Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Evaluation of biosafety cabinets used in microbiology laboratories according to performance qualification tests

Mikrobiyoloji laboratuvarlarında kullanılan biyogüvenlik kabinlerinin performans yeterlilik testlerine göre değerlendirilmesi

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ABSTRACT

Objective: The biosafety cabinet is one of the important devices used in microbiology laboratories to provide personnel, environment and product protection. For this reason, performance characterization tests should be performed both at the installation stage and routinely. There are inspection bodies established for this purpose in our country. In this study, the findings were examined based on the last three years' data by the Sterility Control Laboratory (accredited-Inspection institution) accredited by TS EN ISO/IEC 17020 under the Ministry of Health of Türkiye, Department of Public Health Reference Laboratories of the General Directorate of Public Health.

Methods: In this study, nonconformities in biosafety cabinets, which were inspected based on TS EN ISO 12469 "Microbiological Safety Cabinets Performance, Classification and Verification" and ANSI/NSF-49 "Biosafety Cabinet Design, Production, Performance and Field Certification" standards between 2019-2021, were investigated. Within the scope of these standards, leakage in the main filter, leakage in the exhaust filter, inlet air flow velocity, downstream air velocity

ÖZET

Amaç: Biyogüvenlik kabini; personel, çevre ve ürün korumasını sağlamak amacıyla mikrobiyoloji laboratuvarlarında kullanılan önemli cihazlardan biridir. Bu sebeple hem kurulum aşamasında hem de rutin olarak performans yeterlilik testlerinin yapılması gerekmektedir. Ülkemizde bu amaçla kurulan muayene kuruluşları mevcuttur. Bu çalışmada T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü Halk Sağlığı Referans Laboratuvarları Dairesi Başkanlığı bünyesinde TS EN ISO/IEC 17020 akreditasyonuna sahip Sterilite Kontrol Laboratuvarı (Akreditasyona sahip-Muayene kuruluşu) tarafından son üç yıllık veriler incelenmiştir.

Yöntem: Bu çalışmada 2019-2021 yılları arasında teste tabi tutulan biyogüvenlik kabinleri ; TS EN ISO 12469 "Mikrobiyolojik Güvenlik Kabinleri ile ilgili performans özellikleri ve Sınıflandırması ve Doğrulaması" ve ANSI/NSF-49 "Biyogüvenlik Kabini Dizayn, Üretim, Performans ve Alan Sertifikalandırması" standartları baz alınarak muayeneleri yapılan biyogüvenlik kabinlerindeki uygunsuzluklar araştırılmıştır. Bu standartlar kapsamında ana filtreda kaçak, egzoz filtrede kaçak, içeri hava

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Geliş Tarihi / Received : 22.05.2023
Kabul Tarihi / Accepted : 01.11.2023

DOI ID : 10.5505/TurkHijyen.2023.94220

Namlı Bozkurt EN, Öznur AS. Evaluation of biosafety cabinets used in microbiology laboratories according to performance qualification tests. Turk Hij Den Biyol Derg, 2023; 80(4): 483 - 490

measurement, air flow direction and visualization tests were applied. In addition, the cabins have undergone preliminary inspection.

Results: The leakage rates were found as 22.9% in the main filter and 23.6% in the exhaust filter. The nonconformity rates due to air flow velocities and flow visualization tests were found as 19.1% for inflow airflow velocity, 15.3% for downstream airflow velocity and 19.1% for flow visualization tests.

Conclusion: It was observed that the most frequently observed non-compliance was leakage in the filters. In addition, it has been determined that users do not have a filter certificate. In the preliminary examination, it was determined that there were also cabins that did not give visual and audible alarms when the flow was blocked. Since providing a healthy working environment in microbiology laboratories is one of the main objectives, we believe that it is important to have the performance qualification tests of the biosafety cabinets done at certain intervals in terms of employee health.

Key Words: Biosafety cabinet, inspection, performance qualification tests

akış hızı, aşağı hava akış hızı ölçümü, hava akış yönü ve görselleştirmesi testleri uygulanmıştır. Bu esnada kabinlerin ön incelemesi de yapılmıştır.

Bulgular: Ana filtrede kaçak oranı %22,9 ve egzos filtresinde kaçak oranı %23,6. Hava akış hızları ile akış görselleştirme testlerinden kaynaklanan uygunsuzluk oranları içeri hava akış hızı için %19,1, aşağı hava akış hızı için %15,3 ve akış görselleştirme testleri için ise %19,1 olarak bulunmuştur.

Sonuç: En sık gözlemlenen uygunsuzluğun filtrelerde meydana gelen kaçak olduğu görülmüştür. Ayrıca kullanıcılarda filtre sertifikasının bulunmadığı tespit edilmiştir. Ön incelemede akış engellendiğinde görsel ve işitsel alarm vermeyen kabinlerin de bulunduğu belirlenmiştir. Mikrobiyoloji laboratuvarlarında sağlıklı çalışma ortamı sağlanmasının ana amaçlardan biri olması sebebiyle çalışan sağlığı hususunda biyogüvenlik kabinlerinin performans yeterlilik testlerinin belirli zaman aralıklarında yaptırılmasının önemli olduğu kanaatindeyiz.

Anahtar Kelimeler: Biyogüvenlik kabini, muayene, performans yeterlilik testleri

INTRODUCTION

Biosafety cabinets are the cabins in which an air barrier is created to protect the personnel working inside and the air is drawn from the outside into the device, releasing the air into the environment after filtering in order to protect the environment during operation, and reduce the risk of contamination of the sample/product (1). Although it is divided into various classes according to the purpose of use and the sample/product to be studied, Class II A2 type is mostly used in microbiology laboratories (2). So as a primary engineering control biosafety cabinets are used, and the laboratory design is the second engineering control (3) (Figure 1). Since the placement of the biosafety cabinets in the laboratory will affect the performance of the device, attention should be paid to its positioning. It should be away from open windows, doors, ventilation grilles, air supplies, areas with heavy staffing, and equipment that generates large amounts of heat where as cabin airflow may be disturbed (4). Many studies done about the relationship between crossdraft, people walkinng by, sash movement and operatör hands movement and the performance of biosafety of cabinet. It is found that although sash movement and operator hand movement are effecting containtment, walk-bys cause more (5). Biosafety cabinets' ability to attract contaminants is dependent on the airflow pattern within the room, the level of turbulence within the room, and the rate of air entry into the cabinet. When the entry speed to the device decreases, also the contamination withdrawal speed decrease (4). There should be enough space between

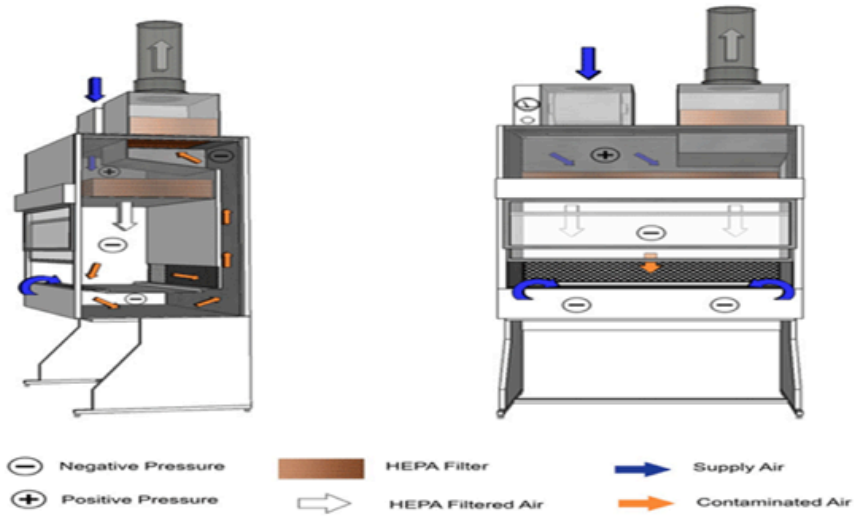

Figure 1. Class II A2 Cabinet (3)

the exhaust filter on the cabin and the ceiling so that the air outlet is not blocked. Disassembly and assembly of HEPA filters should be done by competent persons. This both protects the filter and protects the person from any contamination who is changing the filter (6). In addition, it should be noted that the filter is at least H14 based on the sample to be studied in the biosafety cabinet. Class II A2 cabinets are mostly used in microbiology laboratories. In Class II A2 cabins, approximately 30% of the air inside passes through the filter before leaving the cabin. The remaining 70% air is recycled and filtered before being sent back to the work area (7). Although HEPA filters are not effective to remove gasesand vapors, they can remove particulate contamination like virus particules (8).

Periodic inspections of these devices, which are used for the protection of the working personnel, the product and the environment, must be carried out. Inspection is the determination of the conformity of the device to the requirements by performing the performance qualification tests of the device within the framework of the relevant standards, procedures, instructions and valid methods (9). Certification documentation is also important side of inspection; calibration reports of test equipment that used for inspection biosafety cabinet have to be sent, while as the technician/inspector has to be trained nad know the cabinet and standarts. Therefore the qualifications of technician/inspector should be checked as looking at training records, education backgrounds (10).

In order for microbiological safety cabinets to work in accordance with its purpose, it is necessary to have a CE certificate and to standardize at the production stage, to carry out installation tests, to perform periodic maintenance, performance tests and repairs, and to comply with the rules of use (11). Decontamination of biosafety cabinet after usage is important to work safely. Disinfectants like ethanol (62%-71%), sodium hypochlorite (1%), phenolic compounds etc. can be used (12). For the surface contamination before and after work ultraviolet light can be used. Be careful about the lambs; they must be clean and replaced in recommended periods (13).

Three main points for biosafety cabinets; effective usage, maintenance and performance tests save life. Microbiological laboratory workers have to know how to operate in cabinet and putting large amonut of material in it cause containtment. To protect environment from infectious agents during replacing HEPA filter special and proper procedures have to be applied (14). Performance tests can be

name classified as installation tests and routine tests. Installation tests are carried out by the manufacturer to confirm the compliance of all functions of the biosafety cabinet with the standards. Performance qualification tests are the evaluation of conformity; after maintenance/repair or once a year (the period of which may change according to the frequency of use) during the use of the device. Organizations that perform installation tests and periodic inspections are called inspection bodies. The competence of these organizations is important. One of the important indicators of competence is accreditation. Accreditation of inspection bodies is carried out in accordance with TS EN ISO / IEC 17020 Conformity Assessment - 'Requirements for the operation of various types of inspection bodies' standard. There are 15 inspection bodies accredited by the Turkish Accreditation Agency in our country. Only one of the 15 accredited inspection institutions is a public laboratory, the Sterility Control Laboratory under the Ministry of Health, General Directorate of Public Health, other institutions are private companies.

In our study, it is aimed to evaluate the compliance of the biosafety cabinets, which must be used in every microbiology laboratory to protect the environment, product and person, with international standards by performing performance qualification tests.

MATERIAL and METHOD

In this study, 160 biosafety cabinets for which performance qualification tests were carried out by the Sterility Control Laboratory of the General Directorate of Public Health, Department of Consumer Safety and Public Health Laboratories between 2019-2021 were examined (Figure 2). Inspections of the said biosafety cabinets were carried out in line with the demands received from microbiology laboratories in public and private institutions and organizations.

Since 3 of them are defective, data could not be obtained. While performing performance qualification tests, TS EN 12469 and NSF / ANSI 49 standards are taken as basis. Of the 157 biosafety cabinets examined, 13 are Class II B2 cabins and the others are Class II A2 cabins. Class II A2 cabinet differs from B2 air circulation; a recirculating cabinet A2 and fully exhaust cabinet B2. About 30% of the air in A2 cabins passes through the filter before leaving the cabin. The remaining 70% is turned back and passed through the filter before being sent once more towards the work area. For B2 cabinet, building exhaust system draws air through the front and rear grills into a contaminated plenum and then into a filter before it is expelled directly into the cabin's outer atmosphere. The B2 cabinet is powered by 100% fresh air and there is no recirculation (15). While making this

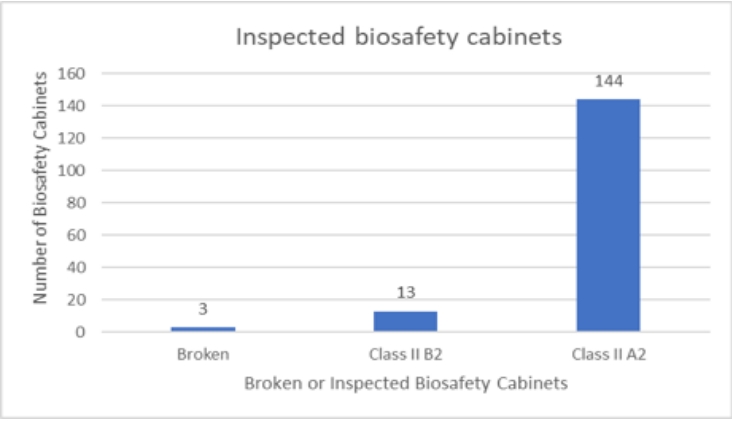

Figure 2. Inspected biosafety cabinets

study it is observed that the number of A2 cabinet are much more. The reason of this A2 cabinets are more economic design and their versatility in usage (16). While performing performance qualification tests, attention was paid to the fact that a staff member from the laboratory that owns the device is a test observer and that the measuring inspection devices are within the calibration periods. Measuring devices calibration is an important issue also, as some values can be very near to international limits and the making a decision if safety cabinet is suitable for working or not can cause a big diference for health. To overcome this and rely on calibration metrological traceability come into account (17).

Preliminary examination

It is done to confirm whether the biosafety cabinet has any malfunctions that will prevent it from being inspected. The occupancy/space ratios of the working area and whether it is sterile are taken into account. The presence of any broken parts, the operation of the control panel elements, the readability of the control panel display are confirmed. The stability of the windshield in accordance with the standards, the presence of a filter certificate is checked. Initial start and recoil alarms and exhaust system control alarm are reviewed.

Downstream Airflow Velocity Measurement

During the calibration period, downstream airflow velocity measurement is applied with anemometer. While taking measurements, attention is paid to the fact that the height level is on the same line and 10 cm above the level of the windshield opening. The entire surface is divided into equal intervals and measurements are taken from the front line and back line and recorded. The probe of the anemometer should be fixed for at least one minute, since the average of the air currents must be taken while the measurements are being taken.

Inflow Air Rate Velocity Measurement

A measurement is taken at least for a minute at 5 separate points 10 cm above the exhaust filter. The arithmetic averages of the measurements are found and the inflow velocity is found by multiplying the active area of the filter and dividing it by the area of the front opening. For these measurements, attention is paid to the fact that the ceiling height with the device is at least 30 cm.

Another measurement method is the test with a balometer. The operating december of the biosecurity cabinet is opened to the appropriate height, and the mouth of the balometer is positioned to close the front opening. The device is fixed and measurements are taken until the flow rate of the balometer is stable (Figure 3). The obtained data is recorded in m3/hour and divided by the area of the mouth of the balometer to obtain the flow rate in meters /second.

Filter Leakage Measurement

Filter leakage measurements are made with a photometer and an aerosol generator. The filter system of the biosafety cabinet, that is, the top of the main filter and the bottom of the exhaust filter, is adjusted so that the generator is exposed to aerosol between 1 mg/m3 and 100 mg/m3, and the reading value is introduced to the photometer as 100% and the measurement is started. While taking the measurement, the photometer probe tip is held perpendicular to the measurement point and the filter surface scanning is started. While scanning, after the measurement of the connection seals and mounting parts of the filter is completed, the entire surface is passed. In order to show that the air discharged from the biosafety cabinet is clean when high-pathogenic studies are carried out, after the main filter of the biosafety cabinet is completely screened from the inner surface, the upper outlet exhaust filter located on the top of the biosafety cabinet is also checked.

Air Flow Direction And Visualization

This test is performed with a flow visualization tube (smoke tube). The flow visualization test includes checking the downstream, air leakage from the windshield, air leakage from the edges and corners, and inlet airflow. It is recorded on video while the relevant controls are being made.

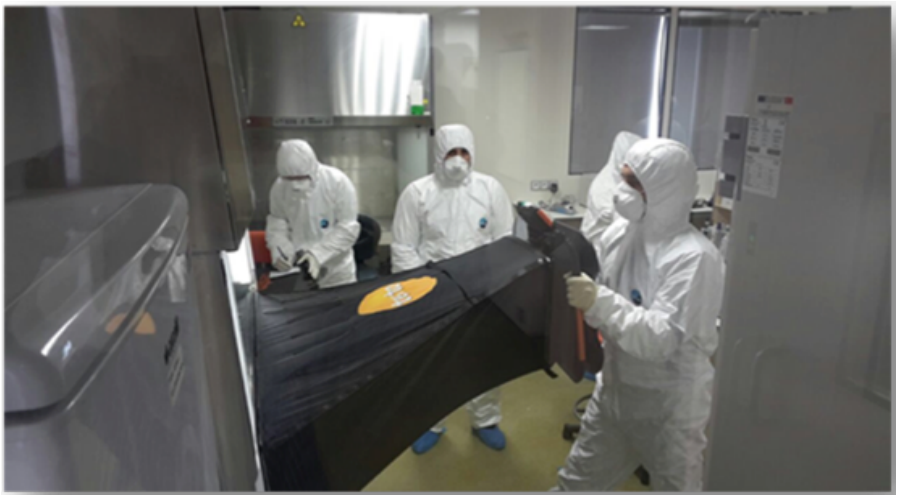

Figure 3. In air flow rate measurement of biosafety cabinet with the balometer device in P3 Laboratory

RESULTS

As a result of the preliminary examination, no certificates were found for the filters used in any of the 157 biosafety cabinets. It has been determined that 39 cabins do not give backfire alarms, and 7 cabins do not give an audio/visual alarm when air draft is blocked. It was observed that 104 did not give an alarm when the exhaust system was covered witth a airtight paper by 20%.

During the filter leakage measurement, 36 cabin main filters and 37 cabin exhaust filters were not found suitable. Leakage was found in the connection seals or main surface areas of the filters in question. It was found that the filters were not suitable during the scanning in 2 cabins. 2 bombs were seen in the main filter laminator in the cabin

The acceptance criteria for measuring the air flow rate inside are divided according to the origin of the device's production. The average speed of Class II A2 and Class II B2 cabins should be greater than 0.40 m/s according to TS EN 12469, and greater than 0.51 according to NSF 49. In addition, each measurement value should not differ more than 20% from the average. As a result of the tests, 30 biosecurity cabins were not found suitable.

According to the TS EN 12469 Standard for downstream airflow velocity measurement, the average velocity in class II biosafety cabinets should be between 0.25 m/s-0.50 m/s. Maximum and minimum speed values cannot be more than ±20% of the average speed value. According to the NSF/ANSI49 standard, the acceptance criterion is that the measured value cannot be ±0.081 m/s higher than the manufacturer's declared flow rate value. If the manufacturer's declaration cannot be reached, the speed values for class II A2 and B2 cabin types should be between 0.25-0.40m/s. According to the downstream air flow test, 24 cabins were not suitable.

According to the air flow direction and visualization test, 30 cabins are not suitable. It has been observed that the smoke given in these cabins is not absorbed, remains in the cabin, leaks from the edges of the frame, and the entering air is not absorbed from the front grilles.

DISCUSSION

The absence of a filter certificate in the users of the biosafety cabinet may cause illness if an unsuitable filter is installed until the device is inspected. The fact that it does not give an alarm when the exhaust system is covered by 20%, that it cannot be noticed when there

Table 1. Number of cabins found to be unsuitable as a result of the tests applied

Applied Tests	Unsuitable Cabin Numbers
Main Filter Leakage	36 unit
Exhaust Filter Leakage	37 unit
Inflow Air Rate Measurement	30 unit
Downstream Airflow Velocity Measurement	24 unit
Air Flow Direction And Visualization	30 unit

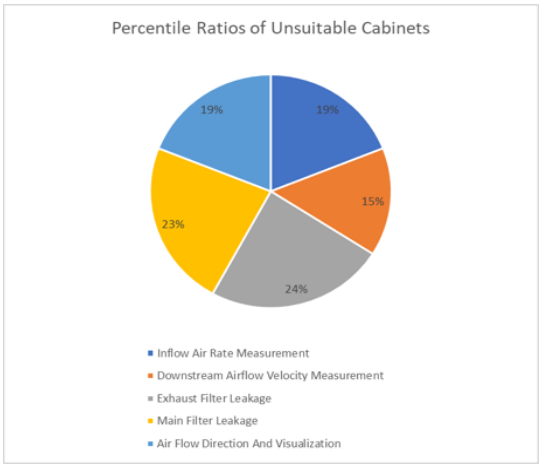

Figure 4. Percentages of cabins that were found unsuitable as a result of the tests applied

is a change in air flow values, is proof that the cabin does not show this when the filter reaches saturation or the formation of tears. Treatment. Preventing air intake and covering the exhaust system while the personnel are working in the cabin will also cause changes in air flows. In such cases, if the cabin does not give an alarm, users will not be able to take any precautions.

Leakage that occurs in the exhaust filter will cause high levels of pathogenic microorganisms to be distributed to the environment and damage the environment, spreading infections. Leaks occurring in the main filter also cause contamination of the sample being studied, which can lead to incorrect laboratory results and damage to patients as a result of incorrect treatment.

High downstream airflow causes turbulence in the cabinet and also damages the durability and permeability of the HEPA filter, while low airflow can cause contamination in the sample being studied and cross contamination when there is another sample in the cabinet.

A low inflow air rate also causes a low downstream air flow rate. Microorganisms are released from the biosafety cabinet. In addition, not absorbing the suspended air in the cabinet causes microorganisms to be released into the laboratory environment when the device is turned off. A high inflow air velocity causes turbulence and therefore contamination.

When we look at the percentile ratios, the fact that the most detected incompatibility occurs in filters, and the air flow rates are close to the flow visualization rates shows that these tests interact with each other.

The Ministry of Health, as the only authorized and accredited public laboratory throughout the country, provides services by providing necessary guidance and information sharing, making an extremely important contribution in terms of biosecurity.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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