

Effectiveness of HEPA Filters in Reducing Airborne Bacteria in Public Health Laboratory Rooms in Bontang City, Indonesia

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Abstract: Air is a basic human need whose quality needs to be maintained, especially in closed environments such as laboratories. Several factors, such as temperature, humidity, lighting, occupancy density, and ventilation systems, affect the presence of microorganisms in the room. Laboratory users can control microorganisms that cause air contamination by using High Efficiency Particulate Air (HEPA) filters. This study aims to determine the effect of HEPA filter use on the number of airborne germs in laboratory rooms. This type of research is an analytical observational study with a cross-sectional design, with nine rooms as research objects. Air samples were taken six times in each room, namely twice before the use of the HEPA filter (0 hour), twice after the use of the HEPA filter for 3 hours without activity, and twice after the use of the HEPA filter for 3 hours with laboratory service activities. Statistical tests used one-way ANOVA to analyze the data. The results showed that the average number of airborne germs before the use of the HEPA filter was 357,667 CFU/m³. After 3 hours of use of the HEPA filter without activity, the number decreased to 177,444 CFU/m³. After 3 hours of active HEPA filter use, the number of airborne bacteria decreased to 124 CFU/m³. The statistical test results showed a significance value of 0.000 ($p < 0.05$), which means there was a significant difference between groups. The conclusion is that the use of HEPA filters has a substantial effect on reducing the number of airborne bacteria in the Bontang City Health Laboratory room. Future researchers are advised to increase the duration of HEPA filter use and identify the types of bacteria in the laboratory room.

Keywords: Indoor air germ count; high efficiency particulate air filter; air quality; laboratory.

INTRODUCTION

Air is a vital component of the environment, crucial for the survival of humans and other living creatures. Oxygen in the air is needed for respiration, and poor air quality can disrupt bodily functions and even cause damage to vital organs¹. Currently, attention to indoor air quality is increasing, given the tendency for most human activities to be carried out indoors. According to Bluyssen (2009), individuals, especially children and older people, can spend up to 19–20 hours per day indoors, significantly increasing their risk of exposure to indoor air pollutants².

Indoor airborne microorganisms include both pathogenic and non-pathogenic bacteria, and their numbers reflect the level of microbial contamination in the indoor

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environment. Airborne microbial concentrations are influenced by human activity and user density, as well as humidity and ventilation conditions, which determine the efficiency of air circulation^{3,4}. In the context of healthcare facilities, particularly laboratories, this issue becomes even more crucial. Laboratories are public facilities that must comply with Environmental Health Quality Standards, which set 700 CFU/m³ as the maximum allowable level of airborne germs⁵. Microbiological contamination of laboratory air also contributes to the occurrence of nosocomial infections, especially in work environments that involve interaction with biological specimens⁶. HEPA (High Efficiency Particulate Air) filter is an air purification technology that effectively filters micro air particles measuring ≥ 0.3 microns, including bacteria and allergens, with an efficiency of up to 99.97%, so it is widely used in hospitals and laboratories to maintain air quality and reduce the risk of infection^{3,4}. Previous studies have shown that the use of HEPA filters can significantly reduce the number of airborne germs, as reported by Wicaksono (2021), with a reduction of 91.14% after the use of HEPA filters and portable UV⁷. However, most previous studies have been limited to waiting rooms in healthcare facilities (Fatma & Ramadan, 2020) or using a combination of air purification methods, so it has not provided a specific picture regarding the effectiveness of HEPA filters alone in various types of functional spaces in the laboratory⁸.

On the other hand, there are not many studies that examine the variation in the number of airborne germs in various activity spaces in one laboratory institution, such as sampling, sterilization, microbiology, and administration rooms. Therefore, this study aims to determine the effect of the use of HEPA filters on the number of airborne germs in the Health Laboratory room of Bontang City by comparing the number of germs before use, after 3 hours of use without activity, and after 3 hours of use with Laboratory service activities.

MATERIALS AND METHODS

This study was an analytical observational study. The design used was cross-sectional, where variables were measured simultaneously during a single observation period. The study was conducted at the Bontang City Health Laboratory Technical Implementation Unit, located in Api-Api Village, North Bontang District, from February 10 to 26, 2025.

The population in this study was all 45 rooms in the Bontang City Health Laboratory Technical Implementation Unit. Sampling was conducted using a purposive sampling technique, which involves selecting samples based on specific considerations by the researcher. The sample in this study was nine rooms on the first floor: the registration room, sampling room, lactation room, clinical pathology room, sterilization room, media room, laboratory room, microbiology room, doctor's room, and administration room. Six air samples were taken from each room: twice before the HEPA filter was used (0 hours), twice after the HEPA filter was used for three hours without any service activity, and twice after the HEPA filter was used for three hours with laboratory service activity. Rooms excluded from this study, in accordance with the exclusion criteria, were the consumables supply warehouse, toilets, BSL-2 (Biosafety Level 2) room, and temporary shelter.

The variables in this study consist of the independent variable, namely the use of HEPA filters, and the dependent variable, namely the number of airborne germs. The use of HEPA filters is distinguished based on three conditions, namely before use, after three

hours of no activity, and after three hours with service activity. The number of airborne germs is measured based on the results of bacterial colony growth on PCA media from air samples taken using a Microbiology Air Sampler (MAS). The tools and materials used in this study include MAS, HEPA filters (air purifiers), scales, 1000 mL Erlenmeyer flasks, stirrers, hot plates, autoclaves, sterile petri dishes, incubators, colony counters, Plate Count Agar (PCA) media, and sterile distilled water.

This study received ethical approval from the Health Research Ethics Commission of the Banjarmasin Ministry of Health Polytechnic with certificate number: 1175/KEPK/PKB/2024, and a permit application was submitted to the Bontang City Health Laboratory Technical Implementation Unit. The research procedure began with the preparation of PCA media, namely by weighing 23.5 grams of media, dissolving it in 1000 mL of distilled water, then heating until homogeneous and sterilizing at 121°C for 15 minutes using an autoclave. The media was poured aseptically into petri dishes and cooled until ready for use. Determination of sampling points was carried out at two points in each room, namely in the center and corners of the room, with a height of approximately 100–150 cm from the floor. Sampling was carried out before using the HEPA filter, then the HEPA filter was turned on for three hours in a room with no activity, followed by a second sampling. After that, the HEPA filter was turned on again for three hours in a room with service activity, and then a third sampling was carried out.

Air samples were taken using MAS with a sampling volume of 1000 L. Petri dishes containing PCA media were installed into the device. After collection, they were labeled with an identity and incubated at 35°C ± 0.5 for 48 hours. The number of colonies that grew was counted using a colony counter, then corrected using the Feller Table to obtain the number of airborne bacteria in CFU/m³ units, with the formula $CFU/m^3 = (CFU \text{ Feller Table} \times 1000) / \text{volume of air sampled}^9$.

Data analysis was conducted descriptively to illustrate differences in airborne bacterial counts across the three treatment conditions. Statistical analysis using the One-Way ANOVA test was performed to determine significant differences between treatment groups.

RESULTS AND DISCUSSION

The Bontang City Health Laboratory Unit is a Technical Implementation Unit of the Bontang City Health Office, specializing in health laboratory services. To carry out its duties and functions, the laboratory has a total of 45 rooms, consisting of 28 on the first floor and 17 on the second floor. This research focused on nine rooms on the first floor, namely the registration room, sampling room, lactation room, clinical pathology room, sterilization room, media room, microbiology room, doctor's room, and administration room.

Table 1 shows the research was conducted in nine different rooms with varying sizes, ventilation or cooling systems, and functions. The largest room was the Registration room (40.5 m²) with AC 5 kW, serving as a high-activity area. Other rooms, such as Clinical Pathology (36 m², AC 4 kW), Sampling, Media, and Microbiology (each 18 m², AC 2 kW), were used for sample processing and analysis. The Doctor's room (12 m²) and the Administration room (9 m²) were utilized for result validation and verification. The Lactation room (3.75 m²) used fan ventilation and was rarely utilized. The Sterilization room (18 m²) had natural ventilation via windows and an exhaust fan.

Table 1. Characteristics of the Rooms Used in the Research

Room	Wide (m ²)	Facility Ventilation/ Cooling	Function / Description
Registration	40,5	AC 5 kW	Registration, payment, sample receipt, result collection, patient waiting room, access path to the second floor, high activity
Sampling	8	AC 2 kW	Blood sampling, Reitz serum, secretions, rectal swabs
Lactation	3,75	Fan wind and ventilation	For breastfeeding mothers, but rarely used because the majority of patients do not breastfeed their babies.
Clinical Pathology	36	AC 4 kW	Blood chemistry, hematology, urinalysis, serology immunology, and staining examinations
Sterilization	18	2 windows and exhaust fan	Equipment washing, dry and wet sterilization, destruction of culture samples
Media	18	AC 2 kW	Weighing, dissolving, and pouring sterile media into petri dishes
Microbiology	18	AC 2 kW	Microbiology culture services
Doctor	12	AC 2 kW	Validation of examination results by a clinical pathology doctor
Administration	9	Fan wind	Verification of laboratory test results

Maintenance involves cleaning the pre-filter using a vacuum cleaner or a dry cloth, which helps protect and extend the lifespan of the HEPA filter. Unlike pre-filters, HEPA filters cannot be washed and require regular replacement. In this study, HEPA filters in all rooms were last replaced on 22 April 2024 and underwent maintenance on 3 February 2025. Air sampling was then conducted between 10 and 26 February 2025, with sampling times varying by room from early morning to afternoon (Table 2).

Table 2. Filter Replacement and Sampling Times

Room	Date HEPA filter replacement	Date Filter maintenance	Date Sampling Space air	O'clock Sampling (WITA)
Registration	22-04-2024	03-02-2025	10-02-2025	03.40-10.40
Sampling	22-04-2024	03-02-2025	11-02-2025	04.00-11.00
Lactation	22-04-2024	03-02-2025	12-02-2025	06.00-12.50
Clinical Pathology	22-04-2024	03-02-2025	17-02-2025	04.00-11.00
Sterilization	22-04-2024	03-02-2025	18-02-2025	07.00-14.00
Media	22-04-2024	03-02-2025	19-02-2025	07.07-14.07
Microbiology	22-04-2024	03-02-2025	24-02-2025	06.40-13.40
Doctor	22-04-2024	03-02-2025	25-02-2025	07.30-14.30
Administration	22-04-2024	03-02-2025	26-02-2025	04.05-11.05

The results of the examination of the number of air germs in each room before using the HEPA filter (0 hours) can be seen in the Table 3.

Table 3. Number of Germs in Room Air Before Using HEPA Filter (0 Hours)

Room	Wide Room (m2)	Temperature (0C)	Humidity (%)	Activities (People)	Number Germs Air (CFU/m3)
Registration	40,5	23,0	54	0	491
Sampling	8	23,9	53	0	377
Lactation	3,75	25,1	57	0	228
Clinical Pathology	36	20,0	42	0	279
Sterilization	18	24,2	58	0	462
Media	18	21,6	43	0	255
Microbiology	18	20,4	41	0	377
Doctor	12	22,1	54	0	306
Administration	9	24,0	57	0	444
Avarage	18,14	22,7	51	0	357,667

Based on Table 3, the average airborne bacterial count was 357.67 CFU/m². The highest value was found in the Registration Room (491 CFU/m²), due to its relatively large area (40.5 m²) and location, which allows for greater interaction and air circulation from outside. The lowest bacterial count was found in the Lactation Room (228 CFU/m²), which has the smallest room size (3.75 m²) and minimal potential for cross-contamination due to its limited function. The average room temperature was 22.7°C, and the humidity was 51%.

Table 4. Number of Germs in Room Air after 3 Hours of HEPA Use Without Laboratory Service Activity

Room	Room Area (m2)	Temperature (°C)	Humidity (%)	Activities (People)	Germ Count Air (CFU/m ³)
Registration	40,5	23,6	55	0	289
Sampling	8	24,3	53	0	165
Lactation	3,75	25,8	57	0	72
Clinical Pathology	36	20,6	42	0	161
Sterilization	18	24,8	58	0	255
Media	18	21,8	43	0	132
Microbiology	18	20,8	41	0	194
Doctor	12	22,9	54	0	111
Administration	9	24,7	57	0	218
Avarage	18,14	23,3	51	0	174,444

After using a HEPA filter for three hours without any laboratory service activity, microbiological air quality measurements in nine rooms showed an average airborne bacterial count of 174.44 CFU/m². The highest count was found in the registration room, at 289 CFU/m², while the lowest value was detected in the lactation room, at 72 CFU/m². The average room temperature during the study was 23.3°C, with a relative humidity of

51%.

Table 5. Number of Room Air Germs After 3 Hours of HEPA Use with Laboratory Service Activities

Room	Wide Room (m ²)	Temperature (°C)	Humidity (%)	Activities (People)	Number Germs Air (CFU/m ³)
Registration	40,5	24,9	56	53	260
Sampling	8	24,4	53	27	103
Lactation	3,75	25,9	57	2	43
Clinical Pathology	36	20,9	42	7	138
Sterilization	18	25,3	58	4	168
Media	18	22,0	45	3	77
Microbiology	18	21,4	41	3	115
Doctor	12	23,1	55	2	57
Administration	9	24,9	57	15	155
Average	12,14	23,6	52	13	124

After using HEPA filters for three hours during laboratory service activities in the laboratory service rooms, air quality measurements showed that microbiological activity remained in the air. The results showed an average airborne bacterial count of 124 CFU/m³, with the highest value in the registration room at 260 CFU/m³ and the lowest value in the lactation room at 43 CFU/m³.

The average room temperature during the observation period was recorded at 23.6°C with a relative humidity of 52%. The highest activity occurred in the registration room, with 53 people recorded inside during the observation period. The high activity in the registration room was influenced by its function as the initial patient visit area, waiting area, result collection area, and access point to the second floor of the Bontang City Health Laboratory building.

From Table 6, it is known that the number of air germs in the room at the Bontang City Health Laboratory is still in accordance with the requirements of the Decree of the Minister of Health of the Republic of Indonesia No. 2 of 2023 concerning Environmental Health Requirements, namely the number of air germs in public facilities (laboratories) is <700 CFU/m³. The average percentage reduction in the number of airborne germs after using a HEPA filter for 3 hours without activity was 51.59%. The average percentage reduction in the number of airborne germs after using a HEPA filter for 3 hours with activity compared to after using a HEPA filter for 3 hours without activity was 32.91%.

The statistical analysis in this study began with a normality test for the airborne germ count data from three treatment conditions, namely before the use of the HEPA filter, after the use of the HEPA filter for 3 hours without activity, and after the use of the HEPA filter for 3 hours with laboratory service activities. The normality test was conducted using the Kolmogorov-Smirnov and Shapiro-Wilk methods. The results of the Shapiro-Wilk test showed a significance value of 0.531 in the group before the use of HEPA, 0.989 in the group after the use of HEPA without activity, and 0.602 in the group after the use of HEPA with activity. All significance values were greater than 0.05, so the data were normally distributed.

After the data were found to be normally distributed, a homogeneity of variance test was performed to ensure equality of variance between groups as a requirement for using the ANOVA test. Based on the results of the Levene test, a significance value of 0.242 was obtained (based on the average), which means that the value is greater than 0.05 and indicates that the variance between groups is homogeneous. Next, a one-way ANOVA test was performed to determine whether there was a significant difference between the three treatment groups in the number of airborne germs. The results of the ANOVA test showed an F value of 22.048 with a significance value of 0.000 ($p < 0.05$), which indicates that there was a statistically significant difference between the tested groups.

To determine which groups differed significantly, a further test was conducted using the Post Hoc Test with the Least Significant Difference (LSD) method. The results of the LSD test showed that there was a significant difference between the number of airborne germs before using the HEPA filter and after using the HEPA filter for 3 hours without activity ($p = 0.000$), and between before using the HEPA filter and after using the HEPA filter for 3 hours with activity ($p = 0.000$). However, there was no significant difference between the group after using the HEPA filter without activity and the group with activity ($p = 0.160$).

Table 6. Percentage Reduction in Airborne Germ Count Before and After Using HEPA filter

Room	Airborne Germ Count (CFU/m ³)			Percentage Reduction in Airborne Germ Count (%)	
	Before (0 Hours)	After 3 hours of no activity	After 3 hours of activity	After 3 hours of no activity	After 3 hours of activity
	a	b	c	d	(b-c)/b*100 (c-d)/c*100
Registration	491	289	260	41,14	10,03
Sampling	377	165	103	56,23	37,58
Lactation	228	72	43	68,42	40,28
Clinical Pathology	279	161	138	42,29	14,29
Sterilization	462	255	168	44,81	34,12
Media	255	132	77	48,24	41,67
Microbiology	377	194	115	48,54	40,72
Doctor	306	111	57	63,73	48,65
Administration	444	218	155	50,90	28,90
Amount	3219	1597	1.116	464,30	296,23
Avarage	358	174	124	51,59	32,91

Air microbiology sampling was conducted over nine days in February 2025 at the Bontang City Health Laboratory. The sampling procedure was carried out in stages in nine different rooms, adapting to the limitations of the available equipment and HEPA filter units. Although sampling times varied, the intervals between the three sampling conditions were kept consistent in each room. Temperature and humidity measurements were conducted simultaneously to obtain a comprehensive picture of environmental factors that

could influence airborne bacterial counts.

HEPA filters work through four main mechanisms: inertial impact, interception, diffusion, and electrostatic attraction¹⁰. This filter is highly efficient in filtering small particles, but its effectiveness still depends on environmental conditions, the intensity of indoor activities, and periodic maintenance and replacement¹¹.

The average airborne bacterial count before the use of the HEPA filter was 357,667 CFU/m³, with the highest value in the registration room (491 CFU/m³) and the lowest in the lactation room (228 CFU/m³). The sterilization room also recorded a high value (462 CFU/m³), which was likely due to specimen processing activities and the use of culture media that can produce aerosol particles, as it is explained that microbial particles can be spread in the air through indoor activities and mixed in the air flow¹².

After 3 hours of inactivity with the HEPA filter, the airborne bacterial count decreased significantly to 177,444 CFU/m³. This decrease reflects the efficiency of the HEPA filter's air filtration. However, microorganisms remained in the air due to circulation lifting particles from surfaces such as floors, tables, and walls, or due to re-aerosolization of settled particles¹². These results are consistent with the findings of Umami (2020), which recorded a decrease in bacterial colony count from 325 CFU/m³ to 180 CFU/m³ after using a HEPA filter-based air purifier¹³.

Under conditions of 3 hours of HEPA filter use with activity, the average number of airborne germs decreased further to 124 CFU/m³. Although human activity is known to increase the number of microorganisms in the air, HEPA filters remain effective in reducing microbial concentrations even when the room is actively used¹². The re-registration room recorded the highest number of germs (260 CFU/m³), in line with the highest recorded activity level (53 people), reinforcing the findings of Hidayati (2007), which states that the number of airborne germs increases along with the number of visitors and human activity indoors¹⁴. As also reported by Hospodsky et al. (2012), which shows that occupant density and activity have a significant impact on the increase of microorganisms in indoor air¹⁵.

The reduction in airborne germ counts varied across rooms, influenced by area and activity level. The registration room experienced the lowest reduction (10.03%), while the administration room, with lower activity and a smaller area, recorded a 28.90% reduction. The decrease in airborne germ counts varied across rooms, influenced by area and activity level, especially when only one HEPA filter unit was used. Experimental studies have shown that the effectiveness of a single HEPA filter in reducing particle concentration is influenced by increasing room volume, making a single device more effective in small rooms or spaces with limited circulation¹⁶.

The highest percentage reduction in germ counts after 3 hours of inactive HEPA filter use was 68.42%, while the lowest was 41.14%. These results are consistent with research by Wicaksono (2021), which showed a reduction of up to 94.11% after 5 hours of use of a portable HEPUV in an empty room.

One-way ANOVA statistical analysis showed a significant difference between the three conditions ($p = 0.000$), with an F value of 22.048. These results indicate that the use of a HEPA filter significantly affects the number of airborne germs. This finding is supported by research by Umami (2020), which reported a significant reduction in airborne bacterial colonies with the use of a HEPA filter-based air purifier ($p=0.002$)¹³.

Airborne microorganisms consist of both pathogenic and non-pathogenic bacteria,

and their numbers reflect the level of microbial contamination in a closed environment. Several factors that influence the concentration of airborne microorganisms include human activity, temperature and humidity conditions, and the room's ventilation system¹⁷. Based on Minister of Health Regulation Number 2 of 2023, the threshold for airborne germ counts in laboratory spaces is 700 CFU/m³. All measurements in this study were within safe limits, but they still demonstrate the importance of air quality control to prevent potential cross-contamination and nosocomial infections.

The strength of this study lies in its systematic sampling design and the use of HEPA filters under real-world laboratory conditions, providing a realistic picture of the device's effectiveness under varying circumstances. However, there are several limitations, including the limitations of the air sampling equipment used. This resulted in data collection being carried out in stages and inconsistent sampling times between rooms, which could have affected the results. Implications of this study support the use of HEPA filters as part of an air quality control strategy in healthcare facilities, particularly in laboratories. It is recommended that HEPA filters be selected according to the room size and design, used routinely, especially during peak hours, and combined with regular room cleaning and disinfection protocols for optimal results.

CONCLUSION

The results of this study indicate that the use of HEPA filters significantly reduced the number of airborne bacteria in the Bontang City Health Laboratory room. The average number of airborne bacteria before the use of the HEPA filter was recorded at 357,667 CFU/m³, which then decreased to 177,444 CFU/m³ after the use of the HEPA filter for 3 hours without any activity in the room, and further reduced to 124 CFU/m³ with the presence of laboratory service activities during the use of the filter. The results of the one-way ANOVA test showed a significance value of 0.000 ($p < 0.05$), which indicated a significant difference between the three conditions. These findings strengthen the evidence that HEPA filters are effective in improving air quality by reducing the number of microorganisms in the laboratory environment. Agencies should routinely maintain and replace HEPA filters to support the effectiveness of air filtration. Future researchers are advised to extend the duration of filter use and identify the types of bacteria for more comprehensive results.

CONFLICT OF INTEREST

In this study there is no conflict of interest

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