# Microbiological Surveillance of Air Quality: A comparative Study Using Active and Passive Methods in Operative Theater

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## ABSTRACT

It is of great important to offer a clean environment, free from microorganisms or contamination beside the good ventilation and good facilities inside the operating room. The present work was designed to estimate the quality of air and the number of bacterial colonies per cubic meter (CFU/m3) in operating room for ophthalmic surgeries. The level of contamination in the samples was estimated via active and passive methods and also the accuracy between the two methods was determined. In addition, the effect of people numbers in the operating room during surgery on the rate contamination of surrounding air was explored.

Method: The study was performed in 6 turbulent air flow operating rooms of Research Institute of Ophthalmology RIO, Giza, Egypt. Samples of room air was taken through air sampler (active sample), while the another samples were taken in settled plates (passive samples). The Total Viable Count (TVC) was evaluated during surgery (working room). Samples from a total of 100 ophthalmic operations, including both active and passive were taken.

Results: The median TVC in operational was 40.0 CFU/m3 (range = 0 -220) for active samplings while it reached 1415.5 CFU/m2/h (range = 157.2-5976.6) for passive samplings. Statistically, the results revealed that both active and passive methods were correlated similarly with the air quality in the present study. The data showed a non-significant correlation was found between TVC and the number of persons in the operating room for both two methods: IMA passive (r =-054; P =0 .626) and active (r =0 .005; P = 0.965).

Conclusion: We concluded that for estimation of air pollution in OR, both active and passive methods can be applied, such as routine inspection tool and on the other hand, for obtaining specific data one of the two methods can be applied according to the needs.

The results also support interventions which help in the control of contamination in the operating room through limiting of traffic movement inside the operating theater.

**Key Words:** Operating theatres, Air samples, Surveillance, settle plates, index of microbial air contamination IMA

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## INTRODUCTION

Balance There are many sources for occurring of infections inside the operating rooms either arising from outside (exogenous) or from inside (endogenous). One of the symptoms or inflammation which may occur during any intraocular surgery is the endophthalmitis. Adequate quarantine measures and sterilization of the surrounding environment can limit and control most of exogenous sources of infection in the operating rooms. In addition, good ventilation, adequate room temperature, proper circulation of air flow and complete sterilization of tools and equipment can minimize or prevent these infections

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[1].

The minimum needs for obtaining most favorable media for operating room as mentioned by United States Public Health Service are: room temperature (18-24$^0$C), humidity averaged 55-80%, and 25 changes/ hour [2,3].

Restrict quarantine measures in the hospital, beside the control measures of the environment inside the hospital play an important role in decreasing health care associated diseases [3-5]. This is predominantly accurate in operating theatres because of tissue exposure to air [6-8]. Regular microbial air monitoring in operating theatres is of a great importance for evaluation of the quality of air and determined the value useful to measure air quality and identify serious situations. The results are an indicator for monitoring the degree of pollution and the required steps which must be taken for preventing the spread of infection in the operating room. Ventilation systems supplied with HEPA filters allow passing of clean air in the rooms, and restrict measures are required to prevent air-borne infection. Estimation of degree of air pollution in OR is an indicator for operating system efficiency [9]. One of the most recommended conditions in air of the operating room which is used for different types of surgical operations, is that the bacterial counts not exceeding 35.5/m$^3$ (1/ft$^3$) [10]. On the other hand, much of advancement or improvements in the methods used, checking data interpretation and level of contamination are considered [11]. The counting of colony-forming unit cfu is still an accurate and effective tool for measuring air-borne infection and determining the living microorganisms which can be divided and multiplied in the operating rooms [11]. Active and passive methods for determination of colony count in the air are used widely for monitoring of air pollution.

During active air sampling, the degree of bacterial contamination of air can be determined through counting of number of colonies (cfu) per cubic meter (cfu/m$^3$). In this method air samplers are taken in which a known volume of air is collected and cultured by different methods in a Petri dishes or tubes containing enriched medium, the incubation time, humidity and temperature varied according to the of type of microorganisms [12]. While in passive sampler; Plates or Petri dishes are opened for a while of time to permit for growing of live microorganisms which present or circulate in the air. Different types of microorganisms can be grown on the plates under room temperature and on specific nutrient media. The colonies on the surface of plates will be incubated under suitable environment [11] C. Pasquarella2000. Gravity or depositional sampling (settle plates) is considered as a non-quantitative collection method, however they reflect the bacterial load nearest the operative site [13]. The methods of active and passive samplers have advantages and disadvantages. The passive sampler is not violent and may fail to discover serious microorganisms, but needs long period time (up to 4-hour) for obtaining a sample and it is not costly. While, the active method needs special tools and equipment, extra training, device skills, and nearly all devices take a short period for performing the samples (average 10-minutes). The two methods have a beneficial effect and a burden, where, the active sampler tools can be applied efficiently in a places where the pollution is not sever or have a low level of microorganisms in a determined area, this means that most rooms are clean, so they cannot be detected through using the traditional passive method [12].

During designing for a taking air sample from any place, it is not easy to decide the method for collection of sample either active or passive method, because there are many factors governing the perfect sample and the results obtained varied from time to time, place to place, and according to the motion (dynamic or static) of people in the crowded room or during the operation procedures. Also the number of equipment and personals in the same area, is among the factors affecting the level of microbial counts [12]. The judgment on the best method which can be applied depends on your definite needs at least to guard the employers, workers and patients from infection.

Therefore, the present investigation was aimed to estimate the level of contamination with microorganisms in the operating room by using active and passive methods for collection of air samples and also, to compare the efficiency of the two methods for detection of bacteria and studying the influence of some factors as number of persons in OR and the level of air contamination.

## METHODS

The study was performed in 6 OR of Research Institute of Ophthalmology in Giza, Egypt. Following the study protocol, air from all operating rooms per day was sampled with both active and passive methods at the same time. In each room, air sampling was performed *in operational* (during surgery). In addition, the number of personnel present *in operational* was recorded to assess the association between the number of people in the room and the value of Total Viable Count (TVC). At the time of sampling, four operating rooms were equipped with HEPA filters while two rooms were not, with mean room volume 75 m3.

The work has been carried out along three months' period between Februarys - May 2017. During the study period, we obtained 186 environmental samples; 86 active sampling and 100 passive samples.

### Passive sampling

Passive sampling was performed according to the *Index of Microbial Air Contamination* (IMA) [11]. This index corresponds to the number of CFU counted on a Petri dish with a diameter of 9 cm left open to the air and placed according to the 1/1/1 scheme (for 1 hour, 1 m above the floor, about 1 m away from walls or any major obstacles). In our study the IMA plates were placed in the operating theatre approximately 1 m from the operating table, with results expressed in CFU/m$^2$/h. The Swiss Hospital Association standards were considered as maximum levels of IMA in operating theatres with turbulent air flow: ≤3932.1 CFU/m$^2$/h (≤25 CFU/9 cm diameter plate/h) *in operational*[14].

### Active sampling

The active sampling was performed using the same impactor (sieve type) IUR air sampler [15] with air volume (10-9900L) and air flow (100l/m-60l/m). The

device meets the following requirements: sufficient flow rate to collect 1m3 in a reasonable time, without significant drying of the sample medium and appropriate air impact speed to the culture medium. [16] The sampler was placed immediately beside the IMA plates, and active sampling was carried out over the period of the hour that the IMA plates were exposed.

The International Standard Organization (ISO), in its official documents for bio-contamination control in operating rooms, does not provide precise recommendations with regard to the sampling protocol (precise air volume to be sampled, length of sampling time etc.) [17]

In this work; A 90 mm Petri dish of blood agar was inserted and the device's lid was screwed in place. Next, accurate volumes of 200 L of air were sampled in two interrupted minutes in fixed sites, the first one meter from the head side of operative table and the second below the A/C inlet by forcing air through the cover thieves towards the Petri plate's blood agar surface.

CFU counts during the period of research were compared using the Mann–Whitney U test.

In 100 L/min sampling capacity, if 1m3 of air is tested, then it would require an exposure time 15 min [11]. Since we use 2-min sampling time to withdraw 200 L, so to calculate cfu per 1000L or m3, we multiply results of (cfu/plate) by 5. Maximum acceptable levels were taken as the standards determined by ISPESL in 2009 for air microbial contamination in operating theatres with turbulent air flow: ≤ 180 CFU/m$^3$*in operational* [18].

### Laboratory methods

Sealed Blood agar plates were incubated at 37°C for 2 days. The plates were examined for microbial growth and colony forming units CFU enumeration per plate enables to evaluate microbial air quality.

In addition, the number of personnel present *in operational* was recorded to assess the association between the number of people in the room and the value of Total Viable Count (TVC).

### Statistical analysis

The results from the two sampling methods were loaded into a database created with the software *File Maker* and Statistical data analysis was performed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as median and range. Qualitative data were expressed as frequency and percentage. Spearman-rho method was used to test correlation between numerical variables. All tests were two-tailed. A p-value < 0.05 was considered significant. In addition, linear regression was used to analyze the relationship between the results of the two methods of air sampling.

## RESULTS

The total number of samplings, for the active method was 86 and the median TVC was 40.0 CFU/m$^3$ (range = 0 - 220). While the number of samplings for the passive method was 100 and the median TVC was 1415.5 CFU/m$^2$/h (range = 157.2-5976.6) (Table 1).

The Spearman's test shows that a high correlation was found between the data of active and passive methods and when CFU/m$^3$ grew, the IMA also grew. As seen in (Fig.1), the correlation between the two methods as demonstrated by the regression model was ($P$ = 0.001, r = 0.37; n=84).

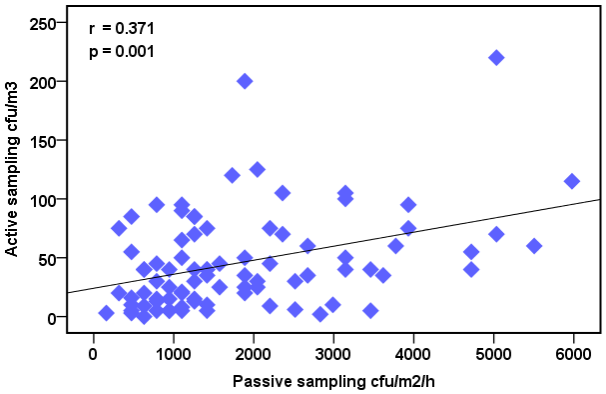


Figure1: Correlation between the TVC values detected simultaneously by IMA (CFU/m $^2$/h) Passive test and active test (CFU/m $^3$) in 84 operative procedures.

The viable count exceeding the acceptable limit (180 CFU/m$^3$) in active method and (3932.1 CFU/m$^2$/h) in passive method, was much less (3 out of 142 samples 2.1%) in OR 1-4 where special air flow arrangements with HEPA filters were present, than OR 5 and 6 (5 out of 44 samples 11.3%) where no HEPA filter was present.

The exceeding cfu count was also less by active method 2 out of 86 (2.3%) than passive sampling 7 out of 100(7%)(Table 1).

There was no significant correlation between the number of personals and the TVC in two methods: IMA passive (r =-054; $P$ =0 .626; n = 100) and active (r =0 .005; $P$ = 0.965; n=86). The median number of persons found in the operating room during the *operational* samplings was 4 (range = 0-8).

## DISCUSSION

The presence of un-seen microbes in the environment is considered as a high risk factor because they are present in the air they cannot be seen by the naked eyes. Good hospital hygiene is an integral part of infection control program. "Microbiological surveillance" provides data about the factors contributing to infection [3,19, 21-23].

In this study, the median TVC by settling plates was1415.5cfu/ m2/h and by active sampling 40.0cfu /m3. In another study, Napoli et al. found the mean TVC by settling plates was 10496.5 cfu /m2/h while active sampling gave 93.8 cfu/m3[14]. Non consistent results given by previous studies which might attributed to dissimilar types of samples used, the source of which the samples was taken (e.g. operating theaters, pharmaceutical clean-rooms, dental clinics, etc.) or due to type and number of parameters estimated such as volume of sample, the duration of sampling (hrs) and the site of sampling etc.[14] Although there are recommended target limits for both active and passive air sampling test, yet there are no specific procedures for collection or

obtaining of the TVC value in air sampling. The external factors such as the site or point of collection of sample from the operating theater influence greatly on the estimated level of microbial contamination [24]; therefore, by using passive technique for estimation of bacterial contamination, it was found that the counts of bacteria in the settled Petri-dishes near the windows was higher than that put in the corners or periphery of OR [13]. With respect to the present study, only one sample was located on a place 1 meter away from the surgical table (as suggested by the guidelines) and, in this site 7/100 of samples exceeded the permissible limit value. This is much less than values obtained by Napoli *et al.* in previous study;14 /19 passive samples were higher than the permissible limit value [14]. This may be explained by the fact that his study was carried out in a university hospital where teaching in the theater results in big number of people near operating table.

Table 1: CFU from day 1 - 18 by settle plate (Passive)& air sampler(Active)

| | Room1 | | Room2 | | Room3 | | Room4 | | Room5 | | Room6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cfu/ m3 | Cfu/m2/h | Cfu/ m3 | Cfu/m2/h | Cfu/ m3 | Cfu/m2/h | Cfu/ m3 | Cfu/m2/h | Cfu/ m3 | Cfu/m2/h | Cfu/ m3 | Cfu/m2/h |
| 1 | 95 | 1100.9 | 90 | 1100.9 | 35 | 1415.5 | 40 | 629.1 | 40 | 3460.1 | 20 | 1887.3 |
| 2 | 75 | 1415.5 | 20 | 629.1 | 10 | 2988.3 | 45 | 2201.9 | 85 | 1258.2 | 75 | 2201.9 |
| 3 | 200# | 1887.3 | 50 | 1100.9 | 30 | 2044.6 | 40 | 1415.5 | | 3617.4 | | 3145.6 |
| 4 | 100 | 3145.6 | 5 | 471.8 | 20 | 314.5 | 120 | 1730 | | 629.1 | | 1258.2 |
| 5 | 75 | 3932 | 50 | 3145.6 | 55 | 4718.4* | 95 | 3932 | | 4718.4* | | 2044.6 |
| 6 | 55 | 471.8 | 15 | 1258.2 | 40 | 4718.4* | 25 | 2044.6 | | 943.6 | | 471.8 |
| 7 | 0 | 629.1 | 25 | 943.6 | 95 | 786.4 | 65 | 1100.9 | | 1730 | | 471.8 |
| 8 | 5 | 786.4 | 15 | 943.6 | 85 | 471.8 | 50 | 1887.3 | 105 | 3145.6 | 70 | 1258.2 |
| 9 | 45 | 786.4 | 10 | 471.8 | 30 | 1258.2 | 20 | 1100.9 | 40 | 1415.5 | 35 | 2673.7 |
| 10 | 5 | 943.6 | 40 | 943.6 | 5 | 1415.5 | 60 | 3774.7 | 60 | 5504.8* | 35 | 3617.4 |
| 11 | 40 | 1258.2 | 35 | 1887.3 | 45 | 1572.8 | 40 | 3145.6 | | | | |
| 12 | 15 | 786.4 | 5 | 943.6 | 30 | 786.4 | 5 | 1100.9 | 10 | 1415.5 | 5 | 3460.1 |
| 13 | 85 | 1258.2 | 40 | 629.1 | 125 | 2044.6 | 105 | 2359.2 | 220# | 5032.9* | 115 | 5976.6* |
| 14 | 70 | 2359.2 | 75 | 314.5 | 150 | | 40 | | | 314.5 | | 1572.8 |
| 15 | 60 | 2673.7 | 25 | 1572.8 | 30 | 2516.4 | 70 | 5032.9* | | 157.2 | | 157.2 |
| 16 | 21 | 1100.9 | 3 | 157.2 | 13 | 1258.2 | 6 | 2516.4 | | 1415.5 | | 2201.9 |
| 17 | 3 | 471.8 | 9 | 629.1 | 8 | 1100.9 | 2 | 2831 | | | | |
| 18 | 16 | 471.8 | 13 | 786.4 | 9 | 2201.9 | 25 | 1887.3 | | | | |

Cfu/m3 = colony forming units per cubic meterCfu/m2/h= colony forming unit per meter square per hour
* The value exceeded the maximum acceptable levels(≤3932.1 CFU/$m^2$/hat operation)
# The value exceeded the maximum acceptable levels (≤ 180 CFU/$m^3$ at operation)



In previous study, the most important factor "the acceptable limit of cfu" did not correlate, that is by settle plate it exceeded whereas by air sampler the cfu was within limit [14]. In our study, higher value exceeding the acceptable limit of cfu was also found by passive method (settling plates) 7 out of 100 samples (7%), while was only 2 out of 86 in active sampling (2.3%). The same was found in a previous study [19]. This discordance is explained by the fact that air sampler calculates suspended particles whereas settle plate calculates the settling large bacteria carrying particles. These settling particles are more prone to cause SSI than the suspended ones [25]. In addition, long sampling periods of time may increase measurement significance and reproducibility in settling plates. [11]

The method of passive sample can provide us with important information about the incidence of infection and it measure the level of dangerous microorganisms (air-born infection) in the area of operating room or which settle on a wound or at the site of operation or transmitted via instruments; thus, passive sample can help in assessment of a risk factor [26-29].

In this study, the number of samples exceeding the acceptable limit of cfu by both active and passive methods was much less (3 out of 142 samples 2.1%) in operative rooms number 1-4, where special air flow arrangements HEPA filters are present, than operative rooms number 5&6 (5 out of 44 samples 11.3%) where no HEPA filter was there. Poongodi *et al.* [19], Geeta Mehta [30] and Dharan *et al.*, [31] also observed that there is less infections in OT with HEPA filters. Knobben BAS *et al.* revealed that laminar flow system will decrease intra-operative contamination during total hip or knee replacements. [27]

The Spearman's test shows high association among the results of the two sampling methods: when CFU/$m^3$ grew the IMA also grew ($P = 0.001$, $r = 0.37$; n=84), as shown in fig (1).

Similar results were found in previous study with correlation between methods ($R^2 = 0.82$; $F = 76.3$; $p < 0.01$) ($\alpha < 0.05$). [14]

In both active and passive methods, a non-significant correlation was found between number of personnel in operative room and the TVC. IMA passive ($r = -054$; $P = 0.626$; n = 100) and active ($r = 0.005$; $P = 0.965$; n=86). The median number of persons were found in the operating room during the *operational* samplings was 4 (range = 0-8) which is much lower than that in another study, where a significant correlation excised between the number of persons and the TVC with two techniques: IMA ($R^2 = 0.610$; $F = 26.3$; $p < 0.01$) and SAS ($R^2 = 0.608$; $F = 26.6$; $p < 0.01$). This may be explained by the high value of the mean number of people present in the operating theatre during samplings 7.4(SD = 3.1;

range = 3-13) which is typical of university hospitals where teaching is done directly in the theatre [14].

## CONCLUSION

The quality of air in operating rooms from bacteriological aspects play an important role in controlling hazard infection and for quality of life. Frequency of air quality surveillance depends on number & type of surgeries, outbreak of post-operative infection and availability of resources. Routine surveillance for OT may be suggested for every two months. Special air flow arrangement (HEPA filter) plays a role in the maintenance of air quality.

Both active and passive air sampling techniques can be used aa a general monitor for air pollution, and for the purposes of routine surveillance programs. Air sampler measures the microbial burden more accurately. Settle plate is a direct indicator of SSI risk.

We can conclude from this work, applying of strict quarantine measures and sharp instruction in the hospital particularly OR is prospect to improve the quality of air and both active and passive sampling are correlated with air quality. On the other hand, the selection of the method depends on the type of specific information we needed. if the sampling is performed to obtain information on the concentration of all inhalable viable particles, the active method should be preferred. On the contrary if the air sampling performed during surgery is carried out to monitor the risk of microbial wound contamination, passive measurement is better than volumetric sampling at predicting the likely contamination rate at the surgical site, as it allows a direct measure of the number of microorganism settling on surfaces [14]. Hence, this economic and simple settle plate method has more practical application in reflecting the risk of infection. The index of microbial air contamination IMA has proved to be a reliable and useful tool for monitoring the microbial surface contamination settling from the air in any environment. Further studies must be undertaken to confirm this result.

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