EFFECTIVENESS OF ANTI-EPIDEMIC MEASURES ON ENSURING INDOOR AIR QUALITY OF CLEANROOMS IN A TERTIARY CARE HOSPITAL

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SUMMARY

Objectives: Maintaining cleanrooms in a hospital is a key part of preventing healthcare-associated infections (HAIs). The aim of this work was to verify the effectiveness of anti-epidemic measures to ensure air quality in cleanrooms in a tertiary care hospital.

Methods: The study was based on the application of anti-epidemic measures and verification of their effectiveness by regular indoor air monitoring between the years 2014–2019. Monitoring was performed according to a recommended procedure based on European standards for cleanrooms.

Results: The results demonstrate a reduction in the number of airborne particles and the number of colonies, as well as the elimination of fungi and conditioned pathogens in air samples.

Conclusions: The authors emphasize the importance of established anti-epidemic measures and regular monitoring for the prevention of HAIs.

Key words: cleanrooms, monitoring of the indoor environment, healthcare-associated infection, airborne dust particles, microbial flora

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INTRODUCTION

Indoor spaces contain a range of microorganisms living in symbiosis with healthy individuals adapted to them (1). Certain closed spaces, however, need to be particularly clean to prevent healthcare-associated infections (HAIs). These so-called "cleanrooms" are intended, for example, for the preparation of pharmaceuticals which are not contaminated with microorganisms present in the air (2). A clean room is a space in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation and retention of particles inside the space, and in which other relevant parameters, for example temperature, humidity and pressure, are controlled as necessary (3). Requirements for cleanrooms are stated in the EU Guidelines to Good Manufacturing Practice and the European standard EN ISO 14644 Cleanrooms and Associated Controlled Environments, which sets out in Part 1 – EN ISO 14644-1 the general standard for classification of air cleanliness and in Part 2 – EN ISO 14644-2 the monitoring specifications (4–6).

The retrospective analytical study aimed to verify the effect of implemented anti-epidemic measures by the long-term monitoring in cleanrooms of drug preparations and tissue banks of a tertiary hospital during a six-year period (2014–2019) with an emphasis on grade A rooms – laminar boxes with vertical air flow that are most demanding on the air quality. Two specific cases of anti-epidemic interventions and their results are described in more detail.

MATERIALS AND METHODS

Cleanrooms were monitored using methods developed by the State Institute for Drug Control – VYR-36 Clean Rooms and recommended for use in the Czech Republic. The methods are based on the European Union's Good Manufacturing Practice (EU-GMP) and on the standard EN ISO 14644 along with Decree No 84/2008 Coll., on good pharmaceutical practice, defining the cleanroom requirements for production of sterile medicinal products and tissue banks (3–5, 7). In accordance with these regulations, the institute also set the rules for cleanroom monitoring including swabs, prints, microbiological air monitoring and airborne particle counts (3).

Air monitoring was carried out in the following departments of University Hospital Olomouc:

- Pharmacy – a section where sterile drugs are prepared;
- Department of Nuclear Medicine (DNM) – laboratories where radiopharmaceuticals are prepared and a PET/CT centre;
- Department of Microbiology – a laboratory where autovaccines are prepared;
- Centre of Assisted Reproduction – an embryology laboratory;
- Department of Haemato-Oncology – a tissue bank;
- Blood Transfusion Department – a section where blood products are prepared.

In all laminar boxes, the air was delivered to the box working area through a HEPA filter type H14 with efficiency > 99.999%

and a pre-filter type EU, which captures dust particles and thus increases the life of the main HEPA filter. The vertical airflow velocity was 0.4 ± 10% m/s.

Indoor air was monitored in laminar boxes using two approaches, airborne particle measurement and microbiological air monitoring. The tests were carried out in the morning during the normal work mode: airborne particle measurements (2 times a year) and microbiological air monitoring (4 times a year). The data were processed and analysed by workers in the hospital's Department of Hospital Hygiene testing laboratory accredited in accordance with EN ISO/IEC 17025 (8). Cleanrooms were monitored "in operation" (or, in case of sensitive processes, during simulated operations) to verify the effectiveness of air conditioning units (ACUs) during processes. The monitoring regime was different only in the embryology laboratory of the Centre of Assisted Reproduction, where the monitoring "at rest" was carried out in parallel with the "in operation" monitoring (3 times a year) both for the airborne particle measurement and for the microbiological air monitoring.

Airborne particle monitoring was performed with APC Smart-Touch airborne particle counter (Merck KGaA, Germany). The number of sampling points in particular area and the sampling times for individual monitored areas classified by cleanliness grades were based on EN ISO 14644-1. The sampling times were derived from the airborne particle counter flow rate. In this case, the flow rate was 28.3 L/min (Table 1).

After monitoring was completed, on-site results were obtained by calculating a 95% upper confidence limit using a software application integrated with the airborne particle counter. Finally, the results were compared with the maximum permitted airborne particle concentrations stated in a decree on good pharmaceutical practice based on EN ISO 14644-1 (Table 2) (7).

Microbiological air monitoring was performed using active air sampling with MAS-100 Eco microbial air sampler (Merck KGaA) and plates with culture media (TRIOS, Czech Republic) which were placed on the work surface of the laminar box. At each sampling point, at least two samples (symmetrically in the middle of the right and left sides of the horizontal work surface) were collected onto Columbia blood agar and Sabouraud agar (based on the recommended procedure of the State Health Institute) (9). The volume of each sample was 1 m^3. The samplers were placed on the technological equipment worktop (sampling point about 100 cm above ground level).

Samples were cultured in an incubator at 30 ± 1 °C for 48–72 hours for bacteriology tests using blood agar or at 25 ± 1 °C for 3–5 days for mycology tests using Sabouraud agar. Colony-forming units (CFUs) were first counted on a plate, then corrected based on Feller's statistical conversion table and converted to particles per cubic meter of air. Finally, the culture test results were compared with recommended limits for microbial contamination of cleanrooms "in operation" (Table 3) (2, 3).

In case of positive findings, the following anti-epidemic measures were implemented:

- training of staff on the compliance with regime measures and principles of good manufacturing practice, including hand hygiene;
- maintaining an aseptic environment with emphasis on the correct use of personal protective work equipment of head, body, hands, and feet (mouthpiece, cap, blouse, trousers, gloves, changing shoes);
- ensuring proper cleaning, including thorough disinfection of areas and surfaces during and after each work task;
- limiting the opening of doors to the cleanroom unless the movement of persons makes it necessary;
- inspection of the ACU and laminar flow boxes by a certified company, including filter replacement and inspection;
- control measurements after the servicing and implementation of preventive measures.

***Table 1**. Sampling times for laser particle counter with air flow rate of 28.3 L/min*

Grade	Minimum sampling time (min)	
	At rest	In operation
A	36	36
B	25	1
C	1	1
D	1	–

***Table 2.** Classification of air cleanliness by particle concentration*

Grade	Maximum permitted number of particles per m^3			
	At rest		In operation	
	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
A	3,520	20	3,520	29
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

RESULTS

Airborne Particle Monitoring

To monitor airborne particles in grade A, a total of 351 samples were collected from 2014 to 2019; of those, only 2 were above limits in 2014, both at the DNM. A declining trend in the total number of particles in all samples taken during monitoring is shown in Figure 1.

***Table 3.** Recommended limits for microbial contamination of cleanrooms in operation*

Grade	Air sample CFU/m^3	Settle plates (diameter 90 mm) CFU/4 hrs	Contact plates (diameter 55 mm) CFU/plate	Glove print 5 fingers CFU/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

CFU – colony-forming unit

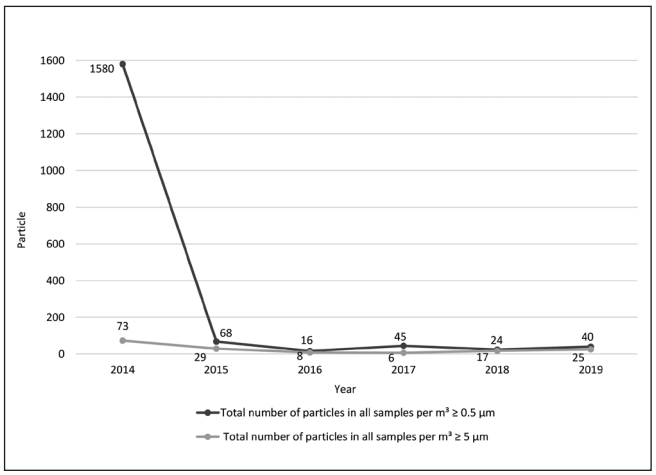

Fig. 1. *Total numbers of airborne particles in all samples in relevant years.*

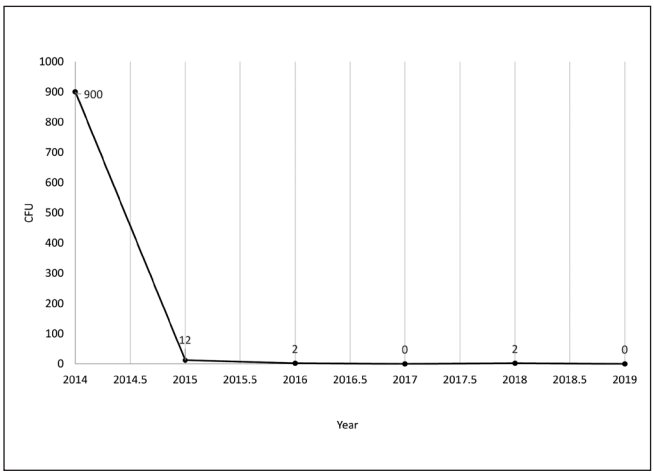

Fig. 2. *A total number of colony-forming units (CFU) converted to m³ in all samples in relevant years.*

Microbiological Air Monitoring

Between 2014 and 2019 in grade A, a total of 256 samples for microbiological tests were collected in the above areas as part of an "in operation" cleanroom air monitoring programme. There were 15 positive samples (Table 4). A declining trend in the total number of colonies in all samples taken in the relevant years is shown in Figure 2.

Figure 3 shows primary and opportunistic pathogens detected in grade A cleanrooms. The most common bacterial pathogens were *Staphylococcus* spp. (species) and *Micrococcus* spp. identified in 7 samples, respectively. An important finding was that fungal colonies were also detected in 5 samples. Since 2016, however, there has been a trend towards fewer bacterial pathogens and fungal colonies.

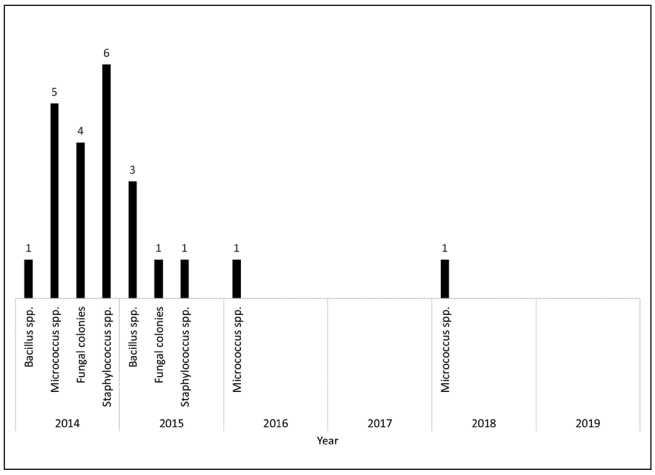

Fig. 3. *Overview of microbial pathogens detected in 2014–2019.*
Numbers above bars denote the numbers of samples in which the pathogen was confirmed

Two Examples of Monitoring Effect and Anti-epidemic Measures

Case One

As part of the above-mentioned cleanroom monitoring, the limits were considerably exceeded in laboratories preparing radiopharmaceuticals and in a PET/CT centre, parts of the DNM in 2014. Cleanroom air samples collected in those areas showed that both contamination with opportunistic pathogens including fungi and numbers of particles were above the limits. Therefore, immediate measures were undertaken which, in addition to the anti-epidemic measures mentioned above, included the following. First, ACU filters were checked. Subsequently, the laboratories for preparing radiopharmaceuticals underwent a major renovation, with a new ACU being installed and set up. Based on the measures taken, these laboratories have reached the relevant indoor air quality limits, which they have maintained for a long time (Table 5).

Case Two

In 2015, contamination of a laminar flow box was detected during the regular monitoring. In this case, undesirable fungi were detected. The contamination was probably related to a not quite professional intervention of service personnel in the laminar equipment, because during the anti-epidemic investigation, service activity of the laminar equipment was detected, during which the filters were manipulated, and which had been performed the day before the regular monitoring. Once again, aforementioned anti-epidemic measures were introduced, mainly laminar flow box filters were checked. Similar to the first case, repeated assessments failed to show the presence of bacterial or fungal pathogens, confirming the relevance of the measures taken (Table 6).

Table 4. *Quantitative results of sample cultures*

	Year						Total
	2014	2015	2016	2017	2018	2019	
Total samples	17	45	44	37	52	61	256
Positive samples n (%)	9 (52.9)	4 (8.9)	1 (2.8)	0	1 (1.9)	0	15 (5.9)

Table 5. *Results of air monitoring prior to and after implementation of measures at the Department of Nuclear Medicine*

Sampling point	Initial monitoring results			Monitoring with measures in place		
	Total number of particles per m^3		Number of microorganisms	Total number of particles per m^3		Number of microorganisms
	≥ 0.5 µm	≥ 5.0 µm	CFU/m^3	≥ 0.5 µm	≥ 5.0 µm	CFU/m^3
PET/CT Laminar flow box (grade A)	7	6	50–224	4	4	0
Laminar flow box 1 (grade A) RP	7	7	262–344	0	0	0
Laminar flow box 2 (grade A) RP	1,393	36	0–4	0	0	0

RP – laboratory preparing radiopharmaceuticals; CFU/m^3 – colony-forming unit converted to 1 m^3 of air

Table 6. *Impact of measures implemented in the Centre of Assisted Reproduction embryology laboratory*

Sampling point	Monitoring after inadequate maintenance	Monitoring with measures in place
	Number of microorganisms CFU/m^3	Number of microorganisms CFU/m^3
Laminar flow box (grade A)	4	0

CFU/m^3 – colony-forming unit converted to 1 m^3 of air

The presence of microbial contaminants has been reported from other hospital departments as well. However, those were only sporadic cases, with the limits being only slightly exceeded (1–2 colonies). These were mostly due to secondary contamination from the outside environment mediated by staff. In these cases, the abovementioned anti-epidemic measures were sufficient.

DISCUSSION

The presented results verified the effectiveness of anti-epidemic measures and the importance of grade A cleanrooms monitoring in the preparation of pharmaceutical products in a tertiary care hospital.

The study identified 3 isolated strains of *Staphylococcus* spp., *Micrococcus* spp. and *Bacillus* spp. in the air of cleanrooms. This is consistent with findings by Bonetta et al. showing that staphylococci and micrococci were most commonly found in the indoor air of a building equipped with a heating, ventilation and air conditioning system (10). Identical results were reported in a study monitoring indoor air in the University Clinical Centre, Ljubljana Pharmacy also assess the cleanroom microbiota (11). Once again, the most common organisms in the indoor air were staphylococci and micrococci. In all the cleanrooms, the distribution of bacterial genera and species was similar to that in the present study.

Other evidence comes from a 2010–2011 study by Matoušková and Holý monitoring microbial contamination of the environment at the Department of Haemato-Oncology transplant unit (12). This department is part of the same hospital in which the present study was conducted. Also, the cleanliness grade was identical to that of the Department of Haemato-Oncology tissue bank. The study showed staphylococci and micrococci as the most numerous in the microbiome of indoor air, followed by *Bacillus subtilis*, results consistent with those from the present study.

Besides the above bacterial genera, our monitoring revealed fungal colonies. These sporadic cases may be attributed to the human factor, namely staff members not adhering to the barrier and aseptic measures. This was illustrated by a 2015 event reported in the Centre of Assisted Reproduction. Both regular staff members and service technicians entering cleanrooms may be a source of indoor contamination (shedding of microbial contaminants and particles from mucosae and clothing). Tršan et al. reported that over 70% of microorganisms isolated from the air in cleanrooms were part of the normal human microbiota (11). In cleanrooms, complete sterility and absence of microbial contamination cannot be achieved. It may be assumed that in any area where air flows, airborne particles and microbial contaminants are present. This is contributed to by physiological acts of moving persons colonized by microbes adapted to this environment (13, 14). It is certainly important to eliminate this microbial flora in cleanrooms as much as possible. Although harmless to healthy people, it can have a negative impact on a vulnerable group of immunosuppressed patients, posing them to a serious health risk (15). ACUs can also become a source of fungal colonies, especially if their filters' validation and replacement are omitted (16). It is important to mention that the recommended requirements for healthcare internal environment quality including increased cleanliness demands are considered met if no pathogens or potential pathogens are present (16). As seen from the present study, opportunistic pathogens were frequently detected during monitoring but only in limited amounts, with the above exceptions.

The 2014 event at the DNM was mainly caused by the age of the facility. In the case of uncomplying findings at the DNM, the risk factor was mainly the age of the building. In this area, the low quality of indoor air was due to an old ACU that was no longer checked. This is mainly a problem of old buildings that have not been renovated (17). In this case, despite all available anti-epidemic measures, the situation required the installation and regulation of a new ACU. The following monitoring confirmed the correctness of the procedure. It must not be forgotten that the ACU system is one of the most important elements in the production process, as it provides adequate protection against the intrusion of contaminants (18). The results of monitoring at DNM

showed a good effect of the measures set. This case confirms the importance of indoor clean air monitoring because, without early detection of adverse events, subsequent organizational and technical measures aimed at optimizing the quality of indoor spaces would not have been possible.

The ideal way to ensure the appropriate quality of cleanrooms is to think about it already when designing new buildings and then to continue with preventive periodic monitoring, which would reveal process and technological deficits (19). Important parts of the monitoring process are its preparation and initiation, with the areas being thoroughly investigated and the technical, spatial and organizational possibilities of the facility being mapped so that potential risks are predicted based on relevant findings and solutions are proposed. To achieve a safe environment for the preparation of medicinal products and to prevent the development and spread of HAIs, both aseptic techniques must be followed and attention to barrier measures must be paid (20).

CONCLUSION

The results of regular monitoring of the indoor air of grade A cleanrooms verify the effectiveness of the implemented anti-epidemic measures, as demonstrated by 6-year experience with the periodic monitoring of these rooms in a tertiary care hospital.

Conflict of Interests

None declared

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