65°C for 10 mins. One hundred microlitres of 5M NaCl and 100μl prewarmed CTAB/NaCl were added, and incubated at 65 °C for 10 mins. Then, 800 μl of Chloroform/Isoamyl alcohol (24:1) was added, mixed well and centrifuged at 14,000 rpm for 15 mins. The upper aqueous phase was transferred into a new 1.5 ml microcentrifuge tube and 800 μl of Phenol/Chloroform/Isoamyl alcohol (25:24:1) was added, mixed well and centrifuged at 14,000 rpm for 15 mins. After that, transferred supernatant into a new tube and added 450 μl of isopropanol, and then placed at -20 °C for 2-3 hrs or overnight. After that, the DNA pellet was spin down by centrifugation at 14,000 rpm for 15 mins. The supernatant was discarded. The pellet was washed with 1 ml of cold 70% ethanol, and centrifuged at 14,000 rpm for 15 mins. Air dried the pellet at room temperature for 15-30 mins, and then resuspended with 20μl of milliQ water and stored at -20 ºC until used.

5.2 Detection of *M. tuberculosis* by nested PCR

For *M. tuberculosis* detection from collected impinger air samples, nested PCR were performed. By this technique, two pairs of primer were used (outer and inner primer). For outer primers (rpoB-f and rpoB-r), this pair of primers was obtained from Kremer *et al*., 1997. This pair of primer was specific for *Mycobacterium* spp. and generated a 435 bp PCR product. For second PCR cycle (nested PCR), the forward primer of this second PCR cycle (nested PCR) was designed by using Primer 3 software (http://www.genome. wi.mit. edu/cgi-bin/primer/ primer3-www.cgi)
and analyzed by oligoanalyzer software. The reverse primer was obtained from Kim *et al*., 2001. The primers of the nested PCR were in the following sequences; rpoB-f (5′-TGG TCC GCT TGC ACG AGG GTC AGA-3′), rpoB-r (5′-CTC AGG GGT TTC GAT CGG GCA CAT-3′), rpoB-7 (5′-GAT CAC ACC GCA GAC GTT GA-3′) and TB-8 (5′-TGC ACG TCG CGG ACC TCC A-3′), respectively, that generate 195 bp of PCR product. Both of PCR reaction were performed in a final volume of 25 μl that comprised of 0.2 μM of each primer, 0.4 mM dNTP (dATP, dCTP, dGTP, dTTP), 0.5 U of Hot start *Taq* polymerase (QIAgen, USA) and PCR buffer (1.5 mM $MgCl_2$, Tris-Cl, KCl, $(NH_4)_2SO_4$).

The reactants were put in thermal cycler with a touchdown 6 steps program using the following scheme. First PCR cycle with primer rpoB-f and rpoB-r; Denaturation at 95 °C 15 mins. 95 °C 1 min., 72 °C 1 min., 72 °C 1 min., 2 cycles. 95 °C 1 min., 71 °C 1 min., 72 °C 1 min., 2 cycles. 95 °C 1 min., 70 °C 1 min., 72 °C 1 min., 2 cycles. 95 °C 1 min., 69 °C 1 min., 72 °C 1 min., 25 cycles. Final extension at 72 °C for 7 mins. Second PCR cycle with primer rpoB-7 and TB-8; Denaturation at 95 °C 15 mins. 95 °C 30 sec., 69 °C 30 sec., 72 °C 30 sec., 2 cycles. 95 °C 30 sec., 68 °C 30 sec., 72 °C 30 sec ., 2 cycles. 95 °C 30 sec., 67 °C 30 sec., 72 °C 30 sec., 2 cycles. 95 °C 30 sec., 66 °C 1 min., 72 °C 30 sec., 30 cycles. Final extension at 72 °C for 7 mins.

5.3 Detection of the amplified products by gel electrophoresis

Ten μl of each amplified PCR products were stained with the gel star (BMA, Rockband, ME, USA) for 15 mins and mixed with loading dye (0.25% Bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) before loading into 1.7% agarose gel (Seakem® LE agarose, Rockland, ME, USA). The electrophoresis was performed in 0.5×TBE buffer for 40 mins at 100 volt at room temperature. The gel was visualized using Dark Reader Transiluminator (Model DR-45M, BMA, Rockland, ME, USA) and photographed with a digital camera. The positive control, and negative control were always loaded to every gels. The presence of a band of expected size was identified by comparison with the Gene Ruler 100 bp DNA ladder plus molecular size marker (Fermentas, USA).

5.4 *M. tuberculosis* H37Ra culture as gold standard

After concentrating the particles of *M. tuberculosis* H37Ra from impinger water sample, 400 μl of resuspended sample was used for culturing. The resuspended sample was spread 100 μl each on four Middlebrook 7H10 agar plates, and incubated at 35-37 ºC for three week. After that colonies *M. tuberculosis* H37Ra were counted.

5.5 Evaluation the efficiency of electronic air filter

To validate the efficiency of electronic air filter for filtrating *M. tuberculosis* H37Ra from contaminated air, the experiment using the HEPA air filter and without air filter were performed by the same procedure in comparison to electronic air filter as mention in material and methods number 5.1-5.4.

5.6 Data analysis

Filtrating efficiency of each filter was calculated based on number of cultured *M. tuberculosis* that passed tested filter, as follow;
$(5\times10^8$ - number of MTB that pass through filter) × 100
divide by 5×10^8

6. T7 bacteriophage detection

6.1 DNA extraction

DNA of T7 was prepared from 35 ml of bioaerosols collected water in impinger air sample. The sterile water was gradually added to 3 ml of 5M NaCl and 6 ml of 50% Polyethylene glycol 8000 (PEG 8000), and mixed, then placed on ice for 30 mins. After that, it was subjected to centrifuge at 7,000 rpm for 20 mins, and carefully discarded supernatant. The phage pellet was resuspended with 400 μl (200 μl was used for plaque count on LB-TOP agar, and 200 μl was used for DNA extraction) phage supernatant buffer (1 M NaCl, 10 mM Tris-Cl, pH 8.0, 1 mM EDTA), then two hundred microliters of phage pellet was added 10 volumes of 10 mM EDTA, pH 8.0, and heat at 65 ºC for 10 mins, and allowed to cool to room temperature. Centrifugation at 14,000 rpm for 10 mins, then transferred the supernatant into a new eppendrof. After that 2.5 volumes of 100% ethanol was added, place at -20 ºC for 20 mins, and centrifuged at 14,000 rpm for 10 mins. The supernatant was removed, and the pellet was rinsed with 1 ml of 70% ethanol, then centrifugation at 14,000 rpm for 10 mins. The supernatant was removed, air dried the pellet at room temperature for 15-30 mins, and then resuspended with 20μl of Tris-HCl (pH 8.5) and stored at -20 ºC until used.

6.2 Detection of T7 by PCR

A PCR technique for amplification of the 10B capsid protein gene of bacteriophage T7 was generated a 133 bp product, using primers T7 select up (5′-AGC TGT

CGT ATT CCA GTC A-3′) and down (5′-ACC CCT CAA GAC CCG TTT A-3′) (T7 select System Manual, Novagen Incorporated, 2002). PCR reaction was performed in a final volume of 25 μl that comprised of 0.5 μM of each primers, 0.5 mM dNTP (dATP, dCTP, dGTP, dTTP), 1 U of *Taq* polymerase (RBC Bioscience) and PCR buffer (1.5 mM $MgCl_2$, Tris-Cl, KCl, $(NH_4)_2SO_4$)). The reactants were put in thermal cycler using the following scheme. Pre-denaturation at 94 °C, 2 mins. Denaturation 94 °C, 20 sec**.** Annealing 50 °C, 20 sec**.** Extention 72 °C, 45 sec**.** Final extension at 72 °C, 7 mins. Total 35 cycles**.**

6.3 Detection of the amplified products by gel electrophoresis

Procedure was performed as same as described in material and method (5.3).

6.4 T7 bacteriophage plaque count as gold standard

T7 phage from air sampling was counted for plaque forming on LB-Top agar plate. After concentrating the particles of T7 phage from each bioaerosols collected water sample, the particles were resuspended with 400 μl of phage supernatant buffer (1 M NaCl, 10 mM Tris-Cl, pH 8.0, 1 mM EDTA). Two hundred microliters of resuspended pellet was added to 300 μl overnight culture of *E. coli* BL21. This BL21-T7 was incubated at room temperature for 15 mins. After that, this infected *E. coli* were added to 3 ml LB-Top agar in 10 ml test tube and mixed by vortexing. The mixture was poured onto prewarmed (37ºC) LB agar plate. After the agar hardens, the plate were turned and incubated at 37 ºC for 3 hrs to observe the clear plaque, and the numbers of plaque in each plate were counted.

6.5 Evaluation the efficiency of electronic air filter

To validate the efficiency of the electronic air filter for filtrating T7 virus from contaminated air, the experiment using the HEPA air filter and without air filter were performed by the same procedure as electronic air filter as mention in material and methods number 6.1- 6.4.

6.6 Data analysis

Filtrating efficiency of each filter was calculated based on number of T7 plaque that passed tested filter, as follow; (5×10^8 - number of T7 plaque that pass through filter) × 100 divide by 5×10^8

Sample design and sample size

Thirty times each of bio-aerosols sampling experiment were performed for validation of electronic air filter, HEPA air filter and without air filter, respectively.

7. Validation of electronic air filter for filtrating other bacteria; *Staphylococcus aureus* and *Escherichia coli*

Since the experiment to validate an efficient of electronic air filter in filtrating *M. tuberculosis* H37Ra could not make conclusive proof, as *M. tuberculosis* was fastidious to grow, even in control experiment without membrane filter, the other microorgamnisms including *Staphylococcus aureus* (gram positive cocci, size 1 μm in diameter) and *Escherichia coli* (gram negative, size 2 μm in long and 0.5 μm in diameter) were thus performed for validation the efficiency of electronic air filter. The methods were performed as follow.

7.1 Experimental chamber model for electronic air filter validation

An experimental chamber model (Figure 1) was set for electronic air filter validation, by using *S. aureus* and *E. coli*, respectively. The experimental chamber model performed as same as described in material and method (1).

7.2 Preparation of inoculums of *S. aureus* and *E. coli*

S. aureus was cultured on Tryptic soy agar while *E. coli* was cultured on MacConKey agar plate, respectively. The single colony from the overnight culture plate was inoculated into 5 ml of Tryptic soy broth, and then shaking overnight at 35-37 °C. The 10 fold serial dilution was performed in 1,000 μl volume. One hundred microliter of overnight inoculum was added to the 900 μl 0.85% NSS (10^{-1}), and serial transfer was performed to obtain all dilution covered 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. A serial dilution series of bacterial strains was prepared. One hundred microliter of *S. aureus* or *E. coli* of each dilution was spreading on Tryptic soy agar or MacConKey agar plate and then incubated overnight at 35-37 ºC. The number of colonies was counted and further calculated using the following formulae; Number of colony × dilution series × 10 = cfu/ml

The overnight culture (approximately 10^9 cfu/ml) of *S. aureus* and *E. coli* were prepared to 10^5 cfu/ml and 10^4 cfu/ml, respectively. Five hundred microlitres of overnight culture was added into 4.5 ml of 0.85% NSS (10^8), and mixed by vortexing. The inoculum dilutions were prepared by this mean to obtain dilution until 10^5 cfu/ml for *S. aureus* and 10^4 cfu/ml for *E. coli*.

7.3. Air sampling and detection of *S. aureus* and *E. coli*

The inoculum of *S. aureus* (10^5 cfu/ml) and *E. coli* (10^4 cfu/ml) were individually sprayed using nebulizer (model Aerofamily, Italy), for 30 mins. The electric fan at the outlet end of the chamber sucked the sprayed air pass through the filter to the other side of chamber to the outlet. This outlet was connected with modified air impinger that further connected with vacuum air pump (with flow rate at 40 L/min). The collecting air sample was trapped in 30 ml of 0.85% NSS in the impinger. The collected bioaerosol sample trapped in 0.85% NSS of the impinger, were further for colony counted.

7.4 *S. aureus* and *E. coli* preparation

The cells of *S. aureus* and *E. coli* were prepared from 30 ml of bioaerosols collected water in impinger air sample. The 30 ml of sample was filtered through 0.2 μm membrane filter (Isopore™, Ireland), using 25 ml syringe, then the cell pellet was rinsed with 1,000 μl of PBS and mixed well.

7.5 *S. aureus* and *E. coli* culture as gold standard

After concentrating the particles of *S. aureus* and *E. coli* from impinger water sample, 100 μl of resuspended pellet was cultured by spreading on Tryptic soy agar and MacConKey agar plate, respectively. The serial dilution was prepared by one hundred microlitre of resuspended pellet added to the 900 μl 0.85% NSS (10^{-1}), and mix by vortexing. Then 100 μl of this dilution was added to 900 μl 0.85% NSS (10^{-2}), and mixed thoroughly. After that 100 μl of dilution 10^{-1} and 10^{-2} was cultured by spreading on Tryptic soy agar and MacConKey agar plate for *S. aureus* and *E. coli*, respectively. Colonies of *S. aureus* and *E. coli* were counted after incubated overnight at 35-37 ºC, and further calculated using the following formulae; Number of colony × dilution series × 10 = cfu/ml

7.6 Evaluation the efficiency of electronic air filter for filtrating *S. aureus* and *E. coli*

To validate the efficiency of electronic air filter for filtrating *S. aureus* and *E. coli* from contaminated air, the experiment using the HEPA air filter and without air filter were performed by the same procedure in comparison to electronic air filter as mention in material and methods (7.1-7.5).

7.7 Data analysis for filtrating *S. aureus* and *E. coli*

Filtrating efficiency of each filter was calculated based on number of cultured *S. aureus* that pass through

tested filter, as follow; (10^5 - number of bacteria that pass through filter) × 100 divide by 10^5

Filtrating efficiency of each filter was calculated based on number of cultured *E. coli* that pass through tested filter, as follow; (10^4 - number of bacteria that pass through filter) × 100 divide by 10^4

Sample design and sample size

Three times each of bio-aerosols sampling experiment were performed for validation of electronic air filter, HEPA air filter and without air filter for filtrating *S. aureus* and *E. coli*, respectively.

RESULTS

1. Validation of electronic air filter for filtrating *M. tuberculosis* H37Ra.

1.1 Detection of *M. tuberculosis* by nested PCR and culture

Thirty reproduced samples were collected from 30 spraying experiments with *M. tuberculosis* H37Ra inoculum of 5×10^8 cells/ml as described earlier. DNAs were extracted from the air-trapped DW using conventional CTAB procedure. The detection of *M. tuberculosis* H37Ra by nested PCR was performed. *M. tuberculosis* H37Ra from air samplings were cultured on Middlebrook 7H10 agar plate. Growth of *M. tuberculosis* H37Ra colony was detected in 4 out of 30 air samples. In each of positive culture plate, there was only one colony (1 cfu) of *M. tuberculosis* H37Ra. Although, twenty six samples were negative for growth, DNA extracted from all 30 samples gave positive reaction to *M. tuberculosis*.

2. Evaluation the efficiency of electronic air filter for filtrating *M. tuberculosis* H37Ra

Validation of the electronic air filter for filtrating *M. tuberculosis* H37Ra from contaminated air was performed in comparison to the experiments using the HEPA air filter and without air filter by the same procedure as electronic air filter.

2.1 Detection of *M. tuberculosis* by nested PCR and culture in condition with HEPA air filter

Thirty reproduced samples were collected from 30 spraying experiments with *M. tuberculosis* H37Ra inoculum of 5×10^8 cells/ml as described earlier. DNAs were extracted from the air-trapped DW using conventional CTAB procedure. The detection of *M. tuberculosis* H37Ra by nested PCR was performed. *M. tuberculosis* H37Ra from air samplings were cultured on Middlebrook 7H10 agar plate. Growth of *M. tuberculosis* H37Ra colony was detected in 1 out of 30 air samples, and there was only one colony (1 cfu) of *M. tuberculosis* H37Ra. Although, twenty nine samples were negative for growth, DNAs extracted from all 30 samples gave positive reaction to *M. tuberculosis*.

2.2 Detection of *M. tuberculosis* by nested PCR and culture in condition without air filter

Thirty reproduced samples were collected from 30 spraying experiments, *M. tuberculosis* H37Ra inoculum of 5×10^8 cells/ml as described earlier. *M. tuberculosis* H37Ra from air samplings were cultured on Middlebrook 7H10 agar plate. Growth of *M. tuberculosis* H37Ra colony was detected in 5 out of 30 air samples and there was only one colony (1 cfu) of *M. tuberculosis* H37Ra. Although, twenty five samples were negative for growth, DNAs extracted from all 30 samples gave positive reaction to *M. tuberculosis*.

3. Sensitivity of nested PCR for *M. tuberculosis* H37Ra detection

The lowest number of *M. tuberculosis* H37Ra DNA that could be detected by nested PCR was determined. DNA of *M. tuberculosis* H37Ra at 1 *f*g, 10 *f*g, 100 *f*g, 1 *p*g, 10 *p*g, 100 *p*g, and 1 *n*g were used as templates. Using nested PCR, as lowest as 10 *f*g (equal to 2 bacill) *M. tuberculosis* H37Ra DNA could be detected.

4. Validation of electronic air filter for filtrating T7 virus.

4.1 Detection of T7 virus by PCR and plaque count as gold standard

Thirty reproduced samples were collected from 30 spraying experiments, T7 virus inoculum of 5×10^8 pfu/ml as described earlier. DNAs were extracted from the air-trapped DW using conventional heat treatment and ethanol precipitation procedure. The detection of T7 virus by PCR was performed. T7 virus from air samplings were cultured on LB agar plate. Plaques of T7 virus were detected in 30 out of 30 air samples. The average plaques of 30 samples were 328 pfu, and DNAs extracted from all 30 samples gave positive reaction to T7 virus**.** The filtrating efficiency of electronic air filter for filtrating T7 virus was shown 99.99% (data not shown).

5. Evaluation the efficiency of electronic air filter for filtrating T7 virus

Validation of the electronic air filter for filtrating T7 virus from contaminated air was performed in comparison to the experiment using the HEPA air filter and without air filter by the same procedure as electronic air filter.

5.1 Detection of T7 virus by PCR and plaque count in condition with HEPA air filter

Thirty reproduced samples were collected from 30 spraying experiments, T7 virus inoculum of 5×10^8 pfu/ml as described earlier. DNAs were extracted from the air-trapped DW using conventional heat treatment and ethanol precipitation procedure. The detection of T7 virus by PCR was performed, and the results were shown in figure 12. T7 virus from air samplings were cultured on LB agar plate. Plaques of T7 virus were detected in 30 out of 30 air samples. The average plaques of 30 samples were 28 pfu, and DNAs extracted from all 30 samples gave positive reaction to T7 virus**.** The filtrating efficiency of HEPA air filter for filtrating T7 virus was shown 99.99% (data not shown).

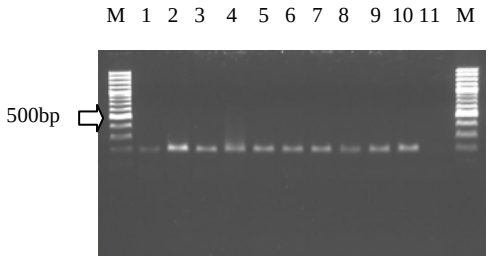

Figure 12. Agarose gel electrophoresis of PCR product of T7 virus (133 bp).

Lane M: DNA ladder 100 bp.
Lane 1: Positive control T7virus DNA concentration 1 *p*g.
Lane 2, 3, 4: Spraying T7 virus in condition without filter number T7C17, T718, T726
Lane 5, 6, 7: Spraying T7 virus in condition with HAPA filter number T7H16, T717, T718
Lane 8, 9, 10: Spraying T7 virus in with condition electronic filter number T7E10, T711, T712
Lane 11: Negative control.

5.2 Detection of T7 virus by PCR and plaque count in condition without air filter

Thirty reproduced samples were collected from 30 spraying experiments, T7 virus inoculum of 5×10^8 pfu/ml as described earlier. DNAs were extracted from the air-trapped DW using conventional heat treatment and ethanol precipitation procedure. The detection T7 virus by PCR was performed. T7 virus from air samplings were cultured on LB agar plate. Plaques of T7 virus were detected in all samples and were uncountable. In addition DNAs extracted from all 30 samples gave positive reaction to T7 virus**.**

Figure 13. Plaques of T7 bacteriophage

6. Sensitivity of PCR for T7 virus detection

The lowest number of T7 virus DNA that could be detected by PCR was determined. Ten-fold serial dilution of T7 virus DNA at 1 *f*g, 10 *f*g, 100 *f*g, 1 *p*g, 10 *p*g, 100 *p*g, and 1 *n*g were used as templates. Using PCR, as lowest as 1 *p*g of T7 virus DNA could be detected.

7. Validation of electronic air filter for filtrating other bacteria; *Staphylococcus aureus* and *Escherichia coli*

Since the experiment to validate an efficiency of electronic air filter in filtrating *M. tuberculosis* H37Ra could not make conclusive proof, as *M. tuberculosis* was fastidious to grow, even in control experiment without filtrating membrane,the other microorganisms including *Staphylococcus aureus* (gram positive cocci, size 1 μm in diameter) and *Escherichia coli* (gram negative, size 2 μm in long and 0.5 μm in diameter) were thus performed for validation the efficiency of electronic air filter for filtrating microorganisms contaminated in the air. In addition, the efficiency of electronic air filter were compared to the efficiency of HEPA air filter and without air filter. The results were shown as below.

7.1 Validation of electronic air filter for filtrating *S. aureus*

S. aureus from air samplings were cultured on Tryptic soy agar plate. Colonies of *S. aureus* were detected in all three samples with the average of 97 cfu**,** and the efficiency of electronic air filter for filtrating *S. aureus* was shown in table 1.

7.2 Validation of HEPA air filter for filtrating *S. aureus*

S. aureus from air samplings were cultured on Tryptic soy agar plate. Colonies of *S. aureus* were detected in all samples with the average of 86 cfu**,** and the filtrating efficiency of HEPA air filter for filtrating *S. aureus* was shown in table 1.

7.3 Detection of *S. aureus* by culture in condition without air filter

S. aureus from air samplings in condition without air filter were cultured on Tryptic soy agar plate. Colonies of *S. aureus* were uncountable in all samples.

Table 1. The efficiency of electronic air filter and HEPA air filter for filtrating T7 virus, *S. aureus and E. coli*

Sample	Electronic air filter		
	Filtrate	**Trapping plaque count**	**Filtrating efficiency (%)**
T7 virus			
Electronic air filter	328 pfu	(5×10^8) - 328 = 4.99999672 $\times 10^8$ pfu	99.9999344
HEPA air filter	28 pfu	(5×10^8)- 28 = 4.99999972 $\times 10^8$ pfu	99.999944
S. aureus			
Electronic air filter	97 cfu	10^5 - 279 = 99,903 cfu	99.903
HEPA air filter	86 cfu	10^5 - 86 = 99,914 cfu	99.914
E. coli			
Electronic air filter	70 cfu	10^4 - 75 = 9,930 cfu	99.30
HEPA air filter	48 cfu	10^4 - 48 = 9,952 cfu	99.52

7.4 Validation of electronic air filter for filtrating *E. coli*

E. coli from air samplings were cultured on MacConkey agar plate. Colonies of *E. coli* were detected in all samples. The average colonies of all three samples were 70 cfu**,** and the efficiency of electronic air filter for filtrating *E. coli* was shown in table 1.

7.5 Evaluation the efficiency of electronic air filter for filtrating *E. coli*

Validation of the electronic air filter for filtrating *E. coli* from contaminated air was performed in comparison to the experiments using the HEPA air filter and without air filter used the same procedure as electronic air filter.

7.6 Validation of HEPA air filter for filtrating *E. coli*

E. coli from air samplings were cultured on MacConkey agar plate. Colonies of *E. coli* were detected in all samples. The average colonies of all three samples were 48 cfu**,** and the efficiency of HEPA air filter for filtrating *E. coli* was shown in table 1.

7.7 Detection of *E. coli* by culture in condition without air filter

E. coli from air sampling were cultured on MacConkey agar plate. Colonies of *E. coli* were uncountable in all samples.

DISCUSSION

In this study, the results of T7 culture could be used for comparing the filtrating efficiency of electronic air filters, since the numbers of T7 plaque could be counted and compared with that of HEPA filter (table 8). Both electronic and HEPA air filters could filter T7 virus (particle size at 0.04 μm) with filtrating efficiency more than 99 % (table 1). The filtrating efficiency of electronic air filter reported in this study is higher than that of the product specification which reported 98% filtrating efficiency (www.alpinefilter.com), and also higher than that of recently validation of electronic air filter (alpine model PT 400) in filtrating *Staphylococcus epidermidis*, *Bacillus subtilis*, *Penicillium citrinum*, *Aspergillus niger* (Limmongkol *et al*, 2008). In that study, the electronic air filter could reduce aerosol *B. subtilis* from 1,600 colonies to be only 20 colonies (98.75% filtrating efficiency) within 1 hr, reduced *P. citrinum* from 1,600 to 1 colony (99.93% filtrating efficiency) within 40 mins, *S. epidermidis* from 1,600 to 1 colony (99.93% filtrating efficiency) within 20 mins and *A. niger* from 1,600 to 1 colony (99.93% filtrating efficiency) within 40 mins (Limmongkol *et al*, 2008).

The filtrating efficiency revealed from this study is also higher than that of Foarde *et al* (1999), who tested the Amway air filter model E2526J for filtrating *B. subtilis, S. epidermidis, P. chrysogenum, Cladosporium sphaerospermum* and bacteriophage MS2, which obtained filtrating efficiency from 95-98 %.

The efficiency of a portable indoor air cleaner in removing pollens (particle diameters ranging from 20-30 μm) and fungal spores (particle size at 8-45 μm) was tested by Cheng *et al* (1998). The result clearly showed that a commercial portable indoor air cleaner with a HEPA filter and activated charcoal prefilter system was effective in removing large pollens and spores > 8O%. In addition, the results suggested that this air cleaner was more effective when doors and windows are closed, especially when there was no activities in the room.

The collection efficiency of bioaerosol may affect to bioaerosol sampler. There are many affecs such as air sampling technique. Grinshpun *et al* (1996) reported that the level of the collection fluid may significantly affect the physical and the biological collection efficiencies of impingers. The liquid evaporation during prolonged impingement may affect the impinger performance. At relatively high flow rates and low levels of collection fluid (which may result from the evaporation of most of the liquid), the liquid under the impinger jet was observed to be removed by the air pressure and pushed against the impinger's walls. Particles may thus bounce from the bottom of the collection vessel and escape with the effluent flow or may impact sidewise into the liquid and remain collected. The bursting of the bubbles rising through the impinger liquid may cause particle re-aerosolization that reduces the collection efficiency of impingers. Limits of impinger are including the sampling time and the initial volume of the collection fluid. While Lin *et al* (1999) compared the efficiency of bioaerosol sampling between Impingement and BioSampler. They found that conventional sampling of bioaerosols into liquid impingers can only be performed with water or another low-viscosity liquid as the collection medium. Since, these liquid impingers evaporate quickly and reaerosolization, sampling is generally limited to short-time periods of 15-30 min.

In general, the conventional PCR result is qualitative which is not quantifiable. However, the detection limit of nested PCR used in this study has been optimized to detect at least 10 *f*g of MTB (equal to 2 bacilli), so it could be assumed that if only 2 MTB bacilli can pass through the filter, the PCR result would be positive. As seen from the result of nested PCR for MTB detection, all samples were positive which was not corresponded to the culture result according to the nested PCR was too sensitive for detecting MTB.

In this study, the concentration of sprayed organisms was too high (5×10^8 cells each of MTB and T7), as electronic filter has filtrating efficiency at 98 %. So it can be calculated that 2% of 5×10^8 cells that can not filter are 2×10^6 cells, which are also high amount numbers. For further study, the amount of sprayed organisms should be lower than this study, and the different concentration of sprayed organisms from 10^6, 10^5, 10^4, 10^3, and 100 cells should be used, then a better interpretable

result may be given. Moreover, the quantitative PCR should be use instead of qualitative PCR.

Eventhough, the suitable media Middlebrook M7H10 agar was used for culture *M. tuberculosis*, but colonies of *M. tuberculosis* were detected in only 10 out of 90 samples. This may be due to the aerosolization stress (dessication and injury of aerosolized bacteria) from collecting air sampler that cause *M. tuberculosis* fail to culture (Schafer *et al*, 1999), and as Wan *et al* (2004) described reasons of failing in culture aerosolized Mycobacteria in that (1) inherent difficulties in culturing aerosolized tubercle bacilli; (2) the tubercle bacilli may be viable and infectious but not culturable; and (3) the content of the airborne mycobacteria may be too low to detect using conventional bioaerosol sampling methods. Moreover, *M. tuberculosis* was fastidious to grow.

Since the experiment to validate an efficient of electronic air filter in filtrating *M. tuberculosis* H37Ra could not make conclusive proof, as *M. tuberculosis* was fastidious to grow, even in control experiment without filtrating membrane, and PCR result could not be quantifiable. So the additional experiment using other microorganisms including *S. aureus* (10^5 cfu sprayed) and *E. coli* (10^4 cfu sprayed) followed by non filtered bacterial cultured, were thus performed for validation the efficiency of electronic air filter.

The culture result of electronic air filter for filtrating *S. aureus* could be used for comparing with the result of HEPA air filter. The result was shown that the electronic air filters could filter *S. aureus* (particle size at 1 μm) with filtrating efficiency at 99.90%, while HEPA air filter showed the filtrating efficiency at 99.91%. The number of *E. coli* colony could be counted and compared between that of electronic air filters and HEPA air filter. Both electronic and HEPA air filters could filter *E. coli* (particle size at 2μm) with filtrating efficiency at 99.30 and 99.52 % respectively. These results also similar to the results reported by Kudo *et al* (2007) who found that air purifiers employing the rectangular columnstructure titaniumoxide photocatalyst for air purifier wall hanging, Model (BF-H201A) showed high performance for bacteria elimination in air. Photocatalysts air purification system was tested that it can elimination Influenza virus A 99.00%, *E. coli* 99.95% and, *S. aureus* 99.94 %.

However, as clearly seen from the culture results of T7, *S. aureus* and *E. coli*, both electronic and HEPA air filter showed more than 99 % in filtrating efficiency. So this study proved that electronic air filter can be used for removing bioaerosal from indoor air, as the well known long time used HEPA filter.

CONCLUSION

The validation of electronic air filter for filtrating *M. tuberculosis*, T7 virus, and the other bacteria like *S. aureus* and *E. coli* from contaminated air since the experiment to validate the efficiency of electronic air filter in filtrating *M. tuberculosis* H37Ra could not make a conclusive proof, as *M. tuberculosis* was fastidious to grow could be concluded as follow, Using T7 plaque count, both electronic and HEPA air filters could filter T7 virus (with particle size at 0.04 μm) with filtrating efficiency more than 99 %. Using colony count, both electronic and HEPA air filters could filter the other bacteria like *S. aureus* (particle size 1 μm) and *E. coli* (particle size 2 μm) with filtrating efficiency more than 99 %. Conventional PCR gave qualitative result and not quantifiable, which may not suitable for this study. To compare the filtrating efficiency of each air filter using PCR, quantitative PCR should be use instead of conventional qualitative PCR.

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