BRITISH STANDARD

BS EN
12296:1998

Biotechnology — Equipment — Guidance on testing procedures for cleanability

The European Standard EN 12296:1998 has the status of a British Standard

ICS 07.080; 07.100.01

National foreword

This British Standard is the English language version of EN 12296:1998.

The UK participation in its preparation was entrusted to Technical Committee CII/58, Biotechnology, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

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Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 7 and a back cover.

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English version

Biotechnology — Equipment — Guidance on testing procedures for cleanability

Biotechnologie — Equipement — Guide des procédures d'essai pour le contrôle de la capacité au nettoyage

Biotechnik — Geräte und Ausrüstungen — Leitfaden für Verfahren zur Prüfung der Reinigbarkeit

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CEN

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Comité Européen de Normalisation
Europäisches Komitee für Normung

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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of an identical text or by endorsement, at the latest by September 1998, and conflicting national standards shall be withdrawn at the latest by September 1998.

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.

Contents

Introduction

The cleaning of plant and equipment is an essential element of biotechnology processes in order to protect the safety of people and the environment and to avoid harmful operational effects through the accumulation of soil.

Testing procedures should be developed and documented to ensure that relevant information on cleanability is available. Standards (e.g. EN ISO 9000 series, see annex C [9]), guidelines (e.g. Good Manufacturing Practice (GMP) see annex C [10]) state general procedures of good practice which facilitate high quality manufacturing if followed. This European Standard refers to assessing the cleanability of equipment used in biotechnology, where additional specific requirements related to safety and to special features of biotechnological processes are required. It should be read in association with the more general standards and guidelines as mentioned above. In particular this European Standard states the principles on which test methodology is based. Informative guidance on selection of test methods is provided in annex A.

The extent to which it is necessary to remove soil from equipment and plant varies substantially with the process. In some cases abundant residues after cleaning do not harm people or the environment or do not cause difficulties in the process. In others very low residues are essential. The complete removal of soil on surfaces cannot be achieved, because for example all surfaces are adsorptive to some degree.

1 Scope

This European Standard gives guidance on general testing procedures to assess the cleanability of equipment (components and units of equipment) used in biotechnological processes.

This European Standard applies primarily if the intended use of equipment includes the use of potentially hazardous micro-organisms. This European Standard also applies to non-hazardous micro-organisms and/or to residual soil which can adversely affect sterilization processes or which can cause cross-contamination of products or processes.

This European Standard applies to plants or components such as, valves and fitting, tanks, pumps, piping, separating and filling devices as well as instrumentation in contact with process fluids.

2 Definitions

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

2.1

clean

condition of (a) product, surface, device, gases and/or liquids with residual soil below a defined threshold level

2.2

cleanability

ability to be made clean

2.3

cleaning

removal of soil

2.4

Cleaning-In Place (CIP)

cleaning without dismantling of components of equipment and/or unit of equipment

2.5

components of equipment

technical entity which forms part of a unit of equipment

NOTE Examples of components of equipment are vessels, valves and sensors.

2.6

contamination

presence of soil

2.7

residual soil

soil left after cleaning

2.8

soil

material, including **micro-organisms**, metabolites and components of process media present on a surface

2.9

unit of equipment

assembly of components used to perform one or more unit operations

3 Testing

3.1 General

The requirements for cleanability vary with process, especially in relation to its assessed risk. Therefore cleanability classes for biosafety are defined in individual equipment standards. This will allow the manufacturers of plant and equipment to state the performance of their equipment using test methods, including visual inspection, developed according to the principles described in **3.2**. It will also allow users of equipment to define their requirements in simple terms. These classes define performance only in relation to a defined indicator substance(s) and one or more defined cleaning protocol(s) and are relevant to the proposed use in the equipment. The cleanability class assigned to the equipment is likely to vary with the indicator and cleaning protocol chosen.

The soil adhering to surfaces at the end of a biotechnology process will contain many constituents. The indicator substance chosen to demonstrate the cleanability should be representative of those constituents that have an impact on safety aspects in relation to the need to protect people, the environment or features of the process. A brief description of indicators and test methods is given in annex B.

3.2 Methodology

To determine the cleanability of plant and equipment, choose and specify an appropriate test method or combination of test methods (see annexes A and B):

a) specify an appropriate indicator related to the proposed use of the equipment;

b) select the sampling procedure and the analytical procedure to be used to determine the quantity of this indicator which is present on relevant equipment surfaces or on surfaces in the plant ;

c) specify a cleaning protocol including, as a minimum, the specification of the constituents of the cleaning material and the mode of application.

NOTE 1 Potential hazards to the operator during cleaning should be assessed.

NOTE 2 Factors such as the duration, temperature and fluid flow rates of cleaning should be included in the protocol.

NOTE 3 The cleaning protocol can consist of a number of successive operations.

3.3 Testing procedure

Carry out the testing procedures as follows:

a) load the equipment or plant with the indicator under normal operating conditions or in a way which simulates these;

b) run the equipment and/or plant under normal or simulated-normal conditions until the load containing the indicator has been discharged from the equipment and the equipment and/or plant is ready to be cleaned;

c) using the analytical procedure selected in **3.2**, determine the quantity of indicator substance present after discharge of the load but before cleaning;

d) apply the cleaning protocol specified in **3.2** to the plant or equipment being tested for cleanability;

e) using the analytical procedure selected in **3.2**, determine the quantity of indicator substance present on the relevant surface(s) of the equipment and/or plant after application of the cleaning protocol;

f) using the data obtained, express the cleanability of the equipment or plant;

g) determine the appropriate cleanability class to the equipment under test as described in the equipment standards with respect to the chosen indicator substance and cleaning protocol.

NOTE The procedure described by European Hygienic Design criteria Group (EHEDG) (see annex C [2]) can be quoted as an example of the application of **3.2** above, in which the indicator substance is the spores of a specific bacterium, the analytical procedure is a culturing method which detects the number of this bacterium present before and after cleaning and in which the cleaning protocol is described (see annex C [3]).

Clearly other approaches can be used for the indicator substances could be another microorganism, a specific member of a chemical group such as a defined protein, carbohydrate or lipid, a specific compound known to be harmful to people or to the environment, or to future processing (see annex B). Potential cleaning protocols can be simple, for example a wash with water applied through a hosepipe or complex, as with sophisticated in-place cleaning involving the use of hazardous chemicals at high temperature.

Many test methods are possible, ranging from the use of a biological indicator to the use of chemical assays, immunological techniques, fluorescence assays and physical test methods, including microscopy (see annex B).

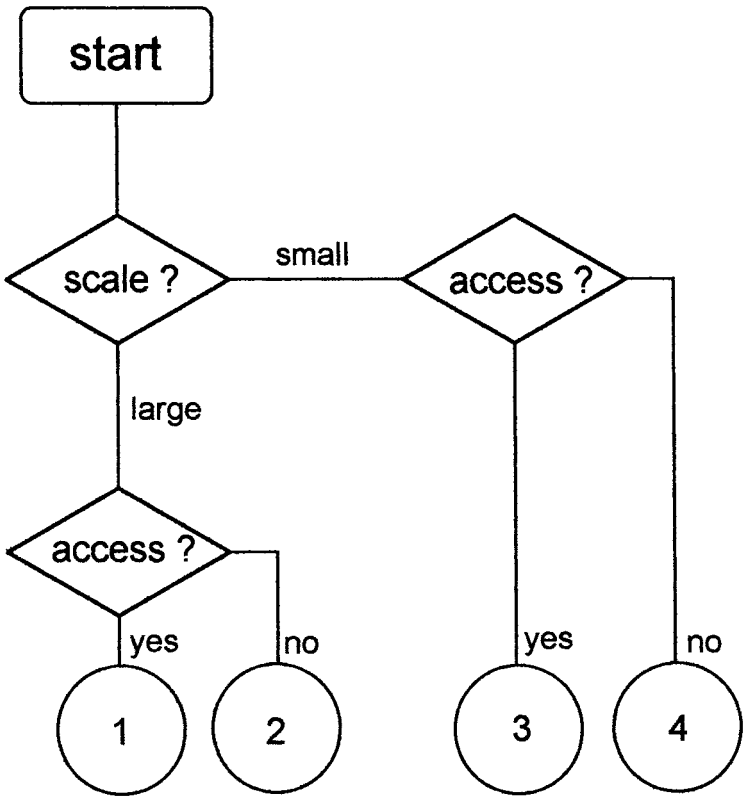
4 Documentation

The equipment manufacturer/supplier and/or the user should establish and document the procedure(s) used for the assessment of the cleanability of the component or unit of equipment. This documentation should include the applied test conditions (testing method, indicator, analytical procedure and cleaning protocol) and the results of the test.

Annex A (informative)

Selection guide on test methods for cleanliness

Figure A.1 gives guidance on the selection of test methods for cleanability. It represents a decision tree for selection of a cleanability test method based on scale, and access of the equipment.



1 rinse (see **B.4**), visual inspection (see **B.2**), swab (see **B.3**), optional

2 rinse, test following batch for contamination (see **B.5**)

3 rinse *, visual inspection, swab *

4 rinse, test following batch for contamination optional

* compare results, if they are consistent sample rinse-fluid only

Figure A.1 — Decision tree for selection of a cleanability test method

Annex B (informative)
Information on test methods for cleanliness

B.1 General

Essentially four test methods are available to determine the level of cleanliness of equipment (see annex C [3]). They are visual inspection, swabbing the inner surfaces, sampling the final rinse and testing the following batch for contamination.

B.2 Visual inspection

Residual soil, adsorbed microbial populations or relevant tracer indicators may be detected by a visual inspection of the equipment under investigation. This detection can be done by the eye or by using microscopic techniques such as light microscopy, scanning or transmission electron microscopy (see annex C [4]). In most cases it will be necessary to gain access to the equipment under investigation in order to be able to use these techniques.

B.3 Swabbing the inner surfaces

Indicator substances (chemical or (micro-)biological) can be measured by swabbing exactly defined areas of equipment that come into contact with the product. Swabbing of easily accessible flat surfaces may cause over-optimistic results with regard to the cleaning. Swabbing poorly accessible spots can produce over-pessimistic results. Vitally important for statistically relevant results are a correct choice of places where to swab in the equipment and the area to be swabbed when using this test method. Swabbing can be done either direct e.g. with agar count plates or indirect with e.g. cotton wool, alginate tips or petrifilms. This test method also requires that access may be gained to the equipment under investigation.

As an example, a very sensitive and simple and inexpensive method for the detection of residual amino acids and proteins on surfaces is described as follows. The ninhydrin method is based on the reaction of amino acids, peptides and proteins with triketohydrindenhydrat. This dye reaction is widely used for thin layer chromatography applications. The advantage of this method with respect to the detection of residual soil from biotechnological processes is the capability to detect a broad spectrum of substances from culture media and from micro-organisms, such as amino acids, peptides and proteins. Hence, a large variety of media, cell culture and protein fluids and suspensions can be detected by using the same performance test method. Due to its simplicity and ease of handling, this method appears suitable for challenge tests and for a proof of efficiency of cleaning methods, especially at equipment manufacturers or companies which do not have laboratories.

The method is used as follows. Sampling from the surface of interest is done by swabbing of a defined area with a wetted cotton wool roll.

The detection of amino acids, peptides and proteins is then done directly on the cotton roll. Some drops of the ninhydrin reagent are put on the roll surface and the cotton roll is developed at 110 °C to 120 °C for 20 min to 30 min. A violet hue will appear on the cotton roll surface if there were ninhydrin-colourable residues. For a proof of efficient cleanability of equipment, the equipment can be challenged with a ninhydrin-colourable substance (e.g. an amino acid like arginine), then be cleaned and analysed for residues of the applied substance. The threshold value of this method for arginine is approximatively 1 mg/m^2.

The ninhydrin reagent is prepared as follows.
Add 0,30 g ninhydrin to 100 ml *n*-butanol and 3 ml glacial acetic acid.

B.4 Sampling of the final rinse

After cleaning the final rinse fluid can be sampled for an appropriate indicator, the product itself or one of the components of the production process.

Examples are:

— viable micro-organisms (see annex C [2]);

— total protein content;

— lipopolysaccharides (e.g. the limulus lysate assay);

— salts, sugars (see annex C [7]);

— TOC (Total Organic Carbon).

Care should be taken that the contaminating substances are soluble or suspendable in the rinsing fluid and that the rinsing fluid can make good contact with all parts of the equipment.

B.5 Testing the following batch for contamination

Checking the next batch for contamination (using for example indicators applied according to the test method mentioned in clause **B.3**) is not the most favourable test method when testing cleanability of equipment but may be used if for some reasons the above mentioned test methods are not applicable or do not produce consistent results. The next batch should be checked in the case that pathogenic micro-organisms, different from the micro-organisms in the previous batch, have been used.

B.6 Direct monitoring test methods for the detection of residual soil

Direct monitoring test methods for detection of residual soil in equipment are largely still under investigation.

Examples are:

— ultrasound (see annex C [5]);

— laser scan microscopy;

— polarimetric methods;

— heat transfer (see annex C [6]).

Annex C (informative)

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