An improved protocol for indoors airborne bacterial enumeration and elimination using a Cold-plasma Air-sanitizer.

Mohammed Ibrahim Saeed.[1]* Ahmed Luay Osman.[1] Abdelgadir Alamin Altoum.[1] Salah-Eldin Omar Hussein.[1] Salma Elnour.[1]

1-Department of Medical Laboratory Sciences, Faculty of Health Science, Gulf Medical University, Jurf1- Ajman, UAE.

Abstract:
Background: Air Sanitizers are devices that eliminate airborne bacteria from indoor settings. **Objectives:** This study introduces an improved approach for monitoring and enumerating air-circulating bacteria following the use of a cold-plasma air-based sanitizer; the study was authorized under Ref. IRB-COHS-FAC-95-MAR-2024. Materials and Methods: A standardized air sample of 500 squire meters was collected using the Sugold FX-100ST (USA) and diectly inoculated into Chocolate, CLED, and Chromogenic culture medium 30 minutes before and during air purification. The medium was incubated and examined for colony forming units (cfu), which represent bacteria circulating in laboratory indoor air with room space of 1000 sq. meters. The total air particle pollution were measured using a particle meter (Atmotech Inc., USA). **Results:** Results revealed a significant bacterial eradication rate of 91-100%. The most often observed air pollutants bacterial species were Micrococcus and Bacillus, with few non-pathogenic gram-negative bacteria; the pathogen identified was co-aggulase positive Staphylococcus. **Conclusion:** The results showed that a cold plasma filter effectively and simultaneously removed both indoor air pollutant particles and airborne microorganisms. This study presents a simplified, standardized approach for sampling and counting indoor air bacteria, which cut down on the steps the expenses, and efforts associated with airborne bacterial testing. Furthermore, using a cold plasma filter to remove airborne bacteria could significantly reduce the incidence of indoor respiratory infections, especially for individuals who are highly susceptible such as immunocompromised people, kidney transplant recipients, and intensive care unit patients who need pathogen free indoor air.

Keyword: bacterial, elemination, enumeration, indoor-air, cold-plamsa filter.

***Correspondence**: Mohammed Ibrahim Saeed, Department of Medical Laboratory, Faculty of Health Science, Gulf Medical University, Jurf1- Ajman, UAE. email: moibsaeed@yahoo.com, ORCID: 0000-0002-3530-1351.

Author's ORCID:
Mohammed Ibrahim Saeed: 0000-0002-3530-1351
Ahmed Luay Osman: 0000-0003-0144-3543
Abdelgadir Alamin Altoum: 0000-0002-2442-2756
Salah-Eldin Omar Hussein: 0000-0002-1819-5948
Salma Elnour: 0000-0001-6439-5062

Airborne bacterial enumeration & elimination protocol

Introductions:

Monitoring airborne microorganisms is critical for maintaining public health and preventing respiratory infections. Potocol testing includes air sampling, microorganisms counting, isolation, and identification applying many approaches, each with its own advantages and limitations.

Several air sampling techniques are commonly employed, including the Andersen impactor, which separates particles by size but can kill microorganisms.[1] Another method is filtering with PTFE filters, which can capture a wider range of sizes but can dry out target organisms.[2] Finally, liquid impingement, such as the SKC BioSampler, maintains pathogens better but must be processed promptly.[3]

The culture-based methods are still considered the gold standard for counting and detecting pathogens, as they allow for the isolation of viable organisms in the form of colony-forming units (CFU). However, these methods can be quite slow,[4] However, these methods can be quite slow. On the other hand, techniques like qRT-PCR, metagenomics, biosensors, and CRISPR-based assays offer quicker results, but they come with higher costs and can't differentiate between live and dead organisms. Additionally, these methods require further optimization.[5,6,7,8]

Airborne microbes pose a severe hazard to public health, particularly infectious diseases. Containment of respiratory infectious diseases caused by various well-known microorganisms is an important technique for minimizing these disorders.[9] A new and increasing subject is the development of improved approaches that use cutting-edge technology to reduce the risk of airborne illnesses and pollution problems.[10]

Air Sanitizers are devices that act on airborne microorganisms, including bacteria, fungi, and viruses, in various environments. Unlike filter-based air purifiers, which only trap particles within an air circulator, Air Sanitizers act on airborne microorganisms in open interior air space. [11,12]

The latest technologies in atmospheric air sanition are becoming more innovative and efficient.[13] The latest innovative designed air purifiers are getting smaller, such as the Luft Duo air purifier.[12,14]

One of the latest introduced air purification technology is the atmospheric Cold Plasma filter technology which generate a plasma field, eliminating various types of polluting molecules and microorganisms circulating in air by a physical reaction, operating with durable, reusable & easily washable filters.[12,15] The method seems to kill the bacterium by a variety of mechanisms, such as increased intracellular reactive oxygen species (ROS), low-level DNA damage, and cell wall destruction.[16]

The airborne microbial enumeration protocols were well established for liquid samples such as water, milk products, and other food products, but it is still not well standardized for bacterial air samples and mainly the amount of air volume in the sample used for bacterial count in colony forming units, so this work provide smart method for collection of equal air sample volume and direct inoculation of the collected air bacteria into the cultivation medium petri-dis.

This study employed standardized collected air sample volumes that were directly injected into a culture medium. The cfu count after overnight incubation was used to determine the amount of bacteria circulating in the air sample. The air was also tested every 30 minutes for particles while a cold plasma filter was running.

Materials and Methods:

This investigation was carried out in the medical lab at Gulf Medical University's Faculty of Health Sciences. The study design entails collecting standardized air samples of 500 squire meters per sample using a Sugold FX-100ST (USA); counting and bio-typing of airborne circulating bacterial isolates before and during air filtration using a Cold Plasma filter machine (TPA Air Technology, Germany) pre-fixed in the teaching microbiology and hematology laboratories.

Simultaneously to the microbiological enumeration by cfu in culture media, the total number of circulating air particles in the laboratory room were quantified using an indoor pollution recorder (Atmotech Inc., USA), and the recorded particle count records were compared to the bacterial count in the air at the time of sample collection, which was measured every 60 seconds throughout the study duration.

Samples collection: The air samples were collected directly and inoculated on the various types of culture media used (Chocolate agar, CLED, and Chrome agar for Gram negative via Microbial air sampler device (Sugold FX-100ST, USA), which was used to control the collected air-sample volume and to have equal amounts of air inoculated, and the experimental condition was unique in all samples tested throughout the study (Figure 1).

Figure 1 : *Left: Atmocube particle recorder* *Right: Cold-plasma Air Sanitizer*

Bacterial Isolation: The collected air samples were directly inoculated onto the various culture media that were placed within the air sampler equipment during air collection. All inoculated plates were incubated at 37°C for 24-48 hours before and after being treated with Cold-Plasma-Technology sanitizer, and the plates were examined for colony count and bacterial biotype.

Isolate enumeration and typing: All air-borne bacteria cultured in cultivation media were microbiologically typed using colonial morphology on the various culture media employed, gram reaction, and biochemical reactions.

Airborne bacterial enumeration & elimination protocol

Results & discussion:

Bacteria cultivation & enumeration results:

Microbial colonies growth in different media cultures; indicates high count in zero samples beofre running air sanitizer, with significant reduction of colony count in the last samples mainly after 24 hours; (Figure 4). The number of bacteria was substantially reduced with the mean of decrease of bacteria count range between 91 - 100 %, (Table 1 & Table 2).

The circulating air-bacteria were removed by half within one hour after using cold-plasma air sanitizers as shown in the cfu count in different cultivation media. Circulating bacteria colonies were almost absent after 24 hours (Table-1 & 2) and culture plates(Figure- 2 & 3).

Circulating air particles count:

The results of the study revealed the existence of a clear correlation between pattern of the two most significant parameters: the counted particles and bacterial cfu levels in air samples across the study duration. Based on this observation, it is logical to conclude that the microbiological count of indoor air is directly proportional to the total air-circulating counted particles, enabling to confidently assert that the concentration of detected particles rises as more microbes are circulating in the air (Table-1 & 2; Figure-2 & 3).

Table 1: Bacteria cultivation results: the rate of reduction in air bacterial count (cfu) by Cold Plasma Filter Air Sanitation in Microbiology laboratory samples inoculated in Chocolate, CLED & Chromogenic agar.

A-**Microbiology laboratory air sample cfu on Chocolate agar**									
Sampling Time	Zero sample	30 min	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	75	41	32	25	22	15	**6**	**4**	94.7%
B- **Microbiology laboratory air sample cfu on CLED agar**									
Sampling Time	Zero sample	30 mi n	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	59	34	22	18	16	16	12	**2**	96.52 %
C- Microbiology laboratory air sample cfu on UTI Chrome agar									
Sampling Time	Zero ample	30 mi n	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	26	15	11	9	**4**	**4**	**4**	**1**	96.16 %

Airborne bacterial enumeration & elimination protocol

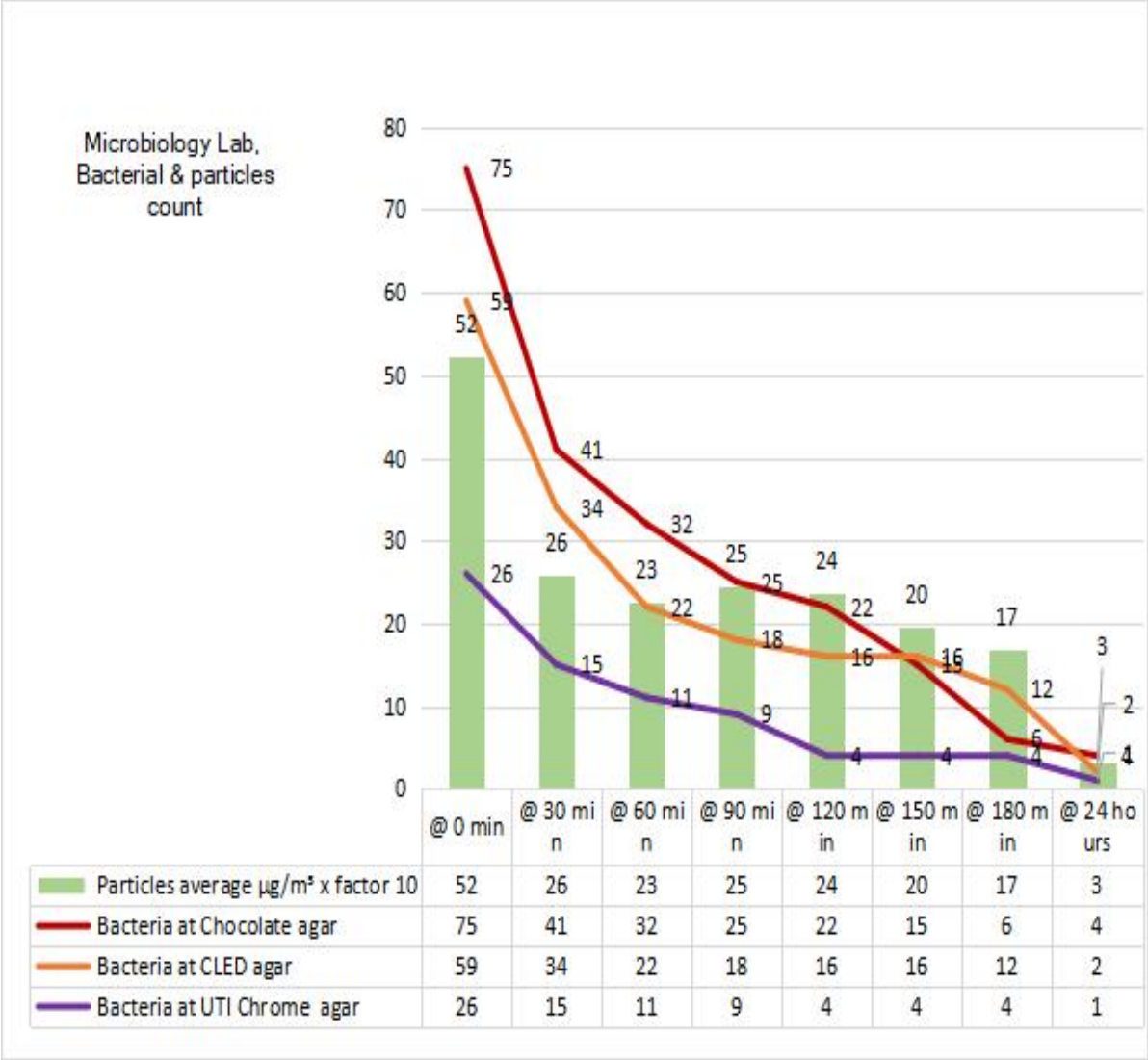

Figure 2. *Particles count VS Colony count in Microbiology Lab, GUM University.*

Table 2: Bacteria cultivation results: air bacterial count & rate of reduction by Cold Plasma Filter Air Sanitation in Hematology laboratory samples inoculated in Chocolate, CLED & Chromogenic agar.

A/ **Hematology laboratory air sample cfu on Chocolate agar**									
Sample Time	Zero sample	30 mi n	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	73	28	26	22	22	17	12	**6**	91.79 %

Airborne bacterial enumeration & elimination protocol

B/ **Hematology laboratory air sample cfu on CLED agar**									
Sample Time	Zero Sample	30 min	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	72	34	29	24	16	16	12	**2**	97.33 %
C/ **Hematology laboratory air sample cfu on Chrome agar**									
Sample Time	Zero sample	30 min	60 min	90 min	120 min	150 min	180 min	24 hours	Reduction %
Bacterial cfu count	20	8	7	7	**3**	**3**	**1**	**0**	95 %

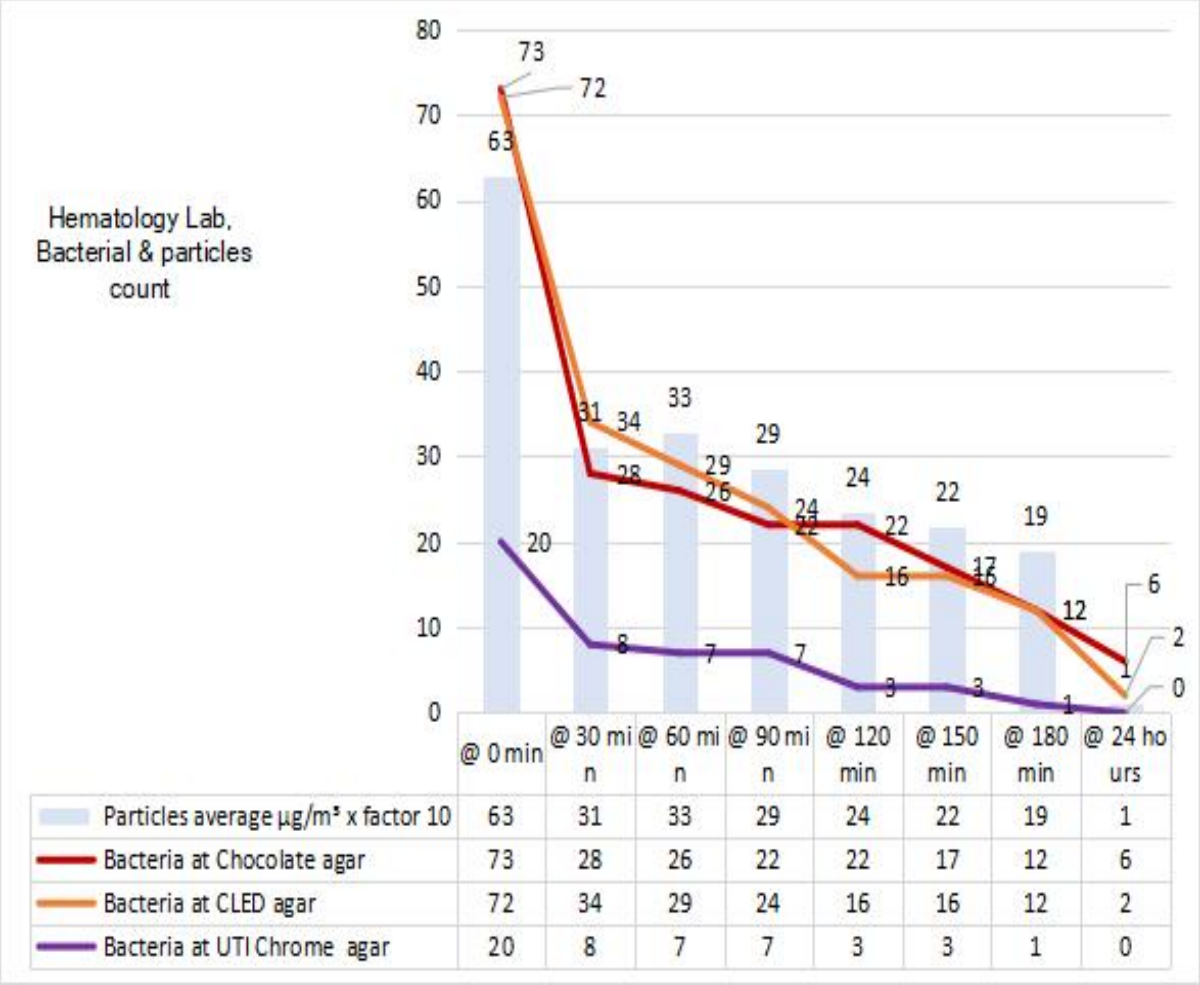

Figure 3. *Particles count VS Colony count in Haematology Lab, GUM University*

Airborne bacterial enumeration & elimination protocol

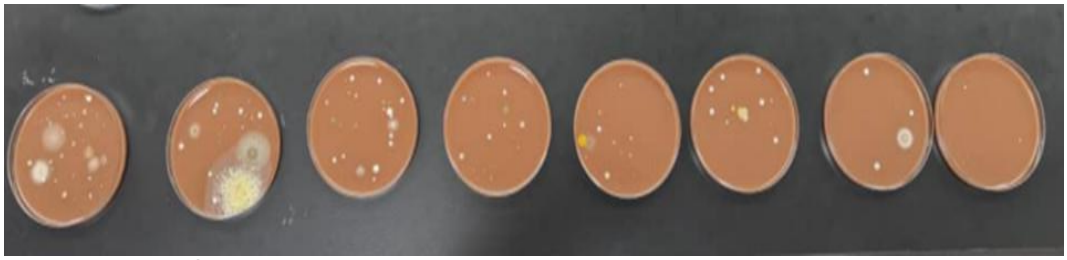

Figure 4-A. Chocolate Agar: the lasl on the right is 24-hour sample culture plate

Figure 4-B. CLED Media: the last on the right is 24-hour sample culture plate

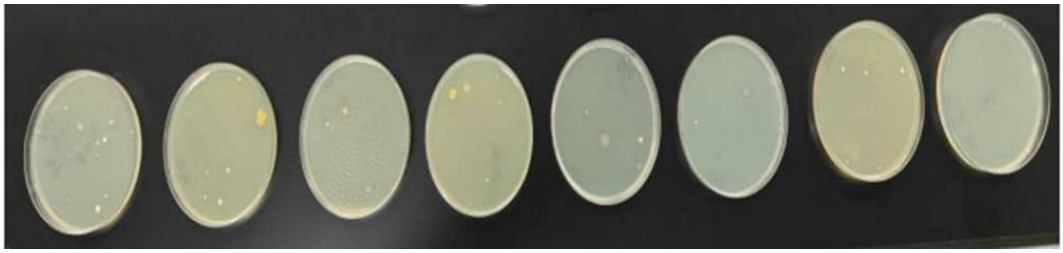

Figure 4-C. CHROME agar for bacteria: the last on the right is 24-hour sample culture plate

Bacterial identification:
Most microorganism growth from air samples was bacteria as shown in the colonies with different gram stain reactions. Most of the bacterial colonies were for the gram-positive cocci, followed by gram positive bacilli, and the least were the gram negative bacilli and there was no gram negative cocci bacterial colony. Most of the gram-positive gown bacteria were cocci including micrococcus and then gram positive bacilli. Few bacterial isolates are observed identified looks-like staphylococci spices isolated from microbiology air samples (Table 3, Figure 5).

The types of bacteria: The dominating morphological types of bacteria were Micrococcus and Bacillus species. Four isolates of staphylococcus species and Five isolates of gram-negative rod growth of bacteria were identified (Table 3, Figure 5).

Biochemical results for Gram positive bacteria: Biochemical test for the gram-positive cocci revealed the following results: Co-agulase positive Staphylococcus aurous were present in Two isolates with one isolate was penicillin-resistant. And Co-agulase negative Two isolates staphylococcus were identified and both were penicillin sensitive. All staphylococcus isolates were vancomycin sensitive (Table 3).

Table 3. **Biochemical test results for Gram positive bacteria:**

Gram stain	Coagulase test	Mannitol Salt Agar test	DNAse test	Penicillin disk	Vancomycin disk
Staphylococcus spices	2 +ve	4 +ve	2+ve	Sensitive 3	Sensitive 4
	2 -ve	-	2-ve	Resistant (1)	Resistant (-)

API test results Biochemical results for Gram negative bacteria: Based on API results and oxidase tests; Four gram negative isolates were found oxidase positive. One gram negative isolate was identified as *Burkholderia Cepacia* and the other was *Stenotrophomonas maltophilia* (Figure 5).

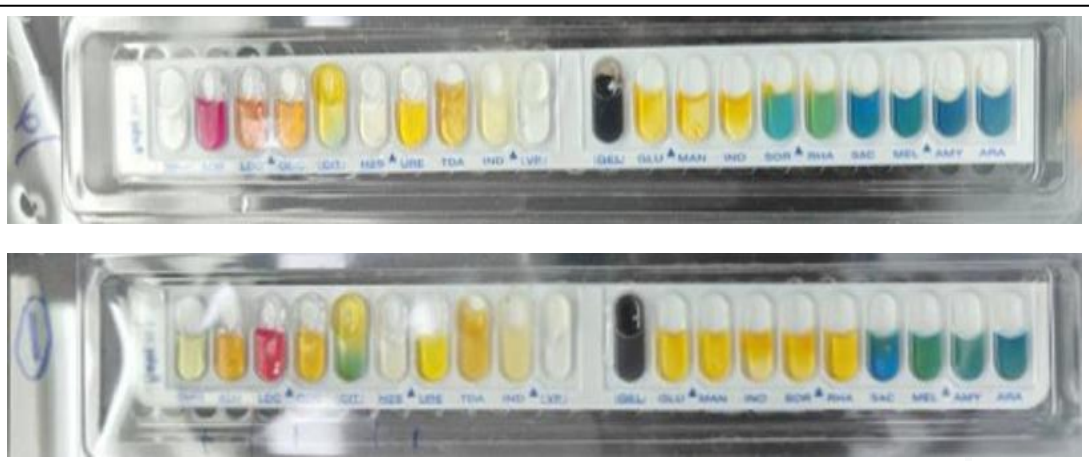

Figure 5. API-test for gram negative bacteria: top (6): *Burkholderia Cepacia*; bottom (1): *Stenotrophomonas maltophili*a

Conclusion:

The cold-plasma-filter-based Air Sanitizer significantly reduced the quantity of air-circulating bacteria such as Micrococcus, Bacillus, and gram-negative bacterial species, including clinically relevant bacterial species such as Staphylococcus aurous. The study demonstrated that microbiological airborne bacterial count is directly correlated with the total number of air particles in general. As a result, cold plasma filters could be used to prevent respiratory infections, particularly in settings where high-risk individuals live, such as immunocompromised patients, intensive care units, transplant recipients, elderly patients, and neonates admitted to intensive care units or those who require special care in pathogen-free indoor air.

Through simple air sample collection and direct inoculation onto culitivation madium, this research provides a prototype protocol for standardized airborne bacterial enumeration in air samples improveming and simplifiyng the current ongoing testing protocols and being one of the best approche for testig the sterility of indoor air from bacterial pollution and validating the capability of atmospheric air sanitizers and other purification systems.

Ethical approval: the study was approved by the Gulf Medical University Ethical Committee Institutional Review Board (IRB) Approval with reference number - Ref. no. IRB-COHS-FAC-95-MAR-2024.

Acknowledgments: Acknowledgement is extend to medical laboratory department, microbiology lab, Gulf Medical University for material support and testing facilities, TPA Air Technology, Germany providing air sampler, sanitizer, and the Institutional Ethical Committee Review Board.

Conflict of interest: All authors were contributed equally and they declared no competing interest.

References:

1-Hinds WC, Zhu Y. Aerosol technology: properties, behavior, and measurement of airborne particles. John Wiley & Sons; 2022 May 24.

2-Fabian, P., McDevitt, J. J., DeHaan, W. H., Fung, R. O. P., Cowling, B. J., Chan, et al. (2011). Influenza virus in human exhaled breath: An observational study. *PLoS ONE*, 6(6), e21481. https://doi.org/10.1371/journal.pone.0021481

3-Lin, K., Marr, L. C., & Li, Y. (2020). Aerosol sampling and detection of airborne viruses. *Environmental Science & Technology*, 54(3), 1583–1591. https://doi.org/10.1021/acs.est.9b05272

4-CDC (Centers for Disease Control and Prevention). (2019). Microbiological Air Sampling.

5-Prussin, A. J., Schwake, D. O., & Marr, L. C. (2017). Ten questions concerning the aerosolization and transmission of Legionella in the built environment. *Building and Environment*, 123, 684–695. https://doi.org/10.1016/j.buildenv.2017.06.024

6-Myhrvold, C., Freije, C. A., Gootenberg, J. S., Abudayyeh, O. O., Metsky, H. C., Durbin, A. F., Kellner, M. J., Tan, A. L., Paul, L. M., Parham, L. A., Garcia, K. F., Barnes, K. G., Chak, B., et al. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. *Science*, 360(6387), 444–448. https://doi.org/10.1126/science.aas8836

7-WHO (World Health Organization). (2021). Laboratory Testing for SARS-CoV-2: Interim Guidance. https://www.who.int/publications/i/item/WHO-2019-nCoV-laboratory-2021.1

8-Jiang, X., Wang, R., Wang, Y., Su, X., Ying, Y., & Wang, J. (2022). Advances in biosensors for airborne pathogen detection: A review. *Biosensors and Bioelectronic*s, 198, 113805. https://doi.org/10.1016/j.bios.2021.113805

9-Gonzalez-Martin, C., 2019. Airborne infectious microorganisms. *Encyclopedia of microbiology*, p.52.

10-Song L, Zhou J, Wang C, Meng G, Li Y, Jarin M, et al. Airborne pathogenic microorganisms and air cleaning technology development: A review. *Journal of Hazardous Materials*. 2022 Feb 15;424:127429.

11-Al-abdalall, Amira Hassan, Sarah Abdullah Al-dakheel, and Hmidah Abdulhadi Al-Abkari. "Impact of air-conditioning filters on microbial growth and indoor air pollution. *Low-temperature technologies* (2019): 179-206.

12-Szczotko, Maciej, Izabela Orych, Łukasz Mąka, and Jolanta Solecka. "A review of selected types of indoor air purifiers in terms of microbial air contamination reduction. *Atmosphere* 13, no. 5 (2022): 800.

13-Joppolo, Cesare Maria, and Jacques Gandini. "Emerging technologies for air cleaning and their integration in HVAC systems: Which role in the increasing quest for healthy and sustainable buildings?. In E3S Web of Conferences, vol. 523, p. 03005. *EDP Sciences*, 2024.

14-Stenström KE, Pédehontaa-Hiaa G, Ramljak B, Rääf C. Alpha spectrometry of radioactive aerosols from the European Spallation Source: an investigation of the possibilities of direct alpha spectrometry, 2024.

15-Brincat JP, Sardella D, Muscat A, Decelis S, Grima JN, Valdramidis V, Gatt R. A review of the state-of-the-art in air filtration technologies as may be applied to cold storage warehouses. *Trends in Food Science & Technology*. 2016, 1(50):175-85.

16-Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P.J. and Bourke, P., 2016. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. Applied and environmental microbiology, *82* (2), pp.450-458.