

Australian Standard[®]

Controlled environments

**Part 3: Biological safety cabinets
Class III—Design**



This Australian Standard® was prepared by Committee ME-060, Controlled Environment. It was approved on behalf of the Council of Standards Australia on 15 June 2011. This Standard was published on 29 June 2011.

The following are represented on Committee ME-060:

- Airconditioning & Refrigeration Equipment Manufacturers Association of Australia
 - Auckland Regional Chambers of Commerce
 - Australian Chamber of Commerce and Industry
 - Australian Institute of Refrigeration Air Conditioning and Heating
 - Australian Society for Microbiology
 - CSIRO Australian Animal Health Laboratory
 - Commonwealth Department of Health and Ageing
 - Engineers Australia
 - Human Factors and Ergonomics Society of Australia
 - International Society for Pharmaceutical Engineering
 - Ministry of Agriculture and Forestry New Zealand
 - NSW Health Department
 - National Association of Testing Authorities Australia
 - Office of the Gene Technology Regulator
 - Therapeutic Goods Administration
-

This Standard was issued in draft form for comment as DR AS 2252.3.

Standards Australia wishes to acknowledge the participation of the expert individuals that contributed to the development of this Standard through their representation on the Committee and through the public comment period.

Keeping Standards up-to-date

Australian Standards® are living documents that reflect progress in science, technology and systems. To maintain their currency, all Standards are periodically reviewed, and new editions are published. Between editions, amendments may be issued.

Standards may also be withdrawn. It is important that readers assure themselves they are using a current Standard, which should include any amendments that may have been published since the Standard was published.

Detailed information about Australian Standards, drafts, amendments and new projects can be found by visiting **www.standards.org.au**

Standards Australia welcomes suggestions for improvements, and encourages readers to notify us immediately of any apparent inaccuracies or ambiguities. Contact us via email at **mail@standards.org.au**, or write to Standards Australia, GPO Box 476, Sydney, NSW 2001.

Australian Standard[®]

Controlled environments

Part 3: Biological safety cabinets Class III—Design

First published as AS 2252.3—2011.

COPYRIGHT

© Standards Australia Limited

All rights are reserved. No part of this work may be reproduced or copied in any form or by any means, electronic or mechanical, including photocopying, without the written permission of the publisher, unless otherwise permitted under the Copyright Act 1968.

Published by SAI Global Limited under licence from Standards Australia Limited, GPO Box 476, Sydney, NSW 2001, Australia

ISBN 978 0 7337 9880 1

PREFACE

This Standard was prepared by the Standards Australia Committee ME-060, Controlled Environment.

The objective of this Standard is to set the minimum performance criteria for safety cabinets for work with micro-organisms and specifies test procedures for microbiological safety cabinets with respect to protection of the worker and the environment, product prevention and cross contamination.

This Standard is identical with, and has been reproduced from EN 12469:2000, *Biotechnology—Performance criteria for microbiological safety cabinets*, while giving due regard to local differences in mechanical and electrical regulations and related standards.

This Standard should be considered only for Class III biological safety cabinets, at this time. It is the intention of the Committee to obtain feedback for consideration and adoption of EN 12469:2000 in its entirety.

As this Standard is reproduced from a European Standard, the following applies:

- (a) Its number appears on the cover and title page while the European Standard number appears only on the cover.
- (b) In the source text ‘this European Standard’ should read ‘this Interim Australian Standard’.
- (c) A full point substitutes for a comma when referring to a decimal marker.

References to European Standards should be replaced by references to Australian or Australian/New Zealand Standards, as follows:

<i>Reference to International Standard</i>		<i>Australian Standard</i>	
EN		AS	
1822	High efficiency air filters (HEPA and ULPA)	4260	High efficiency particulate air (HEPA) filters—Classification, construction and performance
1822-1	Part 1: Classification, performance testing, marking		
12296	Biotechnology—Equipment—Guidance on testing procedures for cleanability	—	
12297	Biotechnology—Equipment—Guidance on testing procedures for sterilizability	—	
12298	Biotechnology—Equipment—Guidance on testing procedures for leaktightness	—	
13091	Biotechnology—Performance criteria for filter elements and filtration assemblies	4260	High efficiency particulate air (HEPA) filters—Classification, construction and performance

The terms ‘normative’ and ‘informative’ are used to define the application of the annex to which they apply. A normative annex is an integral part of a standard, whereas an informative annex is only for information and guidance.

CONTENTS

	<i>Page</i>
Introduction.....	4
1 Scope.....	4
2 Normative references.....	4
3 Definitions	5
4 Hazards	6
5 Performance classes	6
7 Safety requirements	12
8 Marking and packaging.....	13
9 Documentation	14
Annex A (informative) Guidance on materials, design and manufacture	15
Annex B (normative) Test method for leakage of carcass for class I and II MSCs	18
Annex C (normative) Test methods for the retention efficiency at the front aperture	19
Annex D (informative) Aerosol challenge test method for installed HEPA filter system leakage detection	30
Annex E (normative) Test method for product protection for class II MSCs	32
Annex F (normative) Test method for cross contamination protection for class II MSCs.....	34
Annex G (informative) Method of measurement of volumetric airflow rate.....	36
Annex H (informative) Design of MSCs and airflow velocities in MSCs	38
Annex J (informative) Recommendations for decontamination, cleaning and fumigation of MSCs and filters.....	40
Annex K (informative) Recommendations for routine maintenance of MSCs	42
Bibliography.....	44

AUSTRALIAN STANDARD

Controlled environments

Part 3:

Biological safety cabinets Class III—Design

Introduction

Microbiological safety cabinets are intended to reduce the risk to the operator when handling hazardous or potentially hazardous micro-organisms. 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 micro-organisms 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 micro-organisms 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 micro-organisms, with air discharged to the atmosphere being filtered.

3.4 MSC class I

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 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 micro-organisms.

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 micro-organisms 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 micro-organisms/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 micro-organisms during operation for example through the front aperture, exhaust, piping or carcass;
- release of micro-organisms during dismantling or maintenance e.g. filter replacement of MSCs or parts thereof following improper sterilization;
- release of micro-organisms 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 micro-organism not defined
LI-B	leakage ¹⁾ of target micro-organism detected and quantified under defined conditions
LI-C	leakage ¹⁾ of target micro-organism 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 micro-organisms 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 micro-organisms
SI-B	MSC can be treated for a specified reduction of viable target micro-organisms
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 micro-organisms. 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 micro-organisms 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 of 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 micro-organisms 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 bypass 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 recognized 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 m³/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.