KOMISIONI I PROKURIMIT PUBLIK
Nr. 2382 Prot.
Datë,më 28.09.2023

Shtojca 19

FORMULARI I ANKESËS PRANË AUTORITETIT/ENTIT KONTRAKTOR DHE KOMISIONIT TË PROKURIMIT PUBLIK

Ankesë drejtuar: Autoriteti/Entit Kontraktor dhe Komisionit të Prokurimit Publik ☒

Seksioni I. Identifikimi i ankimuesit

Ankimuesi mund të jetë një ofertues ose ofertues i mundshëm (p.sh. individ, operator ekonomik, shoqatë, bashkim operatoresh ekonomike)

BIOMETRIC ALBANIA SH.P.K.

Emri i plotë i ankimuesit (ju lutem shtypeni)

J61827062E

Nuis/Nipt

Bulevardi "Zhan D'Ark", Pallati Nr. 53, Ap. 6B

Adresa

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ARTUR KADAREJA - Administrator

Emri dhe titulli i zyrtarit të autorizuar për lëshimin e ankesës (ju lutemi shkruani)

"BIOMETRIC ALBANIA" SH.P.K. MEDICAL EQUIPMENT TIRANË

	2023/09/28
Nënshkrimi i zyrtarit të autorizuar	Data (viti/muaji/dita)

+355 42364326	**+355 42364 297**
Numri i telefonit (përfshirë kodin e zonës)	Numri i faksit (përfshirë kodin e zonës)

Seksioni II. Informacion mbi procedurën

1. **Numri i referencës së procedurës/Lotit**

Plotësoni numrin e references se kontratës në njoftimin e kontratës ose në dokumentet e tenderit.

REF-80785-09-21-2023

2. **Lloji i Procedurës**

Plotësoni llojin e procedurës së përdorur për prokurimin në fjalë.

Procedurë e hapur	Procedurë e hapur e thjeshtuar
√	☐
Procedurë e kufizuar	Procedurë konkurruese me negocim
☐	☐
Partneritet për inovacion	Dialog konkurrues
☐	☐
Procedurë me negocim me shpallje paraprake të njoftimit	Procedurë me negocim pa shpallje paraprake të njoftimit të kontratës
☐	☐
Shërbim Konsulence	
☐	
Kontratë e lidhur pa zhvilluar ndonjë nga procedurat e prokurimit të parashikuara në LPP	
☐	

3. Autoriteti /Enti Kontraktor

Emri i autoritetit/entit kontraktor që administron procesin e prokurimit.

Spitali Fier

4. Vlera e përllogaritur e prokurimit

(*Vlera e përllogaritur e kontratës/marrëveshjes kuadër) (shuma në shifra dhe fjalë)*

7 392 333 (shtate milion e treqind e nentedhjete e dy mije e treqind e tridhjete e tre) lekë pa TVSH

5. Objekti i kontratës/Marreveshjes Kuadër

(*Përshkrimi i shkurtër i punëve / mallrave / shërbimeve objekt kontrate/marreveshje kuader).*

Lot. 2 " Poltrone aparatura etj per punktin e kemioterapise"

6. Afati i fundit për paraqitjen e ofertës

(*Data (viti/muaji/dita*)

2023/10/09

7. Data e publikimit te Njoftimit te Fituesit

(Data (viti/muaji/dita) nëse është e zbatueshme)

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8. **Data e nënshkrimit të kontratës**

(Data (viti/muaji/dita) në rastet e kërkesave për pavlefshmërinë e kontratës)

Seksioni III. Përshkrimi i ankesës

1.Baza ligjore (Shkelje ligjore, bazuar në vendime, akte, dokumente, etj.)

Ligji Nr. 162/2020 date 23.12.2020 per Prokurimin Publik
VENDIM Nr. 285, datë 19.5.2021 PËR MIRATIMIN E RREGULLAVE TË PROKURIMIT PUBLIK

2. **Objekti i ankesës**

- Modifikim i dokumentave të tenderit

√

- Kundërshtim i vendimit të Komisionit të Vlerësimit të Ofertave lidhur me skualifikimin e ofertës tuaj.

☐

(Citoni këtu arsyet e skualifikimit)

- Kundërshtim i vendimit të Komisionit të Vlerësimit të Ofertave lidhur me kualifikimin e ofertës të një/disa operatori/ve ekonomik pjesëmarrës në procedurën e prokurimit.

☐

(Citoni operatorin/ët ekonomik për të cilin keni pretendime)

- Pavlefshmëri kontrate

☐

(Citoni kontratën për të cilën kërkoni pavlefshmërinë)

- Tjetër

☐

(Citoni këtu objektin e ankesës që nuk përfshihet më sipër)

3. **Rrethanat dhe faktet**
Përshkruani rrethanat e faktit.

Ne daten 22.09.2023 kompania jone eshte njohur ne sistemin elektronik te prokurimit me proceduren e shpallur nga autoriteti "**Spitali Fier**", me objekt: "Aparatura per Okulistike dhe poltrone aparatura etj per punktin e kemioterapise" i ndare ne dy lote perkatesisht – **Lot. 2 " Poltrone aparatura etj per punktin e kemioterapise" : REF-80785-09-21-2023**, dhe pas shqyrtimit te dokumentave standard te tenderit, bazuar ne LPP Neni 110, pika 1, paraqesim pretendimin tone per disa nga kerkesat ne DST.

4. Argumentime mbi shkeljet e pretenduara
Përshkruani në mënyrë koncize shkelejt e pretenduara, duke argumentuar qartë dhe saktë se përse pretendoni për paligjshmëri në veprimet e autoritetit kontraktor.

Ne Shtojcen 5, Formulari i Specifikimeve Teknike, *Specifikime Teknike per " LOT 2 Poltrone aparatura etj per punktin e kemioterapise"* konkretisht per:

Artikulli nr. 7, Kape per citostatiket, eshte kerkuar qe të ketë funksionim silencioz me një nivel zhurme jo më të lartë se 60dB. Kerkojme qe te ndryshohet specifikimi i kerkuar per nivelin e zhurmes.

Argumentim:
Nje nga standardet e perdorura per certifikimin e Kapave (Kabineteve te sigurise biologjike), ne lidhje me kriteret e Bioteknologjise dhe Performances eshte standardi EN 12469:2000. (bashkengjitur). Ne Aneksin A, paragrafi A.3 Sound (Zhurmat/Tingujt), ne faqen 15 te ketij standardi pershkruhet se niveli i zhurmes per nje kabinet te sigurise biologjike te instaluar ne nje laborator nuk duhet te tejkaloje 65dB. Duke qene se niveli i zhurmes se kerkuar nga standardi per nje kabinet te sigurise biologjike eshte $\leq$ 65dB, kerkojme qe edhe ne kete procedure tenderimi, niveli i zhurmes te jete jo me i larte se 65 dB. Ne kete menyre, gara eshte e hapur, brenda standardeve dhe nuk diskriminon asnje prodhues i cili ka pajisje te certifikuara brenda standardeve nderkombetare.

Eshte:
"Të ketë funksionim silencioz me një nivel zhurme jo më të lartë se 60dB"

Te behet:
Të ketë funksionim silencioz me një nivel zhurme jo më të lartë se **65dB**"

Bazuar ne nenin 36 te LPP pika 3, AK duhet te hartoje specifikimet teknike referuar pikave te meposhtme:
a) kërkesave funksionale ose të performancës, përfshirë karakteristikat mjedisore, me kusht që parametrat të jenë të saktë në mënyrë që t'u japin mundësi ofertuesve të përcaktojnë objektin e kontratës dhe autoriteteve ose enteve kontraktore të japin kontratën;

b) standardeve kombëtare, që mbështeten në ato ndërkombëtare, miratimeve teknike ndërkombëtare, specifikimeve teknike të përgjithshme, standardeve ndërkombëtare apo sistemeve të tjera teknike të referimit, të përcaktuara nga organet ndërkombëtare të standardizimit. Kur këto nuk ekzistojnë, ato u referohen standardeve kombëtare, miratimeve teknike kombëtare ose specifikimeve teknike kombëtare, që lidhen me projektimin, përllogaritjen dhe ekzekutimin e punëve ose përdorimin e produkteve;
c) kërkesave në terma funksionalë sipas shkronjës "a", referuar specifikimeve teknike sipas shkronjës "b" të kësaj pike, si mënyrë që nënkupton pajtueshmëri me kërkesat funksionale;
ç) të dyja metodave të përcaktuara në shkronjat "a" dhe "b" të pikës 3 të këtij neni, për mallra, shërbime ose punë të ndryshme, të përfshira në të njëjtin objekt kontrate. Çdo referencë duhet të shoqërohet nga fjalët "ose ekuivalenti i tij/saj".

Gjithashtu per kete artikull eshte kerkuar qe "Dera të jetë me hapje elektrike", kerkojme qe ky specifikim te hiqet.

Argumentim:
Per kete artikull eshte kerkuar specifikimi "Kapa të jetë e pajisur me me dritare sigurie qelqi me lëvizje vertikale elektrike" dhe njekohesisht me poshte eshte kerkuar "Dera të jetë me hapje elektrike". Duke qene se dritarja e sigurise eshte me levizje vertikale elektrike, e cila krijon akses te plote tek zona e punes se kapes, nuk eshte e nevojshme qe kapa te kete edhe nje dere me hapje elektrike. Per sa me siper, kerkojme qe ky specifikim **te hiqet**.

Artikulli nr. 9, Frigorifer professional 4 - 8° C, eshte kerkuar me kapacitet jo me pak se 190 litra dhe me permasa 585 – 595 x 640 – 650 x 1425 – 1435 mm. kerkojme qe te ndryshohet specifikimi i kerkuar per dimensionet e frigoriferit.

Argumentim:
Duke qene se ne kete procedure tenderimi mund te ofrohen edhe frigorifere me kapacitet me te madh se 190 litra, te cilet rrjedhimisht do te kene edhe permasa pak me te medha ose me te vogla ne gjeresi / gjatesi / lartesi, vendosja e diapazoneve me vetem 10 mm tolerance, kufizon garen dhe mundesine e ofrimit te nje frigoriferi me kapacitet pak me te larte qe do te jete me efektiv per autoritetin kontraktor. Per sa me siper, kerkojme qe specifikimi te ndryshohet "Dimensionet **e peraferta: 550 – 650 x 600 – 700 x 1450 – 1550 mm**"

Eshte:
"Dimensionet 585 – 595 x 640 – 650 x 1425 – 1435 mm"

Te behet:
"Dimensionet **e peraferta: 550 – 650 x 600 – 700 x 1450 – 1550** mm"

Artikulli nr. 10, Pompe infuzioni, eshte kerkuar qe te jete pompe elastomerike per perdorim ne kemioterapi dhe tek **TË TJERA** eshte kerkuar qe te jete e pershtatshme per rrjetin elektrik 220V – 240V/ 50 – 60 Hz. Kerkojme qe te hiqet specifikimi "Te jete e pershtatshme per rrjetin elektrik 220V – 240V/ 50 – 60 Hz",

Argumentim:
Pompat e infuzionit elastomerike, ne parimin e tyre te punes nuk kane nevoje per energji elektrike. Parimi themelor i punës së pompës së infuzionit elastomerike është i thjeshtë dhe nuk ka ndonjë përdorim elektronik ose elektrik për të kontrolluar infuzionin e ilacit. Për shkak të kësaj thjeshtësie, ato janë të përshtatshme për përdorim në mënyrë të qëndrueshme dhe për pacientët që kanë nevojë për infuzion të ngadalshëm dhe të qëndrueshëm, si ne rastin e pacienteve qe trajtohen me kemioterapi. Parimi i punës është i bazuar tek elastomerët që janë të përfshirë në strukturën e pompës. Elastomerët janë materiale të cilët kanë aftësi të deformohen dhe pastaj të kthehen në formën e tyre të meparshme. Ky deformim dhe kthim i elastomerëve krijon presion në brendësi të pompës, duke shtyrë ilacin të dalë jashtë në një rritëm të ngadalshëm dhe të përshtatur. Ky presion është i kontrolluar nga dizajni i pompës dhe nga karakteristikat e elastomerëve të përdorur në të.
Per sa me siper, specifikimi: "Te jete e pershtatshme per rrjetin elektrik 220V – 240V/ 50 – 60 Hz" eshte i pavend, ndaj kerkojme qe te **hiqet.**

Eshte:
"Aparaturat ***Pompë infuzioni, kape për citostatikët*** të jenë të përshtatshme për rrjetin elektrik 220V – 240V/ 50 – 60 Hz"

Te behet:
"Aparatura ***Kape për citostatikët*** të jetë e përshtatshme për rrjetin elektrik 220V – 240V/ 50 – 60 Hz"

Gjithashtu per kete artikull eshte kerkuar qe jete me "Minimalisht 2 shtresa membrane silikoni. Kjo përmirëson saktësinë e rrjedhës dhe redukton mbetjet kur infuzioni përfundon", kerkojme qe te ndryshohet specifikimi i kerkuar per materialin e membranes.

Argumentim:
Prodhues te ndryshem te pompave elastomerike, kane zgjidhjet e tyre teknologjike per materialet qe perdoren per membranat e pompes elastomerike. Kerkesa qe pompat te jene te certifikuara dhe te kene nje saktesi +/- 15%, jane mese te mjaftueshme per ti siguruar Autoritetit Kontraktor qe pompat elastomerike qe do te ofrohen ne kete procedure tenderimi do te jene brenda standardeve dhe do te jene funksionale. Eshte e qarte nevoja per te pakten 2 membrana, te cilat mundesojne funksionimin e pompes. Por shtimi i kerkeses per materialin e membranave nuk eshte i justifikuar. Duke qene se asnjera prej membranave, nuk do te kete kontakt me pjeset e trupit, kerkesa per membrane specifikisht silikoni, sherben thjesht per te kufizuar garen dhe pjesemarrjen e disa operatoreve ekonomike. Persa me siper, kerkojme qe ky specifikim te ndryshohet.

Eshte:
"Minimalisht 2 shtresa membrane silikoni. Kjo përmirëson saktësinë e rrjedhës dhe redukton mbetjet kur infuzioni përfundon"

Te behet:
"Minimalisht 2 shtresa membrane. Kjo përmirëson saktësinë e rrjedhës dhe redukton mbetjet kur infuzioni përfundon"

Si perfundim, kerkojme te modifikohen specifikimet teknike ne perputhje me sugjerimet e percaktuara me siper ne respektim, ne fryme dhe permbajtje te LPP dhe VKM.

Gjithashtu, ne Shtojcen 6 Formulari i Sasisë dhe Grafikut të Lëvrimit si dhe ne Shtojcen 5, Formulari i Specifikimeve Teknike, kerkohet:
Afatet e lëvrimit: 30 ditë kalendarike nga momenti i nënshkrimit të kontrates.

Duke qene se disa nga pajisjet objekt prokurimi jane produkte specifike me lende te pare inoksin e standardit 304 dhe prodhohen enkas sipas kerkesave te subjektit, realizimi i tyre kerkon kohe ne sigurimin e komponenteve mekanike dhe elektronike. Gjithashtu procesi i regjistrimit te ketyre pajisjeve mjekesore ne AKBPM kerkon rreth 30 dite, ne rast te shpalljes fitues te operatorit ekonomik.
Per kete arsye, kerkojme qe afati i levrimit te ndryshohet si me poshte:
Afatet e lëvrimit: 45 ditë kalendarike nga momenti i nënshkrimit të kontrates.

5. Kërkesë për ekspertizë të posacme

Po	Jo
□	√

(Nëse po, specifikoni llojin e ekspertizës që kërkoni)

_____

_____

6. Kërkesë për përjashtim të zyrtarëve që do të merren me shqyrtimin e ankesës:

___JO_____

_____

7. **Lista e informacionit konfidencial**:

___JO_____

Përcaktoni se cili informacion është konfidencial, nëse ka. Shpjegoni pse informacioni është ose një version i dokumenteve përkatëse me heqjen e pjesëve konfidenciale dhe një përmbledhje të përmbajtjes.

Kujdes :Ankimuesi duhet t'i bashkëlidhë ankimit, që do të paraqesë në autoritetin/ entin kontraktor dhe Komisionin e Prokurimit Publik, dokumentin bankar që vërteton pagesën e tarifës përkatëse për ankesën pranë Komisionit të Prokurimit Publik **PO, shoqeruar**

Dërgojeni formularin e plotësuar të ankesës së prokurimit, të gjitha shtojcat e nevojshme dhe kopjet shtesë, pranë **Autoritetit /Enti Kontraktor dhe Komisionit te Prokurimit Publik.**

Shënim: Ankimuesi duhet ta dërgojë njëkohësisht ankesën në autoritetin/ entin kontraktor dhe Komisionin e Prokurimit publik

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E-mail: info@biometric.al

Nënshkrimi dhe vula e Ankuesit

ARTUR KADAREJA

Administrator

"BIOMETRIC ALBANIA"
SH . P . K .
MEDICAL EQUIPMENT
REAGENT MATERJALE - TIRANË

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 12469

May 2000

ICS 07.080; 07.100.01

English version

Biotechnology - Performance criteria for microbiological safety cabinets

Biotechnologie - Critères de performance pour les postes de sécurité microbiologique

Biotechnik - Leistungskriterien für mikrobiologische Sicherheitswerkbänke

This European Standard was approved by CEN on 3 January 2000.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 12469:2000 E

Contents Page

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 233 "Biotechnology", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2000, and conflicting national standards shall be withdrawn at the latest by November 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

Microbiological safety cabinets are intended to reduce the risk to the operator when handling hazardous or potentially hazardous microorganisms. They do not necessarily protect the operator from all hazards involved. Some types of safety cabinet can also protect the materials being handled in them from environmental contamination.

1 Scope

This European Standard specifies basic requirements for microbiological safety cabinets (MSCs) with respect to safety and hygiene.

This European Standard sets the minimum performance criteria for safety cabinets for work with microorganisms and specifies test procedures for microbiological safety cabinets with respect to protection of the worker and the environment, product protection and cross contamination. Mechanical, electrical, chemical or radioactive safety precautions are not covered in the standard but are covered in EN 61010-1, EN 292-1 and EN 292-2 (see Bibliography [1], [2] and [3]).

This European Standard does not cover safety precautions for aspects not associated with the use of microorganisms such as those covering mechanical and electrical hazards, which are covered in EN 61010-1 (see Bibliography [1]), nor does it cover safety requirements regarding flammable gas and inert gases.

NOTE Some features of MSCs in addition to those for performance and safety are given for guidance in this European Standard and in EN 12741 (see Bibliography [4]).

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 1822-1	High efficiency air filters (HEPA and ULPA) - Part 1 : Classification, performance testing, marking
EN 12296	Biotechnology - Equipment - Guidance on testing procedures for cleanability
EN 12297	Biotechnology - Equipment - Guidance on testing procedures for sterilizability
EN 12298	Biotechnology - Equipment - Guidance on testing procedures for leaktightness
EN 13091:1999	Biotechnology - Performance criteria for filter elements and filtration assemblies

3 Definitions

For the purposes of this standard, the following definitions apply :

3.1 aperture protection factor (A_{pf})

Ratio of exposure to airborne contamination generated on the open bench, to the exposure resulting from the same dispersal of airborne contamination generated within the cabinet.

3.2 cross contamination

Unintended introduction of impurities of a chemical or microbiological nature from a material or product into another material or product.

3.3 microbiological safety cabinet (MSC)

Ventilated enclosure intended to offer protection to the user and the environment from the aerosols arising from the handling of potentially hazardous and hazardous microorganisms, with air discharged to the atmosphere being filtered.

3.4 MSC class I

Safety cabinet with an front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an inward airflow through the working front aperture and filtration of the exhaust air.

3.5 MSC class II

Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected, the risk of product and cross contamination is low and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an appropriate filtered internal airflow and filtration of the exhaust air.

NOTE A typical way of achieving this is by means of a uni-directional downward laminar airflow inside the cabinet and an air-curtain at the front aperture.

3.6 MSC class III

Safety cabinet in which the working area is totally enclosed and the operator is separated from the work by a physical barrier (i.e. gloves mechanically attached to the cabinet). Filtered air is continuously supplied to the cabinet and the exhaust air is treated to prevent release of microorganisms.

NOTE 1 Relevant provisions of this standard for class III microbiological safety cabinets may apply in respect of performance and construction to rigid or flexible film isolators. Further guidance specific to isolators is given in EN 12741 (see Bibliography [4]).

NOTE 2 The exhaust air can be cleaned by at least double in-line high efficiency particulate air filters and can be conducted through its own exhaust air system into the open air.

3.7 product protection

Ability of the MSC to prevent airborne contamination from outside entering the MSC through the front aperture.

3.8 retention efficiency

Ability of barrier to retain microorganisms and/or aerosols measured as the ratio of the concentration of a given marker substance between a challenged area and an adjacent area.

NOTE For MSCs, the protection of operators, environment and products is achieved by various barriers such as housing, filters or adequate flow patterns. The function of those barriers is to prevent or minimize the transfer of microorganisms/aerosols between adjacent areas being separated by a barrier. Depending on the direction of the transfer to be considered, the challenge area can be the cabinet working space, the upstream side of filters or the laboratory environment.

3.9 working space

Part of the interior of the cabinet within which manipulations are carried out.

4 Hazards

The following hazards shall be taken into account :

- release of microorganisms during operation for example through the front aperture, exhaust, piping or carcass;

- release of microorganisms during dismantling or maintenance e.g. filter replacement of MSCs or parts thereof following improper sterilization ;

- release of microorganisms by removal of contaminated material from the MSC after product- or cross contamination.

5 Performance classes

5.1 Leaktightness

The performance classes for leaktightness of MSCs are given in table 1.

Table 1 - Leaktightness performance

Performance class for leaktightness Leaktightness Index (LI)	Description of performance class
LI-A	leakage of target microorganism not defined
LI-B	leakage[1)] of target microorganism detected and quantified under defined conditions
LI-C	leakage[1)] of target microorganism tested under defined conditions and leakage below detection limit or threshold value[2)]

1) Based on leakage assessment by BATNEEC (Best Available Technique Not Entailing Excessive Costs)*.
2) Prescribed threshold value should be based on the required safety level and can for example be the detection limit of an approved BATNEEC.

5.2 Cleanability

The performance classes for cleanability of MSCs are given in table 2.

Table 2 - Cleanability performance

Performance class for cleanability Cleanability Index (CI)	Description of performance class
CI-A	visible soil or cleanliness not defined
CI-B	cleanability[1)] tested and quantified under defined conditions or MSC designed with regard to specified technical criteria
CI-C	cleanability[1)] tested and quantified under defined conditions and soil below detection limit or threshold value [2)]

1) Based on assessment by BATNEEC (Best Available Technique Not Entailing Excessive Costs)*.
2) Prescribed threshold value should be based on the required safety level and can for example be the detection limit of an approved BATNEEC.

The performance for cleanability shall be CI-B or better for all classes of MSCs.

Cleanability applies as a performance criterion for MSCs where :

- deposits of soil in MSCs could jeopardize the sterilization procedure if the sterilization media do not reach all parts of the MSC or if the required temperature is not reached ;

- cleaning procedures are intended to remove and inactivate microorganisms to make MSCs safe for handling without using any other sterilization or inactivation procedure.

5.3 Sterilizability

The performance classes for sterilizability of MSCs are given in table 3.

* Use of BATNEEC does not mean that financial issues moderate the degree of safety. Where several methods are available, the user can choose the most convenient, provided that it gives results of the necessary quality.

Table 3 - Sterilizability performance

Performance class for sterilizability Sterilizability Index (SI)	Description of performance class
SI-A	MSC not suitable or not tested for reduction of viable target microorganisms
SI-B	MSC can be treated for a specified reduction of viable target microorganisms
SI-C	MSC can be sterilized

NOTE In this table the result (performance) of an inactivation procedure is described and not the way or means of achieving the result.

The performance for sterilizability shall be SI-B or better for all classes of MSCs.

5.4 Minimum requirements for performance

Table 4 gives requirements of performance with respect to leaktightness and microbial containment for the three classes of MSCs.

Table 4 - Minimum requirements of performance for three classes of MSCs

Class	Retention at front aperture[1]	Leaktightness	Product protection	Cross contamination
I	≤ 10 CFU per operator's test[2] and ≤ 5 CFU per non-disturbance test[3]; or $A_{pf} \geq 1 \times 10^5$	LI-C for carcass	Not applicable	Not applicable
II	≤ 10 CFU per operator's test [2] and ≤ 5 CFU per non-disturbance test [3]; or $A_{pf} \geq 1 \times 10^5$	LI-C for carcass	≤ 5 CFU per test	≤ 2 CFU per test
III	Not applicable	≤ 10 % loss of test overpressure of 500 Pa in the whole enclosed system after 30 min	Not applicable	Not applicable

1) Expressed in A_{pf} or egress of microorganisms.
2) At operator's position.
3) At side positions and inward flow at front aperture established by a non-disturbance test.

6 Classification and verification of performance

6.1 General

MSCs covered by this European Standard shall be classified in accordance with the tables 1, 2 and 3. The performance of MSCs is determined and verified by the manufacturer or by the user.

In general three types of performance testing can be distinguished :

- type testing ;

- installation testing after commissioning or at change of installation, or change of environment ; and

- routine maintenance testing.

The design, construction and materials of MSCs shall be adequate for safe operation within the MSC. Illumination, sound level, vibration, stability, temperature, electrical and gas supply shall be adequate for safe operation within the MSC. MSCs shall incorporate continuous monitoring systems linked to alarms, suitable for indicating safe and unsafe conditions.

NOTE Guidance on how to achieve such conditions is given in annex A.

6.2 Leaktightness

6.2.1 General

MSCs shall be tested for leaktightness in accordance with EN 12298. The requirements for leaktightness and retention at front aperture are given in table 4. Tests shall be performed under representative process conditions. The two main aspects for microbial leaktightness for MSCs are described in 6.2.2 and 6.2.3.

NOTE A suitable test method for leakage of the carcass is described in annex B. A suitable test method for retention at front aperture is described in annex C. Any other test method may be used provided a validated correlation is established with these test methods.

6.2.2 Leaktightness for air

6.2.2.1 Leakage of carcass (class I and class II)

MSCs shall fulfil the requirements given in table 4. The carcass of MSCs in which contaminated air is under positive pressure and can leak directly to the outside shall be tested for leaks. MSCs shall be tested for leaks under positive pressure.

6.2.2.2 Leakage of carcass (class III)

The design working pressure in MSCs shall be at least 200 Pa below the air pressure of the laboratory. MSCs shall fulfil the requirements given in table 4.

6.2.3 Leaktightness for liquid spillage

MSCs shall have adequate provisions to collect spilled liquids.

6.2.4 Testing for leaktightness aspects and microbiological contamination

Direct or indirect test methods, for example flow measurement of exhaust or supply air, shall be used for installation testing and routine maintenance testing of MSCs.

NOTE 1 Table 5 summarizes test methods for type testing, installation testing and routine maintenance testing of the various aspects of leaktightness of MSCs.

Installation testing shall be carried out when MSCs are installed or at change of installation, or change of environment. If the ventilation in the room is changed, no (re)installation testing is required if the user can demonstrate, verify and document that the environment and the set up of MSCs are within the conditions of classification after type testing as specified by the manufacturer. In this case filter testing is still required. Measurement of volumetric airflow rate and visualization of airflow patterns are required but can be supplemented by retention testing at front aperture.

NOTE 2 Attention is drawn to relevant National regulations concerning routine maintenance testing.

6.3 Cleanability

MSCs shall be tested for cleanability in accordance with EN 12296. All corners and angles inside the cabinet working space and other normally accessible areas (e.g. during cleaning) likely to come into contact with microorganisms shall be rounded for proper cleaning. When surfaces inside the working space are examined without magnification by normal or corrected vision, there shall be no cracks or surface defects.

6.4 Sterilizability

MSCs shall be tested for sterilizability in accordance with EN 12297.

NOTE Annex J describes a method for fumigation MSCs.

Table 5 - Test methods for type testing, installation testing and routine maintenance testing for class I and II MSCs

Testing	Retention at front aperture	Leaktightness of carcass	Filters*	Product protection (class II only)	Cross contamination (class II only)
Type testing	- microbiological or KI method (see annex C)	- soap solution test method (see annex B)	- aerosol challenge method (see annex D)	- microbiological method (see annex E)	- microbiological method (see annex F)
Installation testing	- check that manufacturer's specification is met ; - check volumetric airflow rate measurements (see for example annexes G and H) ; and - check airflow patterns (visualization) ; - optional : retention testing (microbiological method [see annex E], or alternative methods such as KI, light scattering, after validation)	not applicable	- aerosol challenge method (see annex D) ; or - when appropriate, natural aerosol challenge method*	- check that manufacturer's specification is met ; - check volumetric airflow rate measurements (see for example annexes G and H) ; and - check airflow patterns (visualization) ; - optional : retention testing microbiological method [see annex E], or alternative methods such as KI, light scattering, after validation)	- check that manufacturer's specification is met
Routine maintenance testing (see annex K)	- check manufacturer's requirements for maintenance ; - check volumetric airflow rate measurements (see for example annexes G and H) ; and - check airflow patterns (visualization)	not applicable	- as for installation testing	- check manufacturer's requirements for maintenance ; - check volumetric airflow rate measurements (see for example annexes G and H) ; and - check airflow patterns (visualization)	- check manufacturer's requirements for maintenance ; - check volumetric airflow rate measurements (see for example annexes G and H) ; and - check airflow patterns (visualization)

NOTE National regulations may require risk assessment and may demand additional requirements in special cases, e.g. if highly hazardous microorganisms are to be used or if there is a higher danger of infection via the airborne route.

* Information on filters is given in EN 13091:1999.

7 Safety requirements

7.1 Sealing of openings

It shall be possible to seal all openings of class I, class II and class III MSCs e.g. for fumigation and disinfection.

Provision for sealing the opening for the inlet filter for class III cabinets shall be provided. It shall allow for a pressure test method to be performed.

7.2 Alarm indicators

Auditory and visual indicators of alarm conditions shall be provided for class I, class II and class III MSCs.

NOTE 1 A manometer showing the pressure drop across the filter(s) should not be used for this purpose.

Visual indicators shall be clearly visible from the operating position. The alarm indicators shall be activated when the ventilation parameters deviate from those specified by the manufacturer. The threshold limits of the deviation shall also be specified (see annex K). On starting the cabinet, the alarm indicators shall activate until the correct airflows are reached.

NOTE 2 Audible alarms may be muted during start up.

It shall not be possible for the operator to disable the alarms under operating conditions also in those cases in which the system fails.

7.3 Environmental safety

Each cabinet shall be constructed so that air discharged from the cabinet is filtered through a high efficiency particulate air (HEPA) filter, conforming to the requirements of EN 13091:1999 and of class H14 or higher of EN 1822-1.

NOTE 1 In certain circumstances it may be appropriate to discharge air from a cabinet through two in-line HEPA filters which should be able to be individually tested.

NOTE 2 Cabinets designed to recirculate filtered air to the laboratory should be provided with a suitable means for the dissipation of disinfection gas following fumigation.

7.4 MSCs exhaust and anti blowback system

When the cabinet exhaust(s) are ducted to outside the building, precautions shall be taken to prevent air flowing back into the cabinet. The manufacturer shall provide technical details on the ductwork, necessary to ensure the performance of the cabinet, together with the characteristics of the exhaust system.

NOTE Visible indicators may be used (see annex K).

7.5 Filter assemblies for supply and exhaust air

Filters shall be HEPA filters, conforming to the requirements of EN 13091:1999 and of class H14 or higher of EN 1822-1. Filters shall be mounted in a fail-safe manner.

NOTE 1 Airways containing contaminated air chambers, which are under positive pressure, should be surrounded by internal airways at negative pressure.

Filters shall be mounted in such a way that no air can by-pass the filter medium.

NOTE 2 Air ducts containing contaminated air should be as short as possible and the main exhaust filter should be sited as near to the working space as practicable to facilitate effective disinfection. A suitable pre-filter can be provided to extend the life of the main filter, and should be readily accessible.

Sampling ports, suitable for scanning, shall be fitted for filter testing in such a position that the whole filter, seals, gaskets and construction joints are tested effectively.

If more than one filter is fitted the ports shall permit each filter to be tested independently. The ports shall be sealable.

If cabinets are fitted with double in-line filters provision shall be made to test each filter and relevant seal independently.

The installation shall be tested using a recognised and well documented procedure for installed HEPA filter leak testing by scanning the clean side of the HEPA filters, seals, gaskets, and construction joints. A suitable method is given in annex D.

7.6 Volumetric airflow rate and ventilation ratio (class III)

The airflow velocity through each open glove port, with only one glove removed shall be a minimum of 0,7 m/s. A suitable method is given in annex G.

The volumetric airflow rate through the inlet filter shall be measured and shall be a minimum of 0,05 m^3/s for each cubic metre of cabinet volume when the gloves are attached and the cabinet is at a negative pressure of at least 200 Pa. A suitable method for measurement of mass airflow is given in annex G.

7.7 Cabinet pressure monitoring (class III)

A manometer capable of measuring from -500 Pa to +500 Pa shall be mounted on the cabinet to give a visual indication of the pressure in the interior.

8 Marking and packaging

Each MSC shall be marked in such a way that its performance with regard to the requirements given in this European Standard can be verified. If it is not practical to mark an MSC or a component of it (e.g. due to its size or nature) the packaging shall be marked. MSCs shall be permanently and legibly marked with the following information :

a) the class of the MSC ;

b) the name and address of the manufacturer ;

c) the number and date reference to this European Standard, i.e. EN 12469:2000 ;

d) the model or catalogue number together with a serial number ;

e) the electrical voltage, frequency and power consumption ;

f) the safe working area ;

g) the year of manufacture.

9 Documentation

The manufacturer or supplier shall provide a written manual which details the operating procedures which enable the MSC to perform in accordance with its specified classification.

The user shall keep documentation which demonstrates the current performance of the equipment and its verification, including :

a) a test certificate detailing compliance with all parts of this European Standard; a certificate of HEPA filter/seal leaktightness of the cabinet at the place of use ;

b) methods and equipment used for testing the MSC ;

c) installation and operating manuals ;

d) instructions for maintenance and replacement of filters, including a statement of the need for appropriate decontamination of the cabinet ;

e) a diagram showing the airflow pattern through the cabinet ;

f) the limits of the working space and an indication of any areas (e.g. near the front aperture) that are not protected ;

g) instructions on how to disinfect and clean the cabinet and which disinfectants are suitable ;

h) for class III cabinets, the glove cuff diameter and shape appropriate to the port size.

Annex A (informative)

Guidance on materials, design and manufacture

A.1 Front aperture height

The height of the front aperture(s) should be between 160 mm and 250 mm. Vertical sliding sashes, if provided, should be constructed so that the sash cannot fall and endanger the user if the suspending system fails. If sliding sashes are provided an alarm or locking system should be incorporated to ensure that work can only be carried out within the specified front aperture heights.

A.2 Lighting

Lighting should be adequate for safe working within the cabinet. Illumination measured at the work surface should be at least 750 lux. Ultraviolet (UV) radiation is not recommended for use in safety cabinets. However, if requested, it should be installed in such a manner that it does not affect the airflow and containment performance of the cabinet. Materials which are not affected by UV should be used for the construction of the cabinet. Electrical interlocking should be provided to prevent direct exposure of the worker to UV when working at the cabinet.

A.3 Sound

Sound levels in a cabinet should be low enough not to distract a worker. When tested in accordance with EN ISO 3744 (see Bibliography [5]) using a sound level meter situated 1,0 m from the centre of the front aperture of the cabinet, or 1,0 m from any part of the installation within the laboratory, the A-weighted sound pressure level generated by the cabinet should not exceed 65 dB when the A-weighted sound pressure level of the background is less than 55 dB. If the background noise exceeds 55 dB then the corrected cabinet A-weighted sound pressure level should not exceed 65 dB.

The A-weighted sound pressure level can also be determined according to ISO 11201 (see Bibliography [6]). The measurement is carried out at the workstation which is 0,2 m from the centre of the front aperture of the cabinet and 1,0 m from any part of the installation within the laboratory. If there is a background noise the sound pressure level has to be corrected by A1. The sound pressure level should be declared according to ISO 4871 (see Bibliography [7]) in the instruction handbook and in the technical literature available as information for purchasing, the uncertainty is K = 2 dB.

A.4 Vibration

The net displacement should not exceed 0,005 mm RMS amplitude in the centre of the work surface between 20 Hz and 20 000 Hz when the cabinet is working at the manufacturer's recommended airflow velocities. A suitable method for measuring vibration is given in ISO 5349 (see Bibliography [8]).

A.5 Stability

MSCs should conform to EN 292-1, EN 292-2 and EN 61010-1 (see Bibliography [2] [3] and [1]) for stability. MSCs should be designed to stand on the floor or on a bench in a suitable and secure manner.

A.6 Materials, general

The material of MSCs which is likely to be in contact with microorganisms should be uniformly corrosion resistant, non-flammable and non-adsorbing.

NOTE 1 Manufacturers should ensure that the materials of construction will not be damaged by fumigation and are resistant to disinfectants.

NOTE 2 Chipboard should not be used.

Materials and sealants for joints should be durable and resistant to cleaning and disinfection agents and resistant to general use of MSCs.

NOTE 3 The following have been found to be satisfactory :

a) two component accelerated synthetic temperature resistant high adhesion grade or equivalent used in accordance with manufacturer's recommendation ; or

b) one component sealant compound used in accordance with the manufacturer's recommendations (e.g. silicone or acrylic).

Manufacturers should ensure that the materials of construction will not be damaged by fumigation with formaldehyde.

A.7 Glass and front windows

Laminated safety glass or UV-resistant safety plastics which have the durability to continue to perform satisfactorily for the expected life of the safety cabinet should be used. The external and internal environments to which they will be exposed and the method glazing should be taken into account.

Glass should conform to EN 292-1 and EN 292-2 (see Bibliography [2] and [3]). Means should be provided to hold the front window open securely for loading equipment before starting work.

Vertical sliding sash windows, if provided, should be constructed so that the sash cannot fall and endanger the user if the suspending system fail.

NOTE When in working position, the size position and angle of the window should be such as to give an unobstructed view into the working space when the operator is seated centrally at the cabinet.

An acoustic and optical alarm system should be incorporated into the cabinet in order to ensure that the front window remains well closed in work conditions. This will also ensure that the equipment functions correctly.

A.8 Electrical safety

General requirements for electrical safety are given in EN 61010-1 (see Bibliography [1]).

A.9 Gas supply safety

MSCs should comply with requirements for general aspects of gas supply safety given in EN 292-1 and EN 292-2 (see Bibliography [2] and [3]). If there is provision for gas supply to the inside of the cabinet then the controls should be colour coded as appropriate.

A flammable gas supply to the inside of the cabinet should be controlled by a suitable valve that can be opened by the operator when the cabinet is running and that closes under all other conditions.

NOTE 1 Piezoelectric systems which produce a flame when it is necessary and which can be operated by foot are preferable to Bunsen burners.

NOTE 2 Use of a Bunsen burner within the MSC is not recommended, but if essential, the low profile type operated by foot to give full flame only as required produces the least disturbance to airflow.

Fittings for flammable gas should not be provided in class III cabinets.

A.10 Ergonomics

The design should ensure that maintenance can safely be carried out on the cabinet after installation. The general ergonomic requirements should be considered according to prEN ISO 14738:1997 (see Bibliography [23]).

A.11 Temperature

After 4 h continuous running with the fan(s) working and the lights on, the air temperature inside the cabinet measured at the centre of the working volume should not have risen by more than 8 °C above the ambient laboratory air temperature.

NOTE Buoyancy effects due to high air temperatures can reduce cabinet airflows and can prejudice containment performance.

A.12 Glove ports and gloves (class III)

Gloves should have a conforming fit to the diameter and shape of the glove ports.

It should be possible to replace gloves from the outside of the cabinet in such a way that the old gloves can be pushed inside the cabinet and new ones fitted whilst the fan is still running.

Annex B (normative)

Test method for leakage of carcass for class I and II MSCs

B.1 Principle

The MSC is tested for leakage by subjecting it to an internal pressure and observing bubbles forming in soap applied to welds, gaskets and joints.

B.2 Reagents

Soap solution, comprising 25 g/l soft soap in tepid distilled water prepared in a grease free vessel.

B.3 Apparatus

Manometer, capable of reading in the range 0 Pa to 500 Pa.

Paint brush, 12 mm wide.

B.4 Procedure

Seal all openings in the cabinet by any convenient means and subject the cabinet to an internal air pressure of 250 Pa as measured using the manometer.

Apply the soap solution to all welds, gaskets and joints using the paint brush. Look for any soap bubbles indicating leaks.

B.5 Expression of results

Express the results as the presence or absence of soap bubbles.

B.6 Test report

Include the result of the test and reference to the method used (i.e. annex B of EN 12469:2000) in the documentation specified in clause 9.

Annex C (normative)

Test methods for the retention efficiency at the front aperture

C.1 General

C.1.1 General principle

The MSC is tested for retention efficiency at front aperture by generating within the MSC either an aerosol of microbiological spore suspension or an aerosol of potassium iodide (KI) solution and by counting the number of spores or particles sampled at specific places outside the MSC.

The airflow entering the cabinet is disturbed so as to simulate the effect of a worker's arm by introducing a cylinder through the front aperture.

The test is carried out at nominal set point.

C.1.2 Replicate testing

For cabinets up to 1,5 m wide, 5 replicate tests shall be carried out at the centre of the front aperture. For cabinets from 1,5 m to 2 m wide, 5 replicate tests shall be carried out at the centre of the front aperture and five each at the centre of the right and left halves of the front aperture. For wider cabinets, 5 replicate tests shall be carried out at the centre of each 1 m of the width of the cabinet.

C.2 Microbiological test method

C.2.1 Reagents

Spores of *Bacillus subtilis* var. *niger* (*B. subtilis*), ATCC[1)] 9372, NCTC[2)] 10073 or *B. subtilis* var. *Marburg* ATCC 6051.

Sterile diluent, prepared as follows.

Either :

a) Final diluent phosphate buffer solution (PBS) (step 1)
Dissolve 34 g KH_2PO_4 in 500 ml distilled H_2O
Adjust pH to 7,2 ± 0,5 with 1 N NaOH at 25 °C
Dilute to 1 l with distilled H_2O.
Final diluent PBS (step 2)
Distilled H_2O : 1 l
Stock PBS step 1 : 125 ml
Final pH : 7,2 ± 0,5
Autoclave at 120 °C for 15 min.
Optional : Magnesium sulfate (50 g $MgSO_4.7\ H_2O$ per l distilled H_2O) : 5 ml ;

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1) American Type Culture Collection, Rockville, MD, USA
2) National Collection of Type Cultures, London, England

or :

b) Distilled H_2O : 1 l
Adjust pH to 7,0 ± 0,1 at 25 °C.
Autoclave at 120 °C for 15 min.

NOTE Formula b) is suitable for diluent when spore suspension is prepared for immediate use. When storage of diluent suspension at 4 °C is required, formula a) should be used.

Suspension of *B. subtilis* var. *niger* spores, prepared according to method A or B as follows.

Method A (using previously harvested *B. subtilis* spores)

Aseptically inoculate by streak plating technique several tryptic soy agar Petri plates of size (100 x 15 mm). Incubate for (48 ± 2) h at (37 ± 0,5) °C. Remove characteristic pigmented colonies and transfer to ten 220 ml sterile screw-capped bottles containing approximately 50 ml of tryptic soy agar. Incubate for (48 ± 2) h at (37 ± 0,5) °C. Add 10 ml of PBS to each slant, and gently wash the bacteria from the agar surface.

Transfer the bacterial suspensions to yield approximately 100 ml in a sterile 150 ml screw-cap bottle. If cell debris interferes with nebulizer dissemination, the suspension may be clarified by washing three times in PBS by centrifugation at 1500 r/min for 10 min. Resuspend in PBS to the original volume. Heat stock culture at (65 ± 0,5) °C for 15 min. Determine spore concentration by standard dilution-plate methods using PBS and tryptic soy agar. Spores prepared as above should yield an average count of 2 to 4 x 10^9 spores/ml.

Incubate plates for (48 ± 2) h at (37 ± 0,5) °C. Dilute the spore suspension with PBS to obtain final spore concentration of (5 to 8) x 10^8 spores/ml if spores are to be used immediately. Store the stock spore suspension [(2 to 4) x 10^9 spores/ml] at 4 °C, or divide into aliquots to store in screw-capped vials at -70 °C, making frequent checks of spore viability by surface plating and spore predominance by an acceptable spore staining technique.

Method B

Inoculate 250 ml portions of sterile tryptose broth with aliquots of previously harvested *B. subtilis* spores ; or rehydrated freeze-dried cultures per ATCC or NCTC instructions. Incubate on a reciprocating shaker for (48 ± 2) h at (37 ± 0,5) °C. Heat stock cultures at (65 ± 0,5) °C for 15 min.

Transfer suspensions to screw-cap test tubes and wash at least three times in sterile distilled H_2O by centrifugation at 1500 r/min for 10 min. Use PBS in last washing if storage is required. Determine spore concentration by standard dilution-plate methods using PBS and tryptic soy agar. Spores prepared as described above should average 1,5 x 10^9 spores/ml.

Incubate plates for (48 ± 2) h at (37 ± 0,5) °C. If spore suspension is to be used promptly, dilute the spore suspension with PBS to obtain a final suspension concentration of (5 to 8) x 10^8 spores/ml. To store the stock spore culture, divide into aliquots and store at 4 °C in sterile screw-cap vials, or store in freezer at -70 °C. Before use, check viability of spore suspension as in method A.

C.2.2 Apparatus

Petri plates, of sizes (100 x 15) mm or (150 x 22) mm containing nutrient agar, trypticase soy agar, or other suitable growth medium with no inhibitors or other additives.

Six all-glass raised capillary impinger samplers (see Bibliography [9]), capillary diameter 1,3 mm, flow rate calibrated at 12,5 l/min, containing 20 ml of sterile diluent.

Two slit-type air samplers, operating at a flow of 30 ± 3 l/min[3] .

Refluxing 6-jet modified MRE-type short-form Collison nebulizer, (available as Model CN-38 nebulizer from BGI Inc., Waltham MA, USA[4]) or any other nebulizer that can be demonstrated to produce a bacterial aerosol of equivalent characteristics.

Stainless steel, steel or aluminium cylinder, of outside diameter 63 mm and with closed ends. The cylinder shall be used to disrupt the airflow. The length of the cylinder shall be determined by the size of the cabinet interior. One end butts against the back wall of the cabinet, and the other end protrudes at least 150 mm into the room through the work access opening of the cabinet.

C.2.3 Procedure

Select and calibrate the nebulizer as specified in C.4.

Weigh the nebulizer containing up to 55 ml of spore suspension ((5 to 8) x 10^8 spores/ml of *Bacillus subtilis*) and centre it between the side walls of the cabinet. For class I MSCs ensure that the spray axis is below the cylinder and approximately midway between its lower surface and the work surface. For class II MSCs ensure that the spray axis is level with the upper edge of the front aperture.

Ensure that the opening of the nebulizer is 100 mm behind the front window and that the spray axis is parallel to the work surface and directed towards the front window.

Place the cylinder at the cabinet centre. Ensure that the axis of the cylinder is 69 mm above the work surface. Around the cylinder, position four impingers with the sampling inlets 63 mm outside the cabinet front. Position two impingers so that their inlet axes are 150 mm (6 in) apart, and in a horizontal plane tangent to the top of the cylinder. Position the other two impingers so that their inlet axes are 50 mm apart and lie in a horizontal plane 20 mm below the cylinder. As a positive control, place an agar plate under the centre of the cylinder and ensure that it is supported 10 mm (1/2 in) above or below the front intake grill, to minimize the obstruction of airflow into the grill.

Place two slit-type air samplers so that the horizontal plane of the air inlets is at the work surface elevation, the vertical axes of the inlets are 150 mm in front of the cabinet, and 200 mm from each interior side wall. Place two impingers so that the horizontal plane of the air inlets is 350 mm above the work surface, the vertical axes are 50 mm outside the front edge of the cabinet, and 150 mm on each side of the cabinet centreline.

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[3] Slit-type air samples with an air velocity at the slit of (60 ± 10) m/s. The distance between the lower jet opening and the surface of the agar medium should not be greater than 3 to 6 times the distance between the top and the bottom openings of the slit. Two slit-type samples are needed for each test.

[4] Model CN-38 nebulizer is an example of a suitable product available commercially. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of these products.

Switch on the MSC and allow it to run until normal operation conditions are reached, i.e. at least 30 min before the start of any test.

The duration of the test shall be 30 min. Carry out the test sequence as follows.

- Time 0 : start the slit samplers.
- Time 5 min : start the nebulizer.
- Time 6 min : start the impingers.
- Time 11 min : stop the impingers.
- Time 11,5 min : stop the nebulizer .
- Time 30 min : stop the slit samplers.

Filter the sampling fluid from all the impingers through a 47 mm diameter membrane filter with a pore size of 0,22 µm, remove the filter aseptically and place on appropriate media. Plates containing the filters and plates from the slit-type air samplers shall be incubated at 37 °C. Examine after (24 to 28) h, and if negative, reincubate and read at (44 to 48) h.

NOTE For research and field applications, the sampling fluid may be filtered separately from each impinger to provide information on specific areas within the MSC.

The control plate shall be positive. A plate is "positive" when it contains greater than 300 *B. subtilis* CFU.

C.2.4 Calculation

The amount of liquid sprayed is determined by weighing the nebulizer at the start and at the end of each test. With a known spore concentration, calculate the number of spores sprayed, *N*, as follows.

$$N = c\,(m_1 - m_2)$$

where :

N is the number of spores sprayed, expressed in CFU ;

c is the concentration of the spore suspension, expressed in CFU per gram (CFU/g) ;

m_1 is the mass of the nebulizer with the spore suspension, expressed in grams (g) before the test ;

m_2 is the mass of the nebulizer with the spore suspension, expressed in grams (g) after the test.

Count the number of *B. subtilis* CFU recovered from the six impingers and the number of *B. subtilis* CFU recovered from the two slit-type air samplers.

C.2.5 Expression of results

Express the results as the number of sprayed spores, *N*, and the number of CFU recovered.

C.2.6 Test report

Include the result of the test and reference to the method used (i.e. annex C of EN 12469:2000) in the documentation specified in clause 9.

C.3 Potassium iodide (KI) method

C.3.1 Introduction

The transfer index (see Bibliography [11]) defines the exposure experienced at a given point as $n/(N \times s)$, where N is the number of particles liberated and n the number recovered at a sampling rate of s, the sampling being continued to completion. In a room with turbulent ventilation giving completely uniform mixing throughout the space the transfer index is equivalent to $1/V$ (see Bibliography [12]) where V is the effective volumetric ventilation rate which includes loss by sedimentation. The transfer index has the dimensions time/cubic length.

The ratio of the transfer indices in the two situations (on the open bench and within the cabinet) is the protection factor and is dimensionless. For the reference open-bench conditions V, the room ventilation is taken as 10 m^3/min. The A_{pf} then becomes $(N \times s)/(10n)$, with s, the sampling rate, expressed in cubic metres per minute (m^3/min), or $(N \times s)/(10^4 n)$, if s is expressed in cubic decimetres per minute (dm^3/min). Ideally there should be no escape of hazardous aerosols from a safety cabinet, so n should be zero and the protection factor infinite. However, no open- fronted safety cabinet will give complete protection and the minimum value of the A_{pf} that can be assessed depends on the sensitivity of the test, i.e. the size of the challenge, N, the sampling rate, s, and the smallest number of particles recovered that can be reliably differentiated from background contamination.

Practical values for a test system, are N not less than 3×10^8, s not less than 20 dm^3/min and n not greater than 4, which would lead, for such a system, to a minimum ascertainable value for the A_{pf} of not less than $1{,}5 \times 10^5$.

C.3.2 Reagents

Potassium iodide, 15 g/l solution either in absolute ethanol or industrial methylated spirits with a water content of not more than a volume fraction of 5 %.

Palladium chloride, 1,0 g/l solution in 0,1 mol/l hydrochloric acid.

C.3.3 Apparatus

C.3.3.1 Aerosol generator assembly, comprising a 38 mm diameter spinning disc capable of rotating at 28 000 r/min ± 500 r/min and a nozzle to deliver the potassium iodide solution (C.3.2) to the spinning disc, the gap between the end of the nozzle and the spinning disc being set to 0,1 mm. The assembly also includes a laboratory stand to hold the aerosol generator above the work surface when necessary.

C.3.3.2 Air samplers, working on a centripetal principle with a volume flow rate of air of 100 dm^3/min through the front orifice, and a cone, entraining some 3% of this air (see Bibliography [13]).

NOTE 1 Airflow through the samplers may be provided by a centrifugal fan coupled to the air samplers by a fixed tube.

NOTE 2 The particles, being heavier than air, follow a straight path through the cone and are deposited on a filter membrane located at the base of the cone, while the air is deflected to the outside of the cone.

C.3.3.3 Metal cylinder with a diameter of between 60 mm and 65 mm, a smooth surface and closed at one end, held in an adjustable stand.

C.3.3.4 Petri dishes, of 55 mm diameter.

C.3.3.5 Filter membranes, 25 mm diameter and with a pore size of 3 µm.

C.3.4 Preparation for tests

Set out two Petri dishes away from the cabinet being tested. Half fill one dish with palladium chloride solution, half fill the other with distilled water and replace the lids on each. Set out two filter papers for drying the filter membranes.

Place the cylinder centrally between the side walls of the safety cabinet working space with one end inside the cabinet and the lower edge between 65 mm and 75 mm from the cabinet floor. Using a spirit level, adjust the cylinder so that it is horizontal and protruding a minimum of 250 mm into the laboratory from the plane of the front aperture.

Position four air samplers in front of the centre of the cabinet so that the air inlets are 150 mm to 160 mm from the plane of the front aperture. Ensure that two samplers have their inlets level with the top of the cylinder and 150 mm either side of the front aperture centre line and that the other two samplers are placed with their inlets level with the bottom edge of the window and 155 mm either side of the front aperture centre line.

For class I safety cabinets, place the aerosol generator centrally in the cabinet below the cylinder so that the leading edge of the disc is 100 mm behind the plane of the front aperture.

For class II safety cabinets, place the aerosol generator, on a laboratory stand, centrally in the cabinet so that the centre of the disc is directly above the centre of the cylinder and the leading edge of the disc is 100 mm behind the plane of the front aperture. Adjust the height of the generator so that the disc is level with the upper edge of the front aperture.

NOTE The positions for the aerosol generator are different for class I and class II cabinets in order to test at the most vulnerable part of the air curtain.

Load each air sampler with a filter membrane. Adjust the pressure differences across each sampler according to the manufacturer's instructions, to give an inflow of 100 dm^3/min.

C.3.5 Procedure

Switch on the cabinet and allow it to run until the normal operating condition is reached.

Apply suction to the air samplers and start the spinning disc. Wait 15 s and then allow the potassium iodide to feed on to the centre of the disc. Allow 20 ml of potassium iodide to be

aerosolized. Turn off the air samplers 15 s after aerosolization has stopped. Wait until the suction pump has completely stopped, and then remove the filter membranes.

Place the filter membrane from one sampler in the Petri dish containing the palladium chloride solution, with the surface that has been exposed to the airflow facing upwards. Note the sampler from which the membrane was removed.

Within 30 s to 45 s the membrane will become saturated with palladium chloride and any potassium iodide particles will become visible as brown spots. Remove the membrane and immerse in distilled water for 3 s to 4 s and then place the membrane on a clean filter paper to dry. Repeat this procedure with the filter membranes from the other air samplers. Replace the lids on the Petri dishes.

NOTE 1 In order to avoid contamination, care should be taken to ensure that the tweezers used for placing the membrane in the palladium chloride solution are not used for loading the air samplers.

NOTE 2 The solution of potassium iodide used for the tests is flammable and corrosive to untreated steel. Consequently the cabinet under test should be wiped clean with a wet cloth and scrupulous care should be taken to clean the spinning disc equipment.

Examine each filter with a x10 magnifier and count the number of brown spots on the filter membrane.

NOTE 3 If the number exceeds 50 to 100 spots it will be necessary to use a graticule with the magnifier and to count the spots within a square of a convenient circle and to use an appropriate multiplication factor.

C.3.6 Calculation of the A_{pf} and expression of results

Calculate the number of potassium iodide particles liberated, N using the following equation :

$$N = 3{,}1 \times 10^7 \times M$$

where

M is the volume of potassium iodide solution dispersed by the aerosol generator, expressed in millilitres (ml) ;

$3{,}1 \times 10^7$ is a constant derived from the droplet size, the sampling flow rate and the speed of rotation of the disc.

Then calculate a value for the aperture protection factor, A_{pf}, separately from each filter membrane using the following equation :

$$A_{pf} = NV/10^4 n$$

where

V is the sampling flow rate, expressed in cubic decimetres per minute (dm^3/min) ;

n is the number of spots on the filter membrane.

NOTE 1 In this case $M = 20$ ml and $V = 100$ dm^3/min (see C.3.3.2).

NOTE 2 Using the above equations and the value of M and V given in the NOTE 1, an A_{pf} of $1,0 \times 10^5$ would correspond to 62 spots on the filter membrane.

NOTE 3 When calculating the A_{pf}, if there was one spot on the filter membrane the protection factor would be $6,2 \times 10^6$. If there were no spots on the filter membrane this would indicate that the protection factor was higher than this and the protection factor would be recorded as $A_{pf} > 6,2 \times 10^6$.

C.3.7 Background tests

Place two air samplers loaded with filter membranes in front of the safety cabinet, 150 mm to either side of the front aperture centre line and 100 mm from the plane of the front aperture. Turn the sampler suction fan on and run it for 10 min without any generation of potassium iodide droplets by the aerosol generator.

Remove the filter membranes and develop and examine them in accordance with C.3.5.

NOTE 1 On completion of the background tests in laboratories where no previous tests have taken place within 24 h, the developed membranes should not show any brown spots.

NOTE 2 In laboratories where A_{pf} tests have recently taken place (or where they have resulted in considerable leakage of the aerosol challenge) it is particularly advisable to perform background tests before making any further tests on the cabinets. A count of more than five spots on one of the two filter membranes following a 10 minute test should be regarded as unsatisfactory and further cabinet tests postponed until the background is no longer contaminated.

C.3.8 Test report

Include the result of the test and reference to the method used (i.e. annex C of EN 12469:2000) in the documentation specified in clause 9.

C.4 Nebulizer selection and calibration for microbiological method

C.4.1 Selection criteria

A nebulizer is acceptable when it :

a) delivers (1 to 8) $\times 10^8$ airborne spores of *Bacillus subtilis*, e.g. var. *niger* in 5 min ;

b) delivers (94 ± 6) % single cell spores ;

c) has a spore aerosol discharge velocity of (0,51 ± 0,05) m/s.

NOTE 1 Test performed by First *et al.* (see Bibliography [10]) demonstrated that a stainless steel 6-jet Collison refluxing nebulizer will deliver the bacterial spore aerosol required in paragraph C.2.3 when the following conditions are met :

- the nebulizer is equipped with a glass flask 50 mm in diameter, 90 mm high and a 23 mm ID horizontal discharge spout on top ;

- the nebulizer is operated at 140 kPa (1,4 bar) ;

- 55 ml of a (5 to 8) x 10^8/ml spore suspension is placed in the flask ;

- the bottom of the 6-jet spray head is 18 mm above the bottom of the flask ;

- six rosette patterns created by the air jets form on the inside of the glass flask. (These should be observed frequently for size and contour to verify that the jets are not clogged or obstructed).

NOTE 2 The 6-jet Collison refluxing nebulizer need not be retested for performance before use.

C.4.2 Calibration

C.4.2.1 General

Calibrate the nebulizer in the laboratory where it is being used, prior to first use and periodically thereafter.

C.4.2.2 Reagents

A suspension of (5 to 8) x 10^8 *Bacillus subtilis*, e.g. var. *niger* spores per millilitre.

C.4.2.3 Apparatus

Nebulizer to be calibrated.

One impinger sampler.

Switching timer.

Membrane filter funnel, of filter size 47 mm, with silicone rubber diaphragms sealed to each end with room temperature vulcanizing (RTV). Diaphragms are perforated to insert the outlet of the nebulizer at the wider end of the funnel and one impinger sampler at the other end. Insertions shall be tight on the impinger end. Insertion shall be loose on the nebulizer end so that the impinger is operating in atmospheric pressure, not in a closed system.

Flow meters.

Pressure gauge.

A 37 mm aerosol type membrane filter in sampling cassette with an open face.

C.4.2.4 Calibration procedure

Measure the nebulizer outlet dimensions and calculate the area, expressed in square metres (m^2).

Calculate the airflow, expressed in litres per minute (l/min), through the nebulizer required to result discharge velocity, expressed in metres per second (m/s).

Add the manufacturer's recommended volume of spore suspension to the nebulizer.

Place the outlet of the nebulizer in the rubber diaphragm of the wide end of the filter funnel. Insert the collecting tube of the impinger sampler through the rubber diaphragm at the opposite end of the filter funnel. Insure a tight fit at the impinger end.

> NOTE Two types of impinger samples (chemical corps type) are available: an impinger with tip submerged in liquid 4 mm from flask bottom and passing 6 l/min at a pressure drop of 56 kPa (0,56 bar) or greater (ACE Glass No. 7541 impinger) and an impinger with its tip above the liquid surface and passing 12,5 l/min at a pressure drop of 56 kPa (0,56 bar) or greater, known as AGI-30, (ACE Glass No. 7540 impinger). Either impinger may be used. When the air delivery rate of the nebulizer is not precisely 6 l/min or 12,5 l/min, select the impinger that samples at a higher rate and blead in through an opening around the nebulizer insertion an amount of air equal to the difference in the two airflows. If the nebulizer and the impinger are to be operated at the same flow rate, a snug fit in the diaphragm at both ends is recommended.

Attach a hose to a pressure gauge attached to flow meter, then to nebulizer.

Simultaneously turn on the nebulizer, maintaining the airflow through the nebulizer to result in a calculated 0,51 m/s output velocity based on airflow and diameter of the discharge spout - 12,5 l/min for the 6 jet-collison described in this annex, and the impinger sampler, operating according to manufacturer's instructions. Operate the nebulizer for 5 min (using the switching timer) and the impinger sampler for 5 min 15 s.

Aseptically transfer the impinger sampler collecting fluid to a sterile 500 ml graduated cylinder. Rinse the funnel, impinger stem and bottle with sterile water to insure collection of all spores, and collect all rinse water in the graduated cylinder.

Measure and record the volume of fluid in the graduated cylinder. Transfer all the fluid aseptically to a sterile flask containing a magnetic stirrer and mix thoroughly.

Prepare serial dilutions and quantify spore concentrations by five replicate platings.

Actively sample the cell spore aerosol with the membrane filter located in its design mode. After sampling is completed, stain the membrane with an appropriate dye. Count the number of deposits containing single and more than one cell spore in representative fields under the microscope.

C.4.2.5 Calculations and expression of results

Number of spores delivered in 5 min = (dilution factor) x (average number of CFUs on the 5 plates).

Velocity of air leaving nebulizer = the air volume measured in l/min = 1,67 x 10^{5} m^3/s divided by nebulizer outlet area in m^2. (See also C.4.2.6).

Calculate the percentage of single cell spore in the total aerosol sample.

C.4.2.6 Alternative method for determining the air velocity of the nebulizer

The velocity of the air current leaving the nebulizer can be measured in the middle of the opening using a hot wire anemometer. The nebulizer shall be operated without liquid. The maximum air velocity is at the middle of the opening and the minimum at the sides due to the friction of air molecules at the wall. This can be taken into account with an equation in which the volumetric flow

$$\dot{V} = \pi\left(R - r\right)^2 v$$

and the mean outflow velocity

$$\bar{v} = \frac{\dot{V}}{\pi R^2}$$

can be calculated

where

$\dot{V}$ is the air current, expressed in litres per minute (l/min) ;

R is the inside radius of the outlet, expressed in millimetres (mm) ;

r is the correction constant (1,1), expressed in millimetres (mm) to compensate for loss due to friction ;

v is the velocity measured in the middle of the outlet, expressed in metres per second (m/s) ;

$\bar{v}$ is the calculated mean velocity in the outlet of the nebulizer, expressed in metres per second (m/s).

Annex D (informative)

Aerosol challenge test method for installed HEPA filter system leakage detection

D.1 Principle

The HEPA filter system fitted to the MSC is tested for leakage by subjecting it to an aerosol on the upstream side and measuring passage of the aerosol to the downstream side.

D.2 Reagents

Aerosol material used for dispersion in an aerosol generator.

D.3 Apparatus

Aerosol generator capable of producing test aerosol for HEPA filter leak testing.

Measuring system for detecting the upstream and downstream test aerosol concentrations, capable of detection not less than 5 log rates of concentration.

Suitable measuring systems are :

a) discrete particle counter with a dilution system for detecting a local penetration for particles > 0,3 µm of 0,01 % or less ; or

b) aerosol photometer with an upper measuring threshold of 10 µg/l to 100 µg/l and a range covering not less than 5 log rates.

D.4 Procedure

Operate the MSC and induce the aerosol challenge at a suitable location on the upstream side of HEPA filters, seals and construction joints to be tested. Ensure that the challenge aerosol concentration is uniform on the upstream HEPA filter face.

Measure the average upstream aerosol concentration.

Scan the downstream side of the HEPA filter by passing the aerosol detection probe slightly overlapping over the entire surface of the HEPA filter. Scan the entire periphery of the filter, the junction between the filter, and filter mounting frame.

Determine traverse scan rate, sample acquisition time and the actual particle counts which characterizes a leak.

D.5 Expression of results

Express the result as the upstream aerosol concentration, downstream aerosol concentration and the ratio of concentrations in percentage.

If a discrete particle counter is used, the local penetration of a HEPA filter with 0,005 % integral penetration should be not more than 0,05 % (see Bibliography [14], [15, [16]).

If an aerosol photometer is used, the local penetration of a HEPA filter should be not more than 0,01 % (see Bibliography [15], [16], [17]).

D.6 Test report

Include the result of the test and reference to the method used (i.e. annex D of EN 12469:2000) in the documentation specified in clause 9.

Annex E (normative)

Test method for product protection for class II MSCs

E.1 Principle

The product protection ability of an MSC is tested by determining contamination of culture plates spread on the work surface with an aerosol of microbiological spore suspension from a nebulizer placed outside the MSC.

The airflow entering the cabinet is disturbed so as to simulate the effect of a worker's arm by introducing a cylinder through the front aperture.

The test is carried out at nominal set point.

E.2 Reagents

Spore suspension, comprising spores of a non-pathogenic bacterium, e.g. *Bacillus subtilis* var. *globigii* (NCTC reference number 10073), in sterile distilled water.

Culture plates, comprising Petri dishes of 90 mm diameter containing nutrient agar or trypticase soy agar.

E.3 Apparatus

Nebulizer, capable of generating at least 10^7 spores/min with the velocity of the carrier air measured at the front aperture of the cabinet using the method of Hino and Sato (see Bibliography [18]), not exceeding the inward air velocity of the cabinet.

NOTE Nebulizers of the Collison pattern have been found to be suitable (see Bibliography [19]).

Metal cylinder having a diameter of between 60 mm and 65 mm, with a smooth surface and closed at one end, held in an adjustable stand.

E.4 Procedure

Calibrate the nebulizer as specified in C.4.2.

Cover the work surface with open agar plates with the cylinder at the mid point. Position the horizontal spray axis of the nebulizer containing 55 ml of spore suspension ((5 to 8) x 10^6 *Bacillus subtilis* spores/ml) at the level of the top edge of the work opening, and centre between the two sides of the cabinet, with the opening of the nebulizer 100 mm outside the window. The spray axis is parallel to the work surface and directed towards the open front of the cabinet.

Place a 65 mm outside diameter cylinder, with closed ends, in the centre of the cabinet. Position the cylinder in the cabinet so that one end butts against the back wall of the cabinet, the other end extends at least 150 mm into the room through the front opening of the cabinet, and the axis of the cylinder is 70 mm above the work surface.

As a positive control, place an agar plate under the centre of the cylinder, and support it 10 mm above or below the front intake grill to minimize the obstruction or airflow into the grill.

Switch on the MSC and allow it to run until normal operation conditions are reached, i.e. at least 30 min before the start of any test.

Weigh the nebulizer and operate for 5 min. Five minutes after nebulization is terminated, place lids on the agar plates and reweigh the nebulizer.

Perform three replicates.

Incubate plates at 37 °C and examine them at (24 to 28) h. If negative, reincubate and read after (44 to 48) h.

The control plates shall be positive. A plate is "positive" when it contains greater than 300 CFU of *B. subtilis*.

E.5 Calculation and expression of results

Calculate and express the result as the number of sprayed spores, *N*, as specified in C.2.4.

Count and express the result as the total number of CFU recovered from all agar plates.

E.6 Test report

Include the result of the test and reference to the method used (i.e. annex E of EN 12469:2000) in the documentation specified in clause 9.

Annex F (normative)

Test method for cross contamination protection for class II MSCs

F.1 Principle

The cross contamination protection ability of an MSC is tested by determining contamination of culture plates spread on the work surface with an aerosol of microbiological spore suspension from a nebulizer placed inside the MSC.

NOTE The point where the laminar down flow air splits, with portions moving into the front intake and the rear exhaust grills, is not always located at the centre of the interior side wall. Therefore this split line should be identified for proper placement of the nebulizer. This point can easily be located by using smoke tube test (see Bibliography [18]).

The airflow entering the cabinet is disturbed so as to simulate the effect of a worker's arm by introducing a cylinder through the front aperture.

The test is carried out at nominal set point.

F.2 Reagents

Spore suspension, comprising spores of a non-pathogenic bacterium, e.g. *Bacillus subtilis var. globigii* (NCTC reference number 10073), in sterile distilled water.

Culture plates, comprising Petri dishes of 90 mm diameter containing nutrient agar or trypticase soy agar.

F.3 Apparatus

Nebulizer, capable of generating at least 10^7 spores/min with the velocity of the carrier air measured at the front aperture of the cabinet using the method of Hino and Sato (see Bibliography [18]), not exceeding the inward air velocity of the cabinet.

NOTE Nebulizers of the Collison pattern have been found to be suitable (see Bibliography [19]).

F.4 Procedure

F.4.1 Calibrate the nebulizer as specified in C.4.2.

F.4.2 Weigh the nebulizer containing 55 ml of spore suspension ((5 to 8) x 10^4 *Bacillus subtilis* spores/ml) and position it with the horizontal spray axis of the nebulizer (75 to 125) mm above the work surface, with the back of the nebulizer against the left interior wall at the point where the down flow air splits (smoke test). The spray axis is parallel to the work surface and is directed towards the opposite side wall.

F.4.3 Switch on the MSC and allow it to run until normal operation conditions are reached, i.e. at least 30 min before the start of any test.

F.4.4 Place open agar settling plates on the work surface. Place two rows of control plates with the centreline under the outlet of the nebulizer. Place one row of plates with their centres on a line drawn front to back 355 mm from the side wall being tested. Nest at least one more row of plates beyond the 355 mm row; two rows when there is room.

F.4.5 Start the nebulizer. After 5 min, stop the nebulizer but continue to operate the cabinet for 15 min. Reweigh the nebulizer.

F.4.6 Let the cabinet motor run while placing the covers on the open agar plates. Incubate the plates at 37 °C and examine them at (24 to 28) h. If negative, reincubate and read at (44 to 48) h.

F.4.7 Repeat the procedure in F.4.3 to F.4.6, but place the nebulizer against the appropriate point of the right interior wall.

F.4.8 Perform three replicates from the left and right sides of the cabinet.

F.4.9 Some agar plates, from the challenge sidewall to 355 mm from the sidewall, will recover *B. subtilis* CFU and shall be used as controls.

F.5 Calculation and expression of results

Calculate and express the result as the number of sprayed spores, *N*, as specified in C.2.4.

Count the total number of CFU recovered on agar plates with centres greater than 355 mm.

F.6 Test report

Include the result of the test and reference to the method used (i.e. annex F of EN 12469:2000) in the documentation specified in clause 9.

Annex G (informative)

Method of measurement of volumetric airflow rate

G.1 Principle

The volumetric airflow rate is measured by using either an anemometer in multiplying measurement by the sectional area, or a measurement device suitable for measuring volumetric airflow rate in ducts.

The test is carried out at nominal set point.

G.2 Apparatus

Anemometer, with a suitable range and sensitivity.

NOTE Anemometers should be calibrated at regular intervals in accordance with manufacturer's recommendations.

Measurement device suitable for measuring volumetric airflow rate within the exhaust duct.

G.3 Procedure

G.3.1 Class I MSCs

Turn the MSC on. With the anemometer in the plane of the front aperture, make air velocity measurements over a period of at least 1 min for each measurement at a minimum of five positions, namely at the geometric centre of the front aperture and each of its four corners, with the centre of the anemometer between 50 mm and 55 mm from the right-hand or left-hand edge of the front aperture and from the top or bottom.

G.3.2 Class II MSCs

G.3.2.1 Downflow

Turn the MSC on. With the anemometer inside the MSC, make air velocity measurements in the horizontal plane 50 mm to 100 mm above the top edge of the front aperture. Make measurements over a period of at least 1 min in each position for at least eight positions, namely four along a line 1/4 of the depth of the working space forward of the rear wall and four along a line the same distance behind the front window. Ensure that the measurements are spaced along these lines at 1/8 and 3/8 of the width of the working space from both the left-hand and right-hand sides.

G.3.2.2 Inflow

Turn the MSC on. Measure the mean airflow velocity within the exhaust duct with a suitable measurement device.

Multiply the mean velocity obtained by the area of the exhaust duct to give the volumetric rate of discharge of exhaust air from the MSC. Divide the discharge air volume by the cross-sectional area of the front aperture to give the mean inward air velocity through the front aperture.

NOTE A method for measuring volumetric airflow rate in circular exhaust ducts is given in BS 848-1:1997, clause 23 (see Bibliography [21]).

G.3.3 Class III MSCs

G.3.3.1 Inflow through open glove port

Turn the MSC on. With the anemometer placed at the centre of one open glove port record an air velocity measurement over a period of at least 1 min. Repeat this procedure with the anemometer at the centre of the other open glove port.

G.3.3.2 Inflow through the inlet filter

Turn the MSC on. Measure the mean airflow velocity within the exhaust duct with a suitable measurement device.

Multiply the mean velocity obtained by the area of the exhaust duct to give the volumetric rate of discharge of exhaust air from the MSC which is equal to the inflow through the inlet filter.

NOTE 1 A method for measuring flow rates in circular exhaust ducts is given in BS 848-1, clause 23 (see Bibliography [21]).

NOTE 2 It may be useful to take readings at the face of the inlet filter. Whilst this will not provide an accurate air inflow measurement it will give an indication to the user of filter blockage over a period of time.

G.4 Expression of results

Express the results as the mean volumetric airflow rate or the mean airflow velocity, either in multiplying the mean air velocity by the cross sectional area, or by direct reading of the measurement device.

The mean volumetric airflow rate is expressed in cubic metres per second (m^3/s).

The mean airflow velocity is expressed in metres per second (m/s).

G.5 Test report

Include the result of the test and reference to the method used (i.e. annex G of EN 12469:2000) in the documentation specified in clause 9.

Annex H (informative)

Design of MSCs and airflow velocities in MSCs

H.1 General

Table H.1 describes typical airflow velocities for three classes of MSCs.

Table H.1 - Airflow velocities for three classes of MSCs

Class	Mean inflow velocity to achieve operator protection	Mean downflow velocity to achieve product protection
I	> 0,7 m/s - 1,0 m/s	Not applicable
II	≥ 0,4 m/s	0,25 m/s - 0,50 m/s
III	≥ 0,7 m/s with one glove removed	Not applicable

H.2 Class I MSCs

The direction of airflow, as demonstrated by smoke or other visualization tests, e.g. water fog, should be inward over the whole area of the front aperture (see Bibliography [22]).

When tested in accordance with annex G, the measured air velocities at all points should be in accordance with table H.1 under operating conditions.

NOTE 1 For type testing, testing is performed with unused filters.

NOTE 2 Airflow velocity of > 1 m/s can create unacceptable turbulence.

H.3 Class II MSCs

H.3.1 General

The design should ensure that the front air intake slots cannot be obstructed.

The extent of the intended working space should be suitably indicated on the working surface.

The delivery area for the air to the working space should be without interposed projections or cavities that could interfere with containment performance.

The direction of airflow, as demonstrated by smoke or other visualization tests, e.g. water fog, should be inward over the whole area of the front aperture and downward without undue turbulence over the worksurface (see Bibliography [22]).

H.3.2 Downflow

When tested in accordance with the method given in annex G, the mean velocity of the downward airflow should be in accordance with table H.1 under operating conditions. No individual measurement should differ from the mean by more than 20%.

H.3.3 Inflow

When tested in accordance with the method given in annex G, the mean airflow velocity inward through the front aperture should be in accordance with table H.1 under operating conditions. No individual measurement should differ from the mean by more than 20%.

H.3.4 Stop/start of MSCs

The cabinet should only be turned on and off by means of a special tool (for example, key, magnetic card). Fan(s) that stop due to a power cut should automatically be restarted when the power is reactivated.

Annex J (informative)

Recommendations for decontamination, cleaning and fumigation of MSCs and filters

J.1 Decontamination and cleaning

All MSCs should be kept clean and free of unnecessary equipment. The interior should be swabbed after use with a suitable disinfectant. Phenolics, quaternary ammonium compounds and aldehydes can be used for disinfecting these surfaces. Users of chlorine compounds should be aware of their corrosive nature. The use of alcohols should be undertaken with caution because of the risk of fire. Heavy duty polyvinyl chloride (PVC) or rubber gloves which provide suitable protection for the hands and wrists and which can be disinfected for re-use should be worn when cleaning MSCs .

NOTE 1 In cases where fumigation may be ineffective, it may be necessary to have MSCs designed to allow contained removal for suitable dispersal, for example incineration.

NOTE 2 At the selection of disinfectants occupational health aspects should be considered.

J.2 Fumigation

MSCs should be fumigated in the following circumstances :

a) before any maintenance work on the cabinet where access to potentially contaminated parts is necessary (including filter and pre-filter changes if they have been used for hazardous microorganisms) ;

b) before carrying out filter penetration tests ;

c) after a spillage where inaccessible surfaces may have become contaminated.

NOTE 1 In certain cases it may be necessary to fumigate when the nature of the work changes.

Fumigation should be carried out by a responsible person with adequate knowledge of the procedure and the precautions to be followed. A warning notice that the cabinet is being fumigated should be displayed.

Decontamination of the whole cabinet, including filters, fan unit and work surfaces is most frequently carried out by fumigation with formaldehyde vapour. Alternatives that may be used are for example hydrogen peroxide. However this may not be appropriate in certain cases, when local regulations should be followed.

WARNING Formaldehyde vapour is explosive at a volume fraction of 7,75 % in dry air; its ignition point is 430 °C. National occupational exposure limits should be considered.

The volume of the cabinet should be calculated and sufficient formaldehyde used to produce a persistent airborne concentration of at least 50 mg/m^3.

Formaldehyde vapour can be generated by :

- evaporation of formalin (a volume fraction of ± 36 % in water).

  NOTE 2 : 60 ml of formalin plus 60 ml water should be vaporized per cubic metre of cabinet volume.

- depolymerization of paraformaldehyde by heating, provided adequate humidity is available.

Formaldehyde penetrates poorly and its effectiveness is dependent on temperature and humidity. It is most effective above a temperature of 20 °C and a relative humidity of 65%. Use of excessive amounts can result in polymer deposition within the cabinet and may contribute to filter blockage.

The cabinet should be sealed before fumigation to ensure that no formaldehyde can leak into the laboratory or other rooms. Fumigation is most conveniently done overnight. A shorter exposure period as determined by suitable biological tests can be used but it is unlikely to be less than 4 h.

Procedures for both cabinet and filter fumigation should ensure that the downstream side of the HEPA filters and ductwork is exposed to formaldehyde for a period sufficient to ensure inactivation of microorganisms that have penetrated the filter. This will require an exposure time of over 6 h.

To ensure adequate fumigation after generation of formaldehyde vapour the following procedure should be adopted in the absence of specific recommendations by the manufacturer :

i) after about half the formalin has evaporated, turn the cabinet fans on for 10 s to 15 s to allow formaldehyde to reach all areas ;

ii) after evaporation is completed switch the cabinet fans on for 10 s to 15 s.

Even after this treatment HEPA filters should only be considered as "safe to handle using appropriate protective clothing" rather than sterile, and should be autoclaved or incinerated after removal. After fumigation the seals on the cabinet should be removed and the exhaust fan run for a period sufficient to remove residual formaldehyde vapour before using the cabinet or carrying out maintenance.

For cabinets that recirculate air to the room, the system of fumigation used should ensure that personnel are not exposed to levels above those required by competent authorities, for example by siting the cabinet controls remotely or by using temporary ducting to vent to the outside. Respiratory protective equipment suitable for formaldehyde should be provided for use in the event of an emergency.

Annex K (informative)

Recommendations for routine maintenance of MSCs

K.1 General

Routine maintenance tests should be carried out on class I, class II and class III MSCs at regular intervals or as determined by regulatory authorities.

All equipment used for these tests should be regularly calibrated and certificates to this effect should be available. In addition, the date of the last maintenance should appear in an easily visible position.

Checks should be made to confirm that the anti-blow back valve, if present, is functioning and is clearly visible.

K.2 Class I MSCs

All internal and external surfaces of the safety cabinet should be visually examined to ensure that there are no surface defects, cracks or other damage.

Where practicable, the extraction duct system, if present, should be visually inspected to ensure that it is free from defects, cracks and other damage and that it is clearly labelled.

The airflow indicator(s) and alarm(s) should be examined and checked, and re-calibrated to the manufacturer's specification if necessary.

K.3 Class II MSCs

All internal and external surfaces of the safety cabinet should be visually examined to ensure that there are no surface defects, cracks or other damage.

Where practicable, the extraction duct system, if present, should be visually inspected to ensure that it is free from defects, cracks and other damage and that it is clearly labelled.

The alarm indicators should be checked to the manufacturer's specification. Should the alarm device require calibration, the manufacturer should indicate the recalibration time intervals to prevent tripping of the alarm out of the threshold limits.

The downflow air velocity in the working area of the cabinet should be measured in accordance with the method given in annex G, in accordance with manufacturer's instructions and in accordance with table H.1.

The pre-filter, if fitted, should be replaced prior to airflow measurements being made.

In cabinets that have two exhaust filters each filter should be independently tested.

K.4 Class III MSCs

All internal and external surfaces of the safety cabinet should be visually examined to ensure that there are no surface defects, cracks or other damage.

The manometer should be checked and if necessary re-calibrated to the manufacturer's specification.

Where practicable, the extraction duct system, if present, should be visually inspected to ensure that it is free from defects, cracks and other damage, and that it is clearly labelled.

The alarm indicators should be checked to the manufacturer's specification. Should the alarm device require calibration, the manufacturer should indicate the recalibration time intervals to prevent tripping of the alarm out of the threshold limits.

Filter and seal integrity should be checked using an oil aerosol penetration test in accordance with annex D and the results including those for both inlet and exhaust filters should conform to 7.5. The airflow through each open glove port should be measured in accordance with annex G and should be at least 0,7 m/s.

The airflow through the inlet filter when the gloves are attached and the cabinet is at a negative pressure of at least 200 Pa should be measured in accordance with annex G and should not be less than 3 m^3/min for each cubic metre of cabinet volume. The pre-filter, if fitted, should be replaced prior to airflow measurements being made.

The working pressure in the cabinet should be checked and should be not less than -200 Pa (with respect to the laboratory).

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