Author's response to reviews

Title: Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres

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Version: 2 Date: 12 June 2012

Author's response to reviews: see over
Dear Editor,

on behalf of all authors of the manuscript "Air sampling procedures to evaluate microbial contamination: comparison between active and a passive method in operating theatres" (Ms. Ref. No.: 1651642886671645), I would like to thank you for the chance to consider the manuscript for publication in BMC Public Health after major revisions. Moreover, I thank your qualified Referees for their comments that permitted us to greatly improve the paper. Please find in attachment the revised paper and the cover letters addressed to the Referees. The manuscript was rewritten following - point by point - all the reviewers’ comments (all changes are written in red in the text) and the additional editorial requirements that you kindly requested in your last e-mail. Moreover, some comments of Referees concern the English language. For this reason, the manuscript underwent to a professional English editing, held by:

Dr Atack Stephen Ross  
Professor of Scientific English, Medical School  
University of Bari, Bari Italy

We hope that all our efforts will allow you to accept the article. Please do not hesitate to contact me at anytime for further information. Thank you for your time and best regard.

Christian Napoli

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Dear Prof. Chunchieh Tseng, Referee 1,

thank you for your evaluation and for your important suggestions. We apologize for the confusion in the previous version of the manuscript and we really appreciate your efforts for reviewing it. According to your comments, the manuscript was deeply revised and rewritten in its greatest part. Moreover, the manuscript underwent to a professional English editing, held by:

Dr Atack Stephen Ross
Professor of Scientific English, Medical School
University of Bari, Bari Italy

We are now re-submitting the correct manuscript. A point-by-point description of the changes follows (in black small letters your comments, in red capital letters our reply):

1. This study devoted to research on the relationship between active and passive sampling for bioaerosol in operating theaters. The results obtained from this study may clarify the relationship between active and passive sampling for bioaerosol. However, less constructive conclusions can be made since their results are very limited. Some related results have already been published as reference 18 to 22; however, there is no consistent conclusion among these studies. I suggest the authors to make more discussions, why readers have to believe there is a significant correlation between active and passive sampling results since there are already some opposite findings have been published? What's the same or different among their findings and previous reports? In methods, some descriptions are too rough, the readers may get confused. Detailed comments and questions are given page by page in the section that follows.

IN LIGHT OF YOUR COMMENTS THE ARTICLE WAS COMPLETELY REVISED, WE DEEPLY ANALYZED DATA FROM REPORTED REFERENCES AND WE CLARIFIED THE REASONS FOR THE INCONSISTENCE AMONG PREVIOUS STUDIES AND WHAT IS NEW IN OUR ARTICLE. MOREOVER, TAKING INTO ACCOUNT THE LESSONS LEARNED, WE MADE MORE DISCUSSION AND THE METHODS WERE CLARIFIED. ALL CHANGES WERE POSSIBILITY FOLLOWING YOUR USEFUL DETAILED COMMENTS.
2. Background: Since the major focus is related to active and passive sampling, some issues have to be addressed in this paragraph. What’s the possible reason could explain the inconsistent findings from previous studies? What kind of factor may affect the active and passive sampling results?

BASED ON A CAREFUL READING OF THE REFERENCES, WE HIGHLIGHTED IN THE BACKGROUND THE POSSIBLE REASONS EXPLAINING THE INCONSISTENT FINDINGS FROM DIFFERENT STUDIES. THEY CAN BE REASSUMED IN THE DIFFERENCES IN THE PROTOCOLS OF SAMPLING (TIME, VOLUME OF AIR ETC.), PLACES OF SAMPLING (NOT ALWAYS HOSPITAL ENVIRONMENTS), SAMPLERS USED (DIFFERENT DEVICES ARE AVAILABLE, IN PARTICULAR FOR THE ACTIVE SAMPLING). THIS PARAGRAPH WAS ADDED IN THE BACKGROUND SECTIONS:

Several studies have attempted to compare the values of microbial loads obtained through both active and passive samplings, but with inconsistent results: in some cases there was significant correlation [24-26] while in others there was none [27, 28]. Currently, since air sampling protocols are not standardized, it is difficult to compare results from different studies [18]. In fact, it has been known for some time that different active samplers show high variability giving different results in the same place at the same time [18]. Whyte found a correlation between the active and passive method, comparing settle plates with the Active Casella Slit Sampler [24], while Sayer et al. did not find this correlation using the Andersen Active Sampler [28], and Petti et al. demonstrated that, at low air contamination levels, results provided by active Surface Air System sampler (SAS) and settle plates were not correlated [21]. Sampling was also carried out in different places in the different studies: Whyte studied the clean-room of a pharmaceutical company, while Petti et al. analysed Dentists’ outpatients clinics. Different indoor environments have different levels of bio-contamination, different kinds of airflow, different numbers of people working in them who use different kinds of personal protective equipment, all factors which affect the results of both the sampling and the comparison between methods [18, 22]. Sampling can also be carried out in different moments: Perdelli et al. compared the SAS with the Index of Microbial Air Contamination (IMA) during the surgical activity (in operational) when contamination is higher. Additionally, it could be interesting to also study the bio-contamination before the start of the operation (at rest) when the room is empty, as the ISO norm suggests, in this way checking the performance capabilities of the theatre, especially its air systems [19].

3. Is there anything special in this study that may clarify the relationship and has not been discussed previously?
IN LIGHT OF THE PREVIOUS STUDIES’ METHODS AND RESULTS, WE BELIEVE THAT OUR STUDY COULD COMPLETE THE INFORMATION REGARDING THE COMPARISON BETWEEN TWO SPECIFIC METHODS OF SAMPLING: IMA AND SAS (THAT, TO OUR KNOWLEDGE, ARE THE LARGEST USED METHODS) IN HOSPITAL ENVIRONMENTS THOUGHT A PRECISE PROTOCOL OF SAMPLING THAT TAKES INTO ACCOUNT THE DIFFERENCES EXISTING IN AIR CONTAMINATION BETWEEN THEATERS AT REST AND IN OPERATIONAL. THIS PARAGRAPH WAS ADDED AT THE END OF THE BACKGROUND SECTIONS:

Given this research background it is of fundamental importance that researches continue in order to investigate if there is a real correlation between the two methods, between the results provided by different samplers and in different indoor environments, so using scientific evidence to eventually lead to the proposal of a fixed standard protocol for a correct surveillance procedure.

The aim of the present study is to contribute to the scientific evidence of the previous studies through a comparison between two of the widely used methods (active SAS and passive IMA) in the operating theatres of one hospital in Southern Italy. Bio-contamination surveillance was carried with both methods, to be compared later, at the two moments suggested by the ISO norm: at rest and in operational with a standardized protocol.

4. Methods: I find this aspect of the method is particularly difficult to follow because some descriptions are too rough. The most important thing for me is to check whether active and passive sampling was conducted simultaneously.

WE AGREE WITH THIS COMMENT, THE PREVIOUS VERSION MADE THE READER REALLY CONFUSED. WE TRIED TO CLARIFY ALL ASPECTS; IN PARTICULAR THAT THE ACTIVE AND PASSIVE SAMPLINGS WERE CONDUCTED SIMULTANEOUSLY. THE SENTENCE IS NOW REPORTED AS:

Following the study protocol, air from one operating room per day was sampled with both active and passive methods at the same time.

5. From Figure 1 and Figure 2, it seems active and passive sampling was not parallel, active sampling was only operated in some of operating rooms.

WE FIND THIS COMMENT ONE OF THE MOST USEFUL. THE PREVIOUS FIGURES 1 AND 2 DID NOT MACH WITH THE AIM OF THE STUDY. WE WANTED TO COMPARE ACTIVE AND PASSIVE METHODS BOTH AT REST AND IN OPERATIONAL; TO THIS AIM WE COMPARED IMA AND SAS AT REST IN 32 OPERATING ROOMS SIMULTANEOUSLY AND IN OPERATIONAL IN 19 ROOMS SIMULTANEOUSLY. ON THE
CONTRARY, THE PREVIOUS TWO FIGURES COULD BE MORE USEFUL IN A COMPARISON BETWEEN AT REST VS IN OPERATIONAL USING ACTIVE AND PASSIVE METHODS SEPARATELY. THUS, THANKS TO THIS COMMENT, WE CHANGED THE FIGURES AND REPORTED THE RESULTS OF LINEAR REGRESSION THAT SHOWS THE CORRELATION BETWEEN THE TWO METHODS BOTH AT REST (FIG. 1) AND IN OPERATIONAL (FIG.2). MOREOVER, AS YOU SUGGESTED WE USED The Unit “CFU/m2/h INSTEAD OF “CFU/9 cm plate/h.

6. Active sampling. How many sampling apparatus were used in the theater?
THANK YOU! WE USED ONLY ONE SAS APPARATUS AND WE SPECIFIED THIS IN THE TEXT.

7. What’s the volume of theater?
THIS IS A VERY IMPORTANT PARAMETER TO BE CONSIDERED WHEN AN AIR MONITORING IS GOING TO BE PLANNED. WE ENROLLED DIFFERENT ROOMS, WHOSE VOLUMES DOES NOT VARY SIGNIFICANTLY; THUS, WE ADDED IN THE FIRST PARAGRAPH OF METHODS THE MEAN VALUES PLUS THE STANDARD VARIATION AND THE RANGE OF THE OPERATING ROOMS VOLUMES: 136.9 M³ (SD: ± 15.2; RANGE=112.1-158.7).

8. Why the active sampling protocol at rest was different from sampling in operational? For active sampling, sampling time is important because airborne microbes directly impacted onto the agar surface will damage its culturability. The damaged culturability for microbes impacted onto the agar surface at once is different from microbes impacted onto the agar in batches. Is that fair to compare the bioaerosol concentration at rest and in operational by different protocol?
WE DO APOLOGIZE FOR THE UNCLEAR TEXT OF THE ARTICLE. WE AGREE: IT IS NOT POSSIBLE COMPARE SAMPLING AT REST VS IN OPERATIONAL USING DIFFERENT PROTOCOLS; BUT, AS ALREADY SPECIFIED IN THE TEXT ACCORDING TO YOUR PREVIOUS COMMENTS, WE DID NOT COMPARE SAMPLING AT REST VS IN OPERATIONAL; BUT IMA VS SAS AT REST AND IMA VS SAS IN OPERATIONAL. IN THIS CASE, WE CONSIDERED FUNDAMENTAL FIND A PROTOCOL, BASED ON SCIENTIFIC EVIDENCES, ALLOWING TO COMPARE THE TWO METHODS IN THE VERY DIFFERENT CONTEXT OF ROOMS AT REST AND IN OPERATIONAL. WE CLARIFIED THIS POINT IN THE TEXT, ADDING THE FOLLOWING PARAGRAPH, BASED ON SCIENTIFIC LITERATURE SOURCES:
As reported by Pasquarella et al., a volume of 500L of air was sampled at rest in one continuous drawing [3], because at rest, when the room is empty of people, the results of the sampling reflect mainly the performance of the CCVS [18, 19]; in this situation, a single continuous drawing can be comparable to one hour of settle plates exposure.

During in operational sampling, when the personnel is in the room, the results of the sampling clearly reflect the team’s hygiene procedures and behaviour, and not only the CCVS performance [18, 19]. For this reason, active sampling was carried out over the period of the hour that the IMA plates were exposed, with 5 separate air draws of 100L each for a total volume of 500L, with intervals of 12 minutes between draws. In fact, Perdelli et al. found that a correlation between the two methods is possible when the active sampling is carried out at regular intervals during the exposure time of the settle plate [26], because a single drawing detects the contamination only during the short time necessary for the drawing and is therefore not able to detect what the IMA plate detected over the complete hour. Even the ISPESL guidelines suggest, only in operational, an active serial sampling carried out at regular intervals [30].

9. In my opinion, their data certainly have to be compared with the standards provided by ISPESL, but if their sampling protocol is different from ISPESL, that’s doesn’t make sense.

WE COMPLETELY AGREE WITH THIS COMMENT. WHAT WE WERE LOOKING TO HIGHLIGHT WAS EXACTLY THAT THERE ARE NO STANDARDIZED OR PRECISE SAMPLING PROTOCOLS PROVIDED NEITHER BY ISPESL NOR BY ISO. IT IS VERY STRANGE THAT OFFICIAL DOCUMENTS, SUCH AS ISPESL GUIDELINES, PROVIDE TARGET LIMITS WITH NO DESCRIPTION OF THE PROTOCOL ALLOWING TO GET THAT LIMIT (NO NUMBER OF SAMPLINGS, NO AIR VOLUMES TO BE SAMPLED, NO LENGTH OF SAMPLING TIME, NO KIND OF SAMPLER ETC). WE ADDED THE FOLLOWING SENTENCE IN THE METHODS:

Both the Italian Institute for Occupational Safety and Prevention (ISPESL) and the International Standard Organization, in their official documents for bio-contamination control in operating rooms, do not provide precise recommendations with regard to the sampling protocol (air volume to be sampled, length of sampling time etc.) [19, 30].

MOREOVER, WE DISCUSS THIS POINT IN THE OTHER SECTIONS.

10. Passive sampling. I recommend using the unit “CFU/m2/h instead of “CFU/9 cm plate/h”. That will be clearer to represent the concentration of settled microbes.

THANK YOU, WE AGREE WITH THIS COMMENT. WE CONVERTED ALL RESULTS IN CFU/M²/H, INCLUDING THE LIMITS PROVIDED BY SWISS HOSPITAL ASSOCIATION.
11. Just like active sampling, authors have to describe how many plates were placed, and the exactly position they placed the plates.

**WE MOVED THE PASSIVE SAMPLING PARAGRAPH BEFORE THE ACTIVE ONE AND WE TRIED TO CLARIFY THE TEXT AS FOLLOW:**

This IMA corresponds to the number of CFU counted on a Petri dish with a diameter of 9 cm 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 (one for TVC and one for filamentous fungi) were placed in the operating theatre approximately 1 m from the operating table, with results expressed in CFU/m²/h.

**THE CULTURE MEDIA USED ARE REPORTED IN THE “LABORATORY METHODS” SECTION.**

12. Regarding TSA agar, do you mean “Tryptic Soy Agar”?

**YES! NOW IT IS CORRECT IN THE TEXT.**

13. In addition, I think the incubated temperatures for yeast and molds are too high, usually 25 °C is recommended. Authors cannot find fungi in the operating theaters may be related to this issue.

**WE AGREE WITH THIS COMMENTS. WE DELETED THE SENTENCES RELATIVE TO THE YEASTS, BECAUSE THE RESULTS WERE NEGATIVE AND IT DOES NOT ADD INTERESTING INFORMATION TO THE PAPER. WITH REGARD TO FILAMENTOUS FUNGI, IN TWO PREVIOUS STUDIES CONDUCTED IN THE SAME HOSPITAL, WE FOUND A LITTLE MOULDS CONTAMINATION EVEN WHEN USING TEMPERATURE OF 27°C (MONTAGNA ET AL. 2012). IN THE PRESENT STUDY WE USED THE TEMPERATURE OF 30°C, BECAUSE AS REPORTED BY “DE HOOG”, TO CULTIVATE SOME SPECIES OF FUNGI CAN BE NEEDED HIGHER TEMPERATURE UP TO 37-40°C. IN OUR OPINION THE DIFFERENCE WITH THE REPORTED REFERENCES COULD BE DUE TO THE PRESENCE OF HEPA FILTERS, THAT ARE MISSING IN THE INDOOR ENVIRONMENTS WHERE A HIGHER FUNGAL CONTAMINATION WAS REPORTED. WE DISCUSSED THIS POINT IN THE DISCUSSION SECTION:**

With regard to fungi contamination, only two different strains of mould were identified, one by IMA and one by SAS. These results are in accordance with those of two previous studies carried out in controlled environments of the same hospital, where an uncommon fungi contamination was found [34, 35]. Our data do not confirm the findings from Verhoeff et al, which showed that active sampling was better at collecting fungal species [36] and from Asefa et al. which found that the
SAS air sampler showed higher numbers of fungi species and mean CFU/plate compared to settle plates [37]. However, the operating rooms in our study were equipped with HEPA filters unlike indoor environments in the studies of Verhoeff et al. and Asefa et al. Other authors have reported that fungal air contamination was never detected in rooms equipped with HEPA filters [38, 39] and that simple protective measures, such as air filtration, are known to be effective against mould complications in hospitalized patients [17].

14. Results and discussion: I highly recommended adding a figure to demonstrate the relationship between surface counts and air counts. Figure 1 and Figure 2 only demonstrated the concentrations in operational is higher than those at rest.

AS PREVIOUSLY REPORTED, WE COMPLETELY AGREE AND THE TWO FIGURES WERE CHANGED ACCORDING TO YOUR COMMENT.

15. In the paragraph 5: With regard to the results at operational……..author tried to explain why the passive results are usually higher (73.7% of passive samples) than the threshold limits. The reason they speculated is related to the higher number of people in the theater. I think this reason is not appropriate. Higher number of people would also result in higher value of active sampling (R²=0.608; p<0.001), why only 5.3% of active samples exceeded the threshold limits? By the way, when passive sampling was operated, were there more people presented in the theater than active sampling was operated? If yes, that means passive and active sampling were not operated simultaneously. How could it possible to evaluate the relationship?

WE DO APOLOGIZE FOR THE UNCLEAR TEXT. OF COURSE, BEING THE IMA AND SAS SAMPLING SIMULTANEOUS CARRIED OUT, THE NUMBER OF PEOPLE WAS THE SAME. WHAT WE TRIED TO EXPLAIN WAS THE REASON WHY THE TVC VALUES BOTH FOR IMA AND SAS TESTED HIGHER IN OPERATIONAL THAN AT REST: BECAUSE THE STAFF WAS IN THE ROOM. WE CORRECTED THE SENTENCE AS FOLLOW:

In operational sampling showed higher values of TVC than at rest with both active and passive methods (93.8 vs 12.4 CFU/m³ and 10496.5 vs 722.5 CFU/m²/h respectively) as would be expected due to the inevitable microbial dispersion from people. Linear regression, in fact, revealed a significant association between the number of people and the TVC with both methods: IMA (R²=0.610; F= 26.3; p<0.01) and SAS (R²=0.608; F= 26.6; p<0.01). The mean number of people present in the operating theatre during the 19 in operational samplings was high at 7.4 (SD=3.1; range=3-13).
16. For reference 28, I think this reference is not related to authors’ study, the reference is about the relationship between total particle counts and microbe counts in air.

WE AGREE WITH THIS COMMENT AND WE DELETED THE SENTENCE AND THE REFERENCE.

17. In the discussion (in the end of page 6 to page 7), author only qualitatively described the findings from these references, no further discussion about what’s the possible reason, mechanism, or phenomenon may explain their findings.

THANK YOU. WE DISCUSSED OUR RESULTS IN LIGHT OF THE FINDINGS REPORTED IN THE REFERENCES LISTED:

A study published in 2012 found that levels of recorded microbial contamination in operating rooms are also influenced by external factors such as the point of collection in the operating room [32]; so confirming previous reports in which, with the passive sampling method, higher counts were found on settle plates nearer the wound than in periphery [33]. Our study investigated only one sampling point located 1 m away from the surgical table (as recommended by the guidelines) and, in this position, 14 of the 19 passive samples exceeded the limit value. In the light of the 2012 study, sampling near the wound would have probably resulted in all plates being over the limit, showing that the situation is even more critical.

Minor Essential Revisions

18. I think a comma between the numerals should be a decimal point; e.g. 12,4 should be 12.4 CFU/m3. Please modify throughout the text.

THANK YOU, WE CHANGED ALL COMMAS TO POINTS.

19. Keywords: Bioaerosol should be listed in the keywords.

DONE, THANK YOU!

Thank very much you for your time and for your comment that permitted us to improve the manuscript.

Best regards

The Authors
Bari, June 12, 2012
Ms. Ref. No.: 1651642886671645
Journal: BMC Public Health

Title: Air sampling procedures to evaluate microbial contamination: comparison between active and a passive method in operating theatres
Authors: Christian Napoli, Vincenzo Marcotrigiano and Maria Teresa Montagna

Dear Prof. Pierluigi Lopalco, Referee 2,

Thank you for your evaluation and for your important suggestions. We really appreciate your efforts for reviewing it. According to your comments, the manuscript underwent to a minor revision. Moreover, the manuscript underwent to a professional editing, held by:
Dr Atack Stephen Ross
Professor of Scientific English, Medical School
University of Bari, Bari Italy

We are now re-submitting the correct manuscript. A point-by-point description of the changes follows (in black small letters your comments, in red capital letters our reply):

1. The sentence in the abstract: "Results of active and passive collection method are comparable" is not clear when compared with the results (active sampling is much more sensitive than passive sampling). It should be better expressed.
   THANK YOU, THE SENTENCE WAS NOT CORRECTLY EXPRESSED. WE CHANGED THE SENTENCE ACCORDING TO YOUR SUGGESTION, AS FOLLOW: Statistical analysis confirmed that the two methods correlate in a comparable way with the quality of air.

2. when the Authors say "Since then, many Authors have underlined the importance of microbial surveillance of environmental matrix", it would be nice to better explain which is the correlation between quality of air and incidence of surgical site infections (or other health care related infections).
THANK YOU, ACCORDING TO THIS COMMENT THE IMPORTANCE OF AIR SURVEILLANCE WILL BE EMPHASIZED. WE ADDED A SENTENCE REPORTED TWO REFERENCES RELATED TO THIS ISSUE:

A special focus has been placed on microbial air surveillance; in fact, it has been demonstrated that periprosthetic infection rates correlate with the number of airborne bacteria within the wound [16] and that, in hospital environments, the use of air filtration through a HEPA system completely eliminated invasive pulmonary aspergillosis in immune-compromised patients [17].

3. what does it mean: "This situation, in Italy, is typical of university hospitals"? maybe it is worth some explanation

THANK YOU, THE SENTENCE WAS NOT CLEAR. WHAT WE TRIED TO SAY WAS THAT IN TEACHING HOSPITAL (SUCH AS UNIVERSITY HOSPITAL) THE NUMBER OF PEOPLE IS HIGHER THAT OTHER NOSOCOMIAL FACILITIES. BECAUSE, A PART FROM SURGEONS AND NURSES, THERE ARE STUDENTS (MD STUDENTS, MD ATTENDING THE SCHOOLS OF SPECIALIZATION, PHD STUDENTS ETC.) IN THE OPERATING ROOMS. THE SENTENCE WAS CHANGED AND CLARIFIED:

This is typical of university hospitals in Italy where teaching is done directly in the theatre.

4. In the conclusion: "Our study proved that the results of the active and passive sampling techniques are comparable", should be better phrased, as the two systems are not comparable in terms of sensitivity. Maybe better "active and passive sampling techniques correlate in a comparable way with the quality of air", or like

THANK YOU, EVEN IN THIS CASE THE SENTENCE WAS NOT CORRECTLY EXPRESSED. WE USED THE SENTENCE YOU SUGGESTED:

Our study has demonstrated that when a strict protocol is followed results of active and passive sampling correlate in a comparable way with the quality of air for both at rest and in operational sampling

5. In the conclusion: "Therefore it seems difficult to develop a protocol for a unique standardized sampling method", should be better phrased in the sense that any standard protocol should take into account the different characteristics of the two techniques and each technique should be used according to the purpose of the air sampling.
THANK YOU, THE PREVIOUS VERSION OF THE CONCLUSION WAS NOT CLEAR. WE TRIED TO REORGANIZED THE SECTION TAKING INTO ACCOUNT YOUR SUGGESTION:

In the meantime, it is possible to conclude that both methods can be used for general monitoring of air contamination, such as routine surveillance programs. However, the choice must be made between one or the other to obtain specific information. In particular, 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 [19, 40, 41]. On the contrary, if the sampling is performed to obtain information on the concentration of all inhalable viable particles, the active method should be preferred [19].

Thank very much you for your time and for your comment that permitted us to improve the manuscript.

Best regards

The Authors