EVALUATION OF AN ELECTRONIC AIR FILTER FOR FILTRATING BACTERIA AND VIRUSES FROM INDOOR AIR

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Abstract. This study compared the filtrating efficiency (FE) of a commercial electronic air filter for filtering bacteria and viruses from contaminated air with a high efficiency particulate air (HEPA) filter. An enclosed chamber was constructed, in the middle of which an air filter was placed for testing. MTB H37Ra and T7 virus at concentrations of $5x10^8$ each were sprayed into one side of the chamber using a nebulizer and the sprayed samples were collected by an impinger air-sampler on the other side. MTB and T7 viruses were detected by PCR and culture. The PCR could detect samples down to 10 *f*g for MTB H37Ra and 1 *p*g for T7 virus. Most MTB H37Ra sprayed failed to culture. *S. aureus* at a concentration of 10^5 cfu and *E. coli* at a concentration of 10^4 cfu along with T7 virus were filtered out with a FE of more than 99%. T7 virus has a particle size of 0.04μ m, *S. aureus* has a particle size of 1μ m and *E. coli* has a particle size of 2μ m.

INTRODUCTION

Aerosol transmission of *Mycobacterium tuberculosis* (MTB) and viruses that cause respiratory infection has been increasing. Airborne transmission occurs when bacteria or viruses adhere to dust particles or on small respiratory droplets that may become aerosolized. Airborne bacteria and viruses are transmitted when people cough, sneeze, talk, laugh, sing or exhale. Pathogenic microorganisms transmitted by air include mycobacteria (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 res-

Tel: +66 (0) 2354 9100; Fax: +66 (0) 2354 9167. E-mail: tmprt@mahidol.ac.th piratory syndrome (SARS) (Drosten *et al*, 2003). TB 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 TB, and new infections occur at a rate of one per second (WHO, 2007) (<u>http:</u>//www.who.int/mediacentre/factsheet/fs104/en/index.htn).

Experiments for detecting TB and viruses in contaminated air have been carried out. *M. tuberculosis* H37Ra, an attenuated tubercle, has been used as a surrogate microorganism representing MTB (Brosch *et al*, 1999), and the bacteriophage T7, a double stranded DNA virus, has been used as a surrogate for mammalian viruses in contaminated air (Tseng and Li, 2005). Other diagnostic teachniques, such as polymerase chain reaction (PCR) and nested PCR have been employed to detect bacteria and viruses in air samples (Alvarez *et al*, 1994; Sawyer *et al*, 1994).

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A novel electronic air filter developed by Alpine company, Thailand, is a non-ionizing electronic air filter system, which is claimed to be able to remove particulates of dangerous microorganisms, such as TB and viruses, from the air. However, no studies have been performed to validate this product. This study aimed to test the filtrating efficiency (FE) of the electronic air filter from Alpine company in filtrating TB, Staphylococcus aureus, Escherichia coli and T7 virus from indoor air. The results of this study provide information regarding the FE of this air filter for both the consumer and the manufacturer. The authors declare no conflict of interest.

MATERIALS AND METHODS

Experimental model

An experimental chamber was made from 1 cm thick transparent plastic board, with dimensions of 28 (W) x 36 (L) x 36 (H) cm (Fig 1). The tested filter was located in the center of the chamber. A nebulizer (Aerofamily, Italy) was used to nebulize the test inoculums at the inlet side. The outlet side was integral with an exhaust fan, which sucked the input air through the filter (40 liters/minute), and the air sample was collected from the outlet side with a modified air impinger, which was connected to a vacuum air pump. This experimental system was located in a biosafety cabinet type II (Molten Maxisafe 2000 Modle 1-2, Denmark).

Preparation of *M. tuberculosis* H37Ra inoculum

M. tuberculosis strain H37Ra (non pathogenic strain) was used as a surrogate for pathogenic TB. A loopful of H37Ra TB was grown in 15 ml Middlebrook 7H9 medium at 37°C for 7 days and the inoculum was adjusted to a concentration of 10⁸ cells/ml.

Preparation of inoculums of T7 bacteriophage inoculum

Amplification and preparation of bacteriophage T7 lysate. The bacteriophage T7 was amplified by infection in an *E. coli* BL21 culture. Fifty mililiters Lubria-Bertani (LB) broth was inoculated with a single colony of *E. coli* BL21, then shaken at 250 rpm over night at 37°C. The overnight culture was di-



Fig 1–A laboratory setting for electronic air filter validation.

luted in 50 ml of LB broth and shaken at 37°C until an optical density of 0.6-0.8 at 600 nm (OD600) was reached. The culture was then infected with 5 μ l of high titer phage and shaken at 37°C until lysis. The infected culture was centrifuged at 4,400*g* for 15 minutes. The amount of phage in the supernatant was determined using a phage titration method.

Titration of T7 phage lysate. One hundred microliters each of T7 phage dilutions 10^{-2} , 10^{-4} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} , were added to 300 µl of *E. coli* BL21 culture. This BL21-T7 mixture was incubated at room temperature for 15 minutes. The infected *E. coli* solution was added to 3 ml of LB-Top and mixed by vortexing. The mixture was poured onto a prewarmed (37°C) LB agar plate. After the agar hardened, the plate was incubated at 37°C for 3 hours and observed for clear plaques. The number of plaques in each plate was counted and used in the following calculation: number of plaques x dilution series x 100 = pfu/ml.

Air sampling and detection

Five mililiters of each MTB H37Ra (10⁸ cells/ml) inoculum or inoculum of T7 phage (10⁸ pfu/ml) was sprayed using a nebulizer for 30 minutes. The air sample was trapped in distilled water in the impinger (20 ml for MTB H37Ra and 35 ml for the T7 bacteriophage).

MTB H37Ra detection

DNA preparation. The 20 ml sample was filter d through a 0.2 μ m membrane filter (IsoporeTM, Ireland) using a 25 ml syringe, then the cell pellet was rinsed with 800 μ l of PBS, 400 μ l of which was spread on a Middlebrook 7H10 agar plate, and the other 400 μ l were used for DNA extraction. Four hundred microliters of cell suspension was heated at 80°C for 20 minutes. Fifty microliters of lytic enzyme was added, and the solution was incubated at 37°C overnight.

Then, 75 µl of Proteinase K solution (5 µl Proteinase K and 70 µl of 10% SDS) were added and the sample was heated at 65°C for 10 minutes. One hundred microliters of 5M NaCl and 100 µl of prewarmed CTAB/ NaCl were added, and the solution was incubated at 65°C for 10 minutes. Then, 800 µl of chloroform/isoamyl alcohol (24:1) was added and the solution was centrifuged at 16,000g for 15 minutes. The upper aqueous phase was removed and 800 µl of phenol/ chloroform/isoamyl alcohol (25:24:1) were added and the mixture was centrifuged at 16,000g for 15 minutes. The supernatant was removed and 450 µl of isopropanol was added. The solution was then stored at -20°C for 2-3 hours or overnight. The DNA was pelleted by centrifugation at 16,000g for 15 minutes. The pellet was washed with 1 ml cold 70% ethanol, sedimented at 16,000g for 15 minutes, resuspended in 20 μ l of milliQ water and stored at -20°C until used.

Detection of MTB by nested PCR. Two pairs of primer were used (outer and inner primers). The outer primers (rpoB-f and rpoB-r) were those specified by Ramasoota et al (2006). This pair of primers was specific for Mycobacterium spp and generated a 435 bp PCR product. For the second PCR cycle, the forward primer was designed using Primer 3 software (http://www.genome.wi.mit.edu/ cgi-bin/primer/primer3-www.cgi) and analyzed with oligoanalyzer software. The reverse primer was specified by Kim et al (2001) and produced a 195 bp amplicon. The sequences of the primers were: 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': TB-8 5'-TGC ACG TCG CGG ACC TCC A-3'. Both PCR reactions were performed in a final volume of $25 \ \mu$ l comprising $0.2 \ \mu$ M of each primer, 0.4mM dNTP, 0.5 U of Hot start Taq polymerase (QIAgen, USA) and PCR buffer [1.5 mM

MgCl₂, Tris-HCl, KCl, (NH₄)₂SO₄]. Thermo cycling was performed in a GeneAmp 2400 PCR system (Perkin Elmer Cetus Corp, Norwalk, CT) with a touchdown 6 step program as follow: in the first PCR cycle using primers rpoB-f and rpoB-r denaturation was performed at 95°C for 15 minutes, followed by 2 cycles of 95°C for 1 minute, 72°C for 1 minute, 72°C for 1 minute; 2 cycles of 95°C for 1 minute, 71°C for 1 minute, 72°C for 1 minute; 2 cycles of 95°C for 1 minute, 70°C for 1 minute, 72°C for 1 minute; 25 cycles of 95°C for 1 minute, 69°C for 1 minute, 72°C for 1 minute and final extension at 72°C for 7 minutes. In the seconds PCR cycle using primer rpoB-7 and TB-8, denaturation was performed at 95°C for 15 minutes, followed by 2 cycles of 95°C for 30 seconds, 69°C for 30 seconds, 72°C for 30 seconds; 2 cycles of 95°C for 30 seconds, 68°C for 30 seconds, 72°C for 30 seconds; 2 cycles of 95°C for 30 seconds, 67°C for 30 seconds, 72°C for 30 seconds, 30 cycles of 95°C for 30 seconds, 66°C for 1 minute, 72°C for 30 seconds, and final extension at 72°C for 7 minutes.

Ten µl of each amplified PCR product was mixed with Gel Star solution (BMA, Rockland, ME) and loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol). Electrophoresis on 1.7% agarose gel (Seakem[®] LE agarose, Rockland, ME) was performed for 40 minutes at 100 volts at room temperature. The gel was visualized using a Dark Reader Transiluminator (Model DR-45M, BMA, Rockland, ME) and photographed. Positive and negative controls were loaded in every gel. 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).

MTB H37Ra culture as gold standard. After concentrating particles of MTB H37Ra from 400 μ l of the impinger water sample, a 100 μ l

aliquot of resuspended sample was spread on 4 Middlebrook 7H10 agar plates, and incubated at 35-37°C for three weeks. Then the MTB H37Ra colonies were counted.

T7 bacteriophage detection. DNAT7 was prepared from 35 ml of bioaerosol - collected water in an impinger air sampler. The collected 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 minutes. After that, it was subjected to centrifugation at 4,400g for 20 minutes. The phage pellet was resuspended in 400 µl (200 µl was used for plaque counting on LB-TOP agar, and 200 µl for DNA extraction) of phage supernatant buffer (1 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA). For DNA extraction, 200 µl of phage suspension was added to 10 volumes of 10 mM EDTA at a pH of 8.0, and heated at 65°C for 10 minutes, and allowed to cool to room temperature. The solution was centrifuged at 16,000g for 10 minutes, then the supernatant was transferred to 2.5 volumes of 100% ethanol. stored at -20°C for 20 minutes, and centrifuged at 16,000g for 10 minutes. The pellet was rinsed with 1 ml 70% ethanol, then centrifugation at 16,000g for 10 minutes, resuspended with 20 µl Tris-HCl (pH 8.5) and stored at -20°C until used.

A PCR technique for amplification of the 10B capsid protein gene of bacteriophage T7 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). The PCR reaction was performed in a final volume of 25 μ l comprised of 0.5 μ M of each primer, 0.5 mM dNTP, 1 U of *Taq* polymerase (RBC Bioscience) and PCR buffer [1.5 mM MgCl₂, Tris-HCl, KCl, (NH₄)₂SO₄]. The reactants were placed in a thermal cycler using the following cycling conditions: pre-denaturation at 94°C for 2 minutes followed by 35 cycles of 94°C for 20 seconds, 50°C for 20 seconds, 72°C for 45 seconds and a final extension at 72°C for 7 minutes. Detection of primers by gel electrophoresis was conducted as described above.

T7 bacteriophage plaque count as a gold standard

Two hundred microliters of resuspended phage were added to 300 μ l of an overnight culture of *E. coli* BL21. This BL21-T7 solution was incubated at room temperature for 15 minutes. The infected *E. coli* was added to 3 ml of LB-Top agar and poured onto a prewarmed (37°C) LB agar plate. After the agar hardened, the plate was incubated at 37°C for 3 hours and the number of plaques in each plate were counted as described above.

Evaluation the efficiency of electronic air filter

To validate the efficiency of the electronic air filter for filtrating MTB H37Ra and T7 virus from contaminated air, experiments using and not using the HEPA air filter were performed.

The filtrating efficiency (FE) of each filter for the T7 that passed through the tested filter was calculated as $(5x10^8 - number of T7 plaques) x 100 divided by <math>5x10^8$.

Sample design and sample size

Thirty bio-aerosol sampling experiments were performed to evaluate the electronic air filter and HEPA air filter.

Validation of electronic air filter for filtrating *Staphylococcus aureus* and *Escherichia coli*

S. aureus was cultured on tryptic soy agar and *E. coli* was cultured on MacConkey agar. The *S. aureus* (10^5 cfu/ml) and *E. coli* (10^4 cfu/ml) were prepared and used for the test. The *S. aureus* and *E. coli* suspensions were sprayed using nebulizer for 30 minutes

3 times each, and the air sample was trapped in 30 ml of 0.85% saline in the air impinger. The S. aureus and E. coli were concentrated by filtering through a 0.2 µm membrane filter (IsoporeTM, Ireland), using a 25 ml syringe, then the cell pellet was rinsed with 1,000 µl of PBS and mixed. After that, the pellet was resuspended in 100 µl and cultured by spreading on tryptic soy agar and MacConkey agar to detect S. aureus and E. coli, respectively. In addition, a ten-fold serial dilution of the resuspended pellet was prepared and 100 µl of the 10⁻¹ and 10⁻² suspensions were cultured. Colonies of S. aureus and E. coli were counted after incubation overnight at 35-37°C, and further calculated using the following formula: number of colonies x dilution series x 10 = cfu/ml. The FE against S. aureus and E. coli with the electronic air filter was compared with the HEPA filter.

RESULTS

Evaluation of the electronic air filter in filtrating MTB H37Ra

Thirty spraying cycles with MTB H37Ra inoculum at a concentration of 5x10⁸ cells/ ml were carried out and evaluated by nested PCR and culturing. MTB H37Ra colony was detected in cultures of 4 out of 30 air samples. In each positive culture plate, only one colony (1 cfu) of MTB H37Ra was found. All 30 samples were positive on nested PCR for MTB.

In the 30 cycles with MTB H37Ra at a concentration of $5x10^8$ cells/ml using the HEPA air filter, growth of MTB H37Ra was detected in 1 sample, and there was only one colony (1 cfu) of MTB H37Ra on that culture plate. Nested PCR amplification gave positive results for MTB in all 30 samples.

Using nested PCR, the lowest concentration of MTB H37Ra DNA detectable is 10 fg (equal to 2 bacilli).

In the 30 cycles with MTB H37Ra at a concentration of 5×10^8 cells/ml without using an air filter, growth of MTB H37Ra was detected in 5 out of 30 samples, there was only one colony (1 cfu) of MTB H37Ra per plate. Nested PCR amplification gave positive results for *M. tuberculosis* in all 30 samples.

Validation of electronic air filter for filtrating T7 virus

Thirty spraying cycle with T7 virus at a concentration of 5×10^8 pfu/ml were carried out and evaluated by PCR and plaque counts. The PCR gave positive results for T7 virus on all 30 samples, and 1 *p*g of T7 virus DNA was detected. Plaques of T7 virus were detected in all samples. The average number of plaques was 328 pfu. The filtrating efficiency of the electronic air filter for filtrating T7 virus was estimated to be 99.9%.

In the 30 spraying cycles with T7 virus at a concentration of 5x10⁸ pfu/ml using a HEPA air filter, PCR gave positive results for T7 virus and plaques of T7 virus were detected in all samples. The average plaque number was 28 pfu. The filtrating efficiency of the HEPA air filter for filtrating T7 virus was 99.9%.

Thirty spraying cycles with T7 virus at a concentration of 5x10⁸ pfu/ml without an air filter were carried out and evaluated by PCR and plaque counts. PCR gave positive results for T7 virus in all 30 samples. Plaques of T7 virus were detected in all samples (too numerous to count).

Validation of electronic air filter for filtrating *Staphylococcus aureus* and *Escherichia coli*

Since the experiment to validate the efficiency of the electronic air filter against MTB H37Ra did not give conclusive results, as MTB is fastidious to grow, *Staphylococcus aureus* (gram- positive cocci, 1 μ m in diameter) and *Escherichia coli* (gram-negative,

 $2~\mu m$ in length and 0.5 μm in diameter) were used to evaluate the FE of the electronic air filter.

Colonies of *S. aureus* were detected with averages of 97 cfu, 86 cfu and too numerous to count cfu with the electronic air filter, HEPA air filter and without air filter, respectively. The efficiencies of the electronic air filter and HEPA air filter for filtrating *S. aureus* were both 99.9%.

Colonies of *E. coli* were detected in all samples with averages of 70 cfu, 48 cfu and too numerous to count using the electronic air filter, HEPA air filter and without air filter, respectively. The efficiencies of the electronic air filter and HEPA air filter for filtrating *E. coli* were 99.3% and 99.5%, respectively.

DISCUSSION

In this study, T7 cultures were used to compare the F.E. of the electronic air filter, with the HEPA air filter. Both the electronic and HEPA air filters filtered out the T7 virus (particle size at 0.04 μ m) with a FE of greater than 99%. The F.E. of the electronic air filter in this study was higher than the reported product specification of 98% F.E. (www.alpinefilter. com), and higher than that of a recent validation of an electronic air filter (alpine model PT 400) for filtering out Staphylococcus epidermidis, Bacillus subtilis, Penicillium *citrinum*, and *Aspergillus niger*. In that study, the electronic air filter reduced aerosolized B. subtilis from 1.600 colonies to 20 colonies (98.7% F.E.) within 1 hour, P. citrinum from 1,600 to 1 colony (99.9% F.E.) within 40 minutes, S. epidermidis from 1,600 to 1 colony (99.9% F.E.) within 20 minutes, and A. niger from 1,600 to 1 colony (99.9% F.E.) within 40 minutes.

The FE in this study was higher than that found by 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, obtaining a F.E. of 95-98%.

The efficiency of a portable indoor air cleaner in removing pollens (particle diameters ranging from 20-30 μ m) and fungal spores (particle size at 8-45 μ m) was tested by Cheng *et al* (1998). The results clearly showed that a commercial portable indoor air cleaner with a HEPA filter and activated charcoal pre-filter system was > 80% effective in removing large pollens and spores. In addition, the results suggested that this air cleaner is more effective when doors and windows are closed, especially when there is no activity in the room.

In general, the conventional PCR result is qualitative rather quantitative. The detection limit of nested PCR used in this study was optimized to detect a minimum of 10 fg of MTB (equal to 2 bacilli), therefore, at least 2 MTB bacilli are needed to give a positive result on PCR. Nested PCR for MTB detection was positive for all samples. The concentration of sprayed organisms may have been too high (5x10⁸ cells each of MTB and T7). In further studies, the numbers of sprayed organisms should be lower than this study, and a quantitative PCR should be used.

Although Middlebrook M7H10 agar was used to culture MTB, colonies of MTB were detected in only 10 out of 90 samples. This may be due to aerosolization stress (dessication and injury of aerosolized bacteria) from collecting the air sample that caused the MTB to fail to culture (Schafer *et al*, 1999). Wan *et al* (2004) described the following reasons for failure in culturing aerosolized mycobacteria: (1) inherent difficulties in culturing aerosolized tubercle bacilli, and (2) tubercle bacilli may be viable and infectious but not culturable.

The results for filtering *S. aureus* and *E.coli.* were similar to those reported by

Kudo *et al* (2007), who found that air purifiers employing a rectangular column titaniumoxide photocatalyst structure wall hanging show high performance in bacterial elimination from the air. As seen from the culture results with T7, *S. aureus* and *E. coli*, an electronic air filter can be used for removing bioaerasol from indoor air, similar to a HEPA air filter.

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REFERENCES

- Alpine Company. Electronic air filters (online). [Cited 2009 July 6]. Available from: URL: http://http://alpinefilter.com
- Alvarez AJ, Buttner PM, Toranzos AG, *et al.* The use of solid-phase polymerase chain reaction for the enhanced detection of airborne microorganisms. *Appl Environ Microbiol* 1994; 60: 374-76.
- Bean B, Rhame FS, Hughes RS, Weiler MD, Peterson LR, Gerding DN. Influenza B hospital activity during a community epidemic. *Diagn Microbiol Infect Dis* 1983; 1: 177-83.
- Brosch R, Philipp JW, Stavropoulos E, Colston JM, Cole TS, Gordon VS. Genomic analysis reveals variation between *Mycobacterium tuberculosis* H37Rv and the attenuated *M. tuberculosis* H37Ra strain. *Infect Immunol* 1999; 67(11): 5768-74.
- Centers for Disease Control and Prevention (CDC). Expanded tuberculosis surveillance and tuberculosis morbidity-United States. *Morbid Mortal Weekly Rep* 1994; 3: 361-366.
- Cheng SY, Lu CJ, Chen RT. Efficiency of a portable indoor air cleaner in removing pollens and fungal spores. *Aerosol Sci Technol* 1998; 29: 92-101.

- Committee on infectious diseases and committee on Hospital Care. The revised CDC guidelines for isolation precautions in hospitals: implications for pediatrics. *Pediatrics* 1998; 101: e13.
- Drosten C, Gunther S, Preiser W, *et al.* Identification of a novel corona virus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003; 348: 1967-76.
- Foarde KK, Hanley JT, Ensor DS, Roessler P. Development of a method for measuring single-pass bioaerosol removal efficiencies of a room air cleaner. *Aerosol Sci Technol* 1999; 30: 223-34.
- Kim BJ, Lee KH, Park BN, *et al.* Detection of rifampin-resistant *Mycobacterium tuberculosis* in sputa by nested PCR linked single strand conformation polymorphism and DNA sequencing. *J Clin Microbiol* 2001; 39: 2610-7.
- Kudo T, Kudo Y, Ruike A, Hasegawa A, Kitano M, Anpo M. The design of highly active rectangular column-structured titaniumoxide photocatalysts and their application in purification systems. *Catal Today* 2007; 122: 14-19.

Ramasoota P, Pitaksajjakul P, Phatihattakorn W,

Pransujarit V, Boonyasopun J. Matations in the rpoB gene of rifampicin-resistant Mycobacterium tuberculosis strains from Thailand and its evolutionary implication. *Southeast Asian J of Trop Med Public Health* 2006; 37: 136-47.

- Sawyer MH, Chamberlain CJ, Wu YN, Aintablian N, Wallace MR. Detection of Vaeicella-Zoster virus DNA in air samples from hospital rooms. *J Infect Dis* 1994; 169: 91-4.
- Schafer MP, Fernbach JE, Ernst MK. Detection and characterization of airborne *Mycobacterium tuberculosis* H37Ra particles, a surrogate for airborne pathogenic *M. tuberculosis. Aerosol Sci Technol* 1999; 30: 161-73.
- Tseng CC, Li CS. Collection efficiencies of aerosol samplers for virus-containing. *J Aerosal Sci* 2005; 36: 593-607.
- Wan GH, Lu SC, Tsai YH. Polymerase chain reaction used for the detection of airborne *Mycobacterium tuberculosis* in health care settings. *Am J Infect Control* 2004; 32: 17-22.
- WHO. Tuberculosis. (online). [Cited 2007 November 10]. Available from: URL: <u>http://</u> <u>www.who.int/mediacentre/factsheet/fs104/</u> <u>en/index.htm)</u>