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BRITISH STANDARD

BS EN
12298:1998

# Biotechnology — Equipment — Guidance on testing procedures for leaktightness

The European Standard EN 12298:1998 has the status of a British Standard

ICS 07.080 07.100.01

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# National foreword

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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 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.

A list of organizations represented on this committee can be obtained on request to its secretary.

**Cross-references**

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**Summary of pages**

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 15 and a back cover.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

# EN 12298

March 1998

ICS

Descriptors: biotechnology, medical equipment, leaktightness, leak tests, contamination, microorganisms, noxious microorganisms, tests, safety, inspection, accident prevention, environmental protection, work safety

English version

# Biotechnology — Equipment — Guidance on testing procedures for leaktightness

Biotechnologie — Equipement — Guide des procédures d'essai pour le contrôle de l'étanchéité

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

This European Standard was approved by CEN on 2 March 1998.

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**CEN**

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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## Foreword

This European Standard has been prepared by the 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of an identical text or by endorsement, at the latest by September 1998, and conflicting national standards shall be withdrawn at the latest by September 1998.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.

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## 1 Scope

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

This European Standard gives guidance on the assessment of the leaktightness of biotechnological equipment with respect to a release of process microorganisms that can affect the safety of the worker (occupational health) and/or that can have adverse effects to the environment.

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

This European Standard applies if the intended use of the equipment includes hazardous or potentially hazardous microorganisms.

## 2 Definitions

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

**2.1**

**components of equipment**

technical entity which forms part of a unit of equipment

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

**2.2**

**direct test method (in biotechnology)**

test method which employs microorganisms for quantification

**2.3**

**indirect test method (in biotechnology)**

test method which employs physical and or chemical means for quantification

**2.4**

**leakage**

egress from equipment

**2.5**

**leak rate**

egress from equipment per unit of time

**2.6**

**leaktightness**

ability of component of equipment or unit of equipment to limit egress

**2.7**

**microorganism**

any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material [EN 1619]

NOTE For the purposes of this standard, the term microorganism covers the term of biological agent according to the Directive EEC/90/679: microorganisms, including those which have been genetically modified, cell cultures and human endoparasites which may be able to provoke any infection, allergy or toxicity.

**2.8**

**process microorganism**

microorganism used for production purposes in a biotechnological process or constituting (part of) the product itself

**2.9**

**target microorganism**

process microorganism and/or other microorganisms relevant for the specific process

NOTE For safety testing procedures, non-pathogenic microorganisms should be used where possible.

**2.10**

**unit of equipment**

assembly of components used to perform one or more unit operations

## 3 Testing

### 3.1 General

The selection of a test method depends on a number of factors, including equipment size, pressurization ability and constraints on intrusion by test fluid. Guidance on selection of test methods is provided in annex A.

To achieve relevant information on leaktightness, the design of the test method should be based on an appropriate risk analysis.

NOTE 1 It can be necessary for the test method to comprise one full cycle of the normal operation of the equipment. More operating cycles and/or extreme conditions such as highest pressure, highest rotational speed, range of temperature on repeated cycle basis can be required.

NOTE 2 In case of overpressure, the equipment can be regarded as a pressure vessel. Appropriate European and national regulations should be followed.

The recommended test method for characterizing and comparing emissions of microorganisms from bioprocess equipment consists of measuring the leak rate. This enables equipment emissions to be compared independently of the microorganism concentration inside the equipment.

As leakage can consist of aerosol and/or liquid, the leak rate comprises both liquid leak rates and aerosol leak rates.

If practicable from a technical and practical viewpoint direct test methods of determining leak rates are used since they are representative of the actual operating conditions. Indirect test methods are often more convenient in terms of speed, lack of contamination, economy, and ability for prolonged testing.

NOTE 3 Data obtained from indirect methods should be correlated with the release of microorganisms. Currently, validated correlations are lacking. Until such validated correlations have been established, results from indirect test methods should be used in accordance with common practice.

NOTE 4 Appropriate testing conditions for components of equipment are given in the relevant standards.

NOTE 5 Additional information on test methods for leak testing can be obtained from annex C [12], [13], [14] and [15].

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### 3.2 Methodology

To determine the leaktightness 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 analytical procedure to be used to determine the quantity of this indicator which is present in the equipment or plant;

c) specify a pressurization protocol including time and pressure.

NOTE Potential hazard to the operator during the pressurization should be assessed.

### 3.3 Testing procedure

Carry out the testing procedures as follows:

a) load the equipment or plant with the indicator under conditions representative of conditions during processing;

b) using the analytical procedure selected in **3.2**, determine the quantity of indicator substance present at the time at which pressurization protocol would be applied;

c) apply the pressurization protocol specified in **3.2** to the equipment or plant being tested for leaktightness;

d) using the analytical procedure selected in **3.2**, determine the quantity of indicator present in the equipment or plant after application of the pressurization protocol;

e) using the data obtained, express the leaktightness of the equipment or plant;

f) determine the appropriate leaktightness class to the equipment under test as described in the equipment standards with respect to the chosen indicator and pressurization protocol.

### 3.4 Choice of test methods

If the results of the test method should be quickly available and with a limited amount of work involved in leaktightness demonstration runs, indirect test methods should be used. Indirect test methods may however only be applied if a validated correlation between the measured effect and the desired performance has been shown. The required correlations are prepared for each unit of equipment or component.

### 3.5 Direct test methods

#### 3.5.1 *Aerosol*

An example of a direct test method of measuring the aerosol emission is quantitative bioaerosol monitoring. This can be carried out as described in **3.5.1.1** and **3.5.1.2**.

##### 3.5.1.1 *Preparation*

Quantitative bioaerosol monitoring is based on using the following:

a) characterized test microorganism preferably non-pathogenic;

b) characterized capture method for aerosolized microorganism;

c) characterized detection method;

d) controlled environment where a representative amount of air should be sampled over the test period;

e) standardized microorganism concentration in a defined medium.

##### 3.5.1.2 *Procedure*

Determine the leak rates for biotechnological equipment as follows:

a) ensure that the equipment under test is located in a controlled environment where emissions can be captured in a bioaerosol monitor;

b) collect and assay a measurable quantity of microorganisms over a known sampling time in the bioaerosol monitor;

c) calculate the airborne microorganism concentration within the controlled environment with the known volumetric sampling rate;

d) calculate the emission rate from the airborne microorganism concentration multiplied by the total rate of removal of air from the controlled environment;

e) calculate the leak rate by dividing the emission rate by the known microorganism concentration inside the equipment under test.

NOTE Details of methods and attributes are described by Behizad *et al.* (see annex C [3]) and Griffiths and DeCosemo (see annex C [4]).

#### 3.5.2 *Liquid*

Direct measurement of small flows of liquid leakage can be achieved semi-quantitatively by surface contact test methods such as swabbing and contact plates. In this case, estimates of the volume of carrier fluid are made. For larger leakage, liquid can be collected and the microorganism concentration determined. In this case estimates of the volume of carrier fluid are also made.

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### 3.6 Indirect test methods

Indirect test methods can be used to determine the leak rate in aerosol or liquid form. If there are validated correlations to the release of microorganisms, these should be used and reported. In the absence of such correlations, the result of the indirect test method should be reported as a leak rate based on the test fluid or tracer used.

The following indirect test methods (see annex B) can be used for quantitative leaktightness measurement:

a) pressure stability test method with gas such as air, helium, sulfur hexafluoride ($SF_6$) tracer gas;

b) pressure stability test method with liquid;

c) transmembrane gas diffusion and bubble point test method (filters only);

d) particle counting;

e) tracer fluids;

f) vacuum test method.

## 4 Documentation

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

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# Annex A (informative)
# Guidance on selection of test methods

## A.1 General

Figures A.1 to A.4 give guidance for the selection of an appropriate test method for the equipment under test. At the bottom of each chart are numbers referring to the suggested test method(s). The test methods and their number are given in Table A.1. The following clauses give information on the selection criteria. If several testing methods are available, one should be chosen with the help of BATNEEC (best available technique not entailing excessive costs)*.

## A.2 Performance classification (PC) or operational pre-check (OPC)

PC refers to a situation ensuring that the equipment meets a specified performance standard. PC can be carried out by an equipment manufacturer or equipment customer (e.g. for commissioning of equipment).

OPC can be carried out by the user often after sterilization, cleaning, equipment maintenance or incident resulting in unplanned release to the workplace or the environment.

## A.3 Rapid results

Some test methods require time before the test results are available. This should not be a great problem for equipment testing generally, but can be a problem if a large number of components need to be tested in a reasonably short time.

If rapid results are required, Figure A.1 (PC), or Figure A.2 (OPC) should be used. Otherwise Figure A.3 (PC) or Figure A.4 (OPC) should be used.

## A.4 Equipment volume

The question of volume, in relation to equipment, is relatively arbitrary but it is an important consideration for certain test methods. For example, when pressure stability testing considerably more accurate results are obtained for small volumes. Also when testing with a gas such as helium, pressurizing a large volume can be expensive.

## A.5 Pressurization of equipment beyond its working pressure

Pressure is also an important consideration; several of the test methods require the equipment to be pressurized above its normal working pressure with test pressures which are adjusted to the equation methods and the coefficients. The maximum permissible working pressure should not be exceeded.

## A.6 Access to the equipment

If a piece of equipment fails a test, the location of a leak can be required so that it can be corrected and retested. Reasonable access to the potential leakage points becomes necessary. Access can be as simple as being able to observe a leak by a bubble emission or it can be necessary to approach the equipment with probes. Access can also be a requirement of other test methods for leaktightness, e.g. access to a pressure gauge can be required when pressure stability testing equipment.

## A.7 Intrusive test method

An intrusive test method requires fluids other than the process fluid to be introduced into the equipment. This can be a tracer gas or fluid and is an important consideration if, for example, GMP (good manufacturing practice) could be compromised.

**Table A.1 – Test methods for leaktightness**

| Number | Test method |
|---|---|
| 1 | Pressure loss — gas/air |
| 2 | Pressure loss — liquid |
| 3 | Helium probe |
| 4 | $SF_6$/Freon probe |
| 5 | Thermal conductivity |
| 6 | Ultrasonics |
| 7 | Sonics (monitoring only) |
| 8 | Tracer liquid dyes |
| 9 | Bubble point (filters only) |
| 10 | Bubble formation (only qualitative) |
| 11 | Electronic particle counting |
| 12 | Tracer aerosol (NaCl) |
| 13 | Product aerosol (non-microbial) |
| 14 | Qualitative bioaerosol monitoring |
| 15 | Quantitative bioaerosol monitoring |
| 16 | Surface swabbing |
| 17 | Surface conductivity |
| 18 | Visual inspection (only qualitative) |
| 19 | Bacteria tightness |

* 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.

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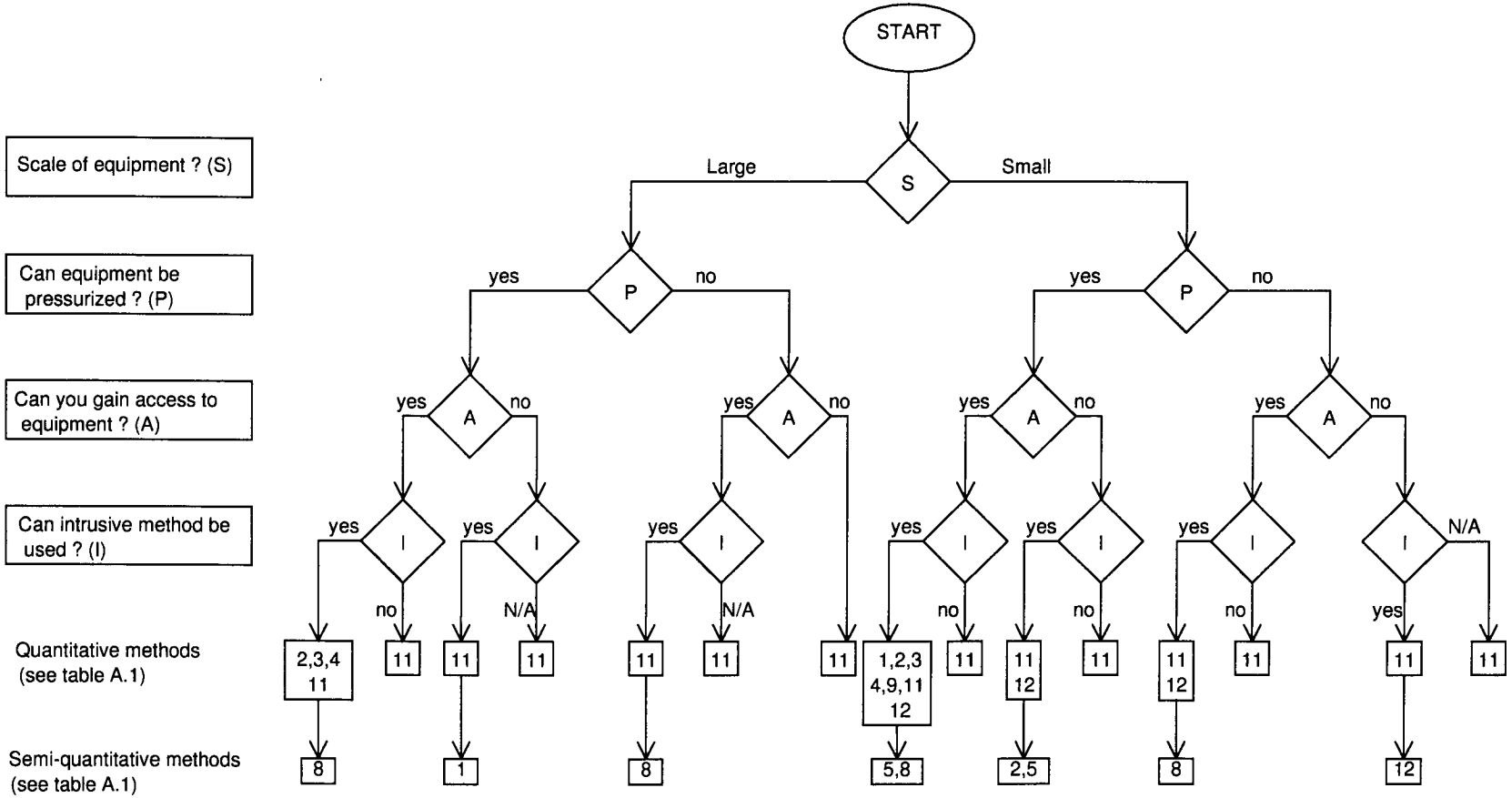


**Figure A.1 — Decision tree for selection of test method for leaktightness — performance classification — rapid results**

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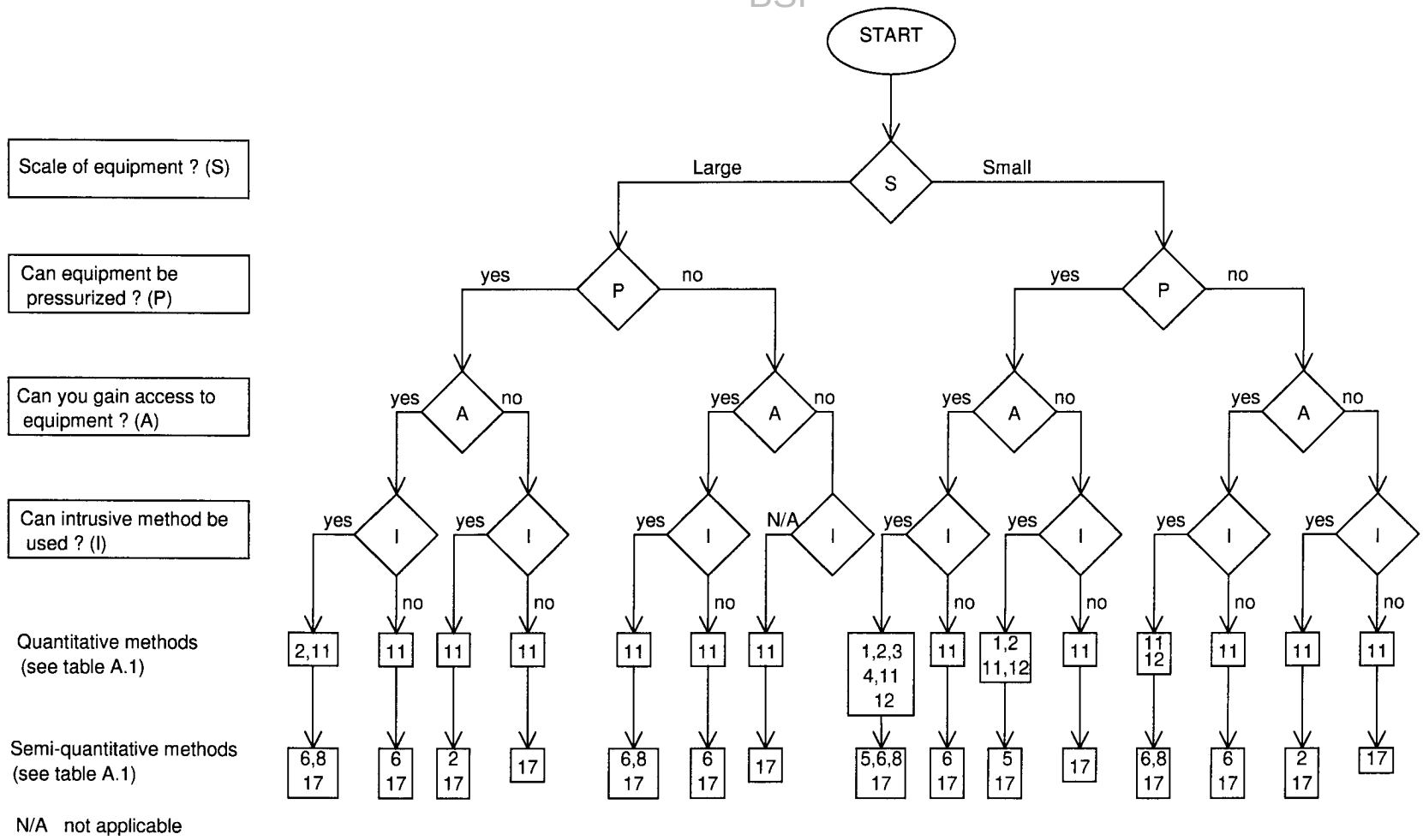


**Figure A.2 — Decision tree for selection of test methods for leaktightness — operational pre-check — rapid results**

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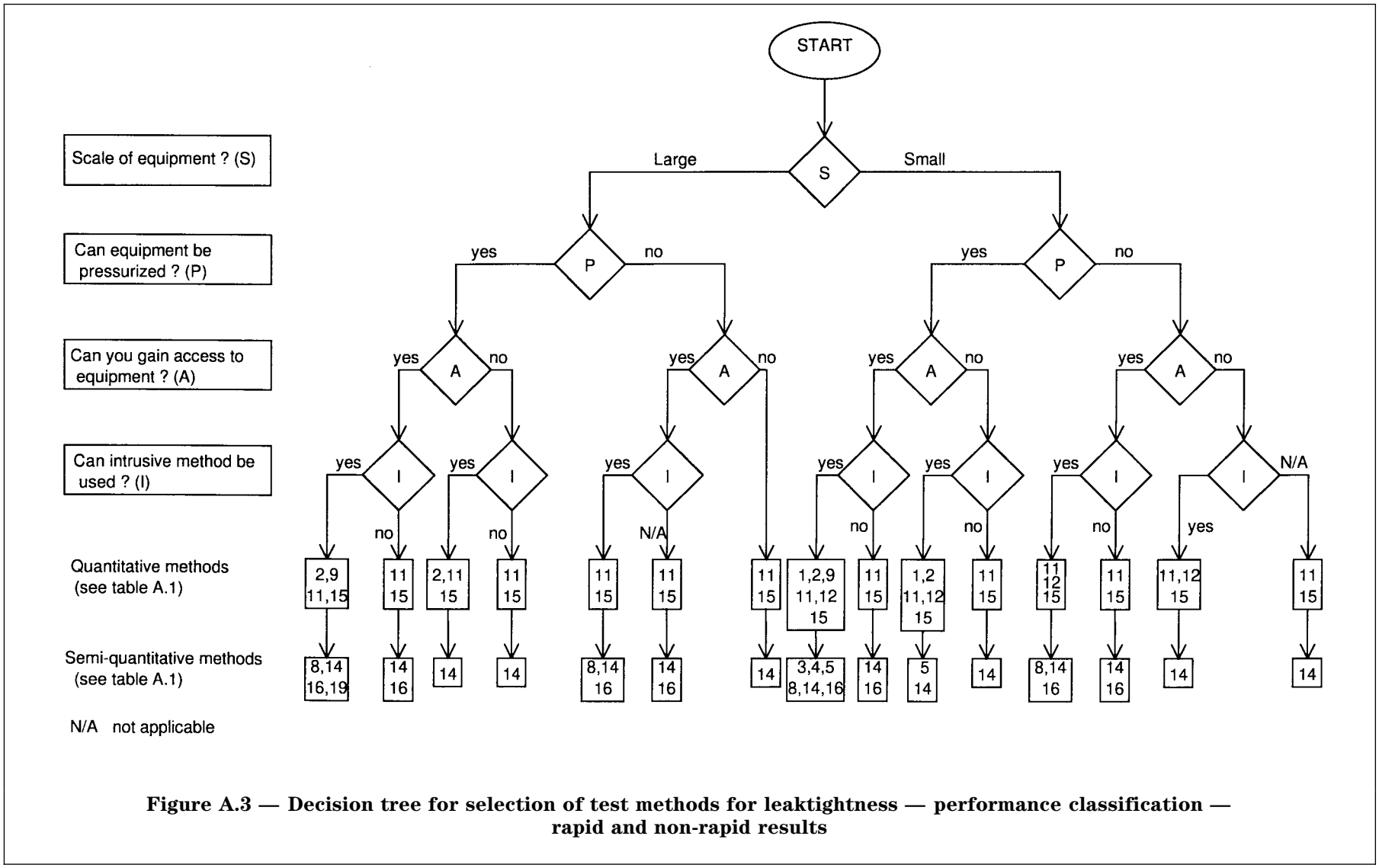


**Figure A.3 — Decision tree for selection of test methods for leaktightness — performance classification — rapid and non-rapid results**

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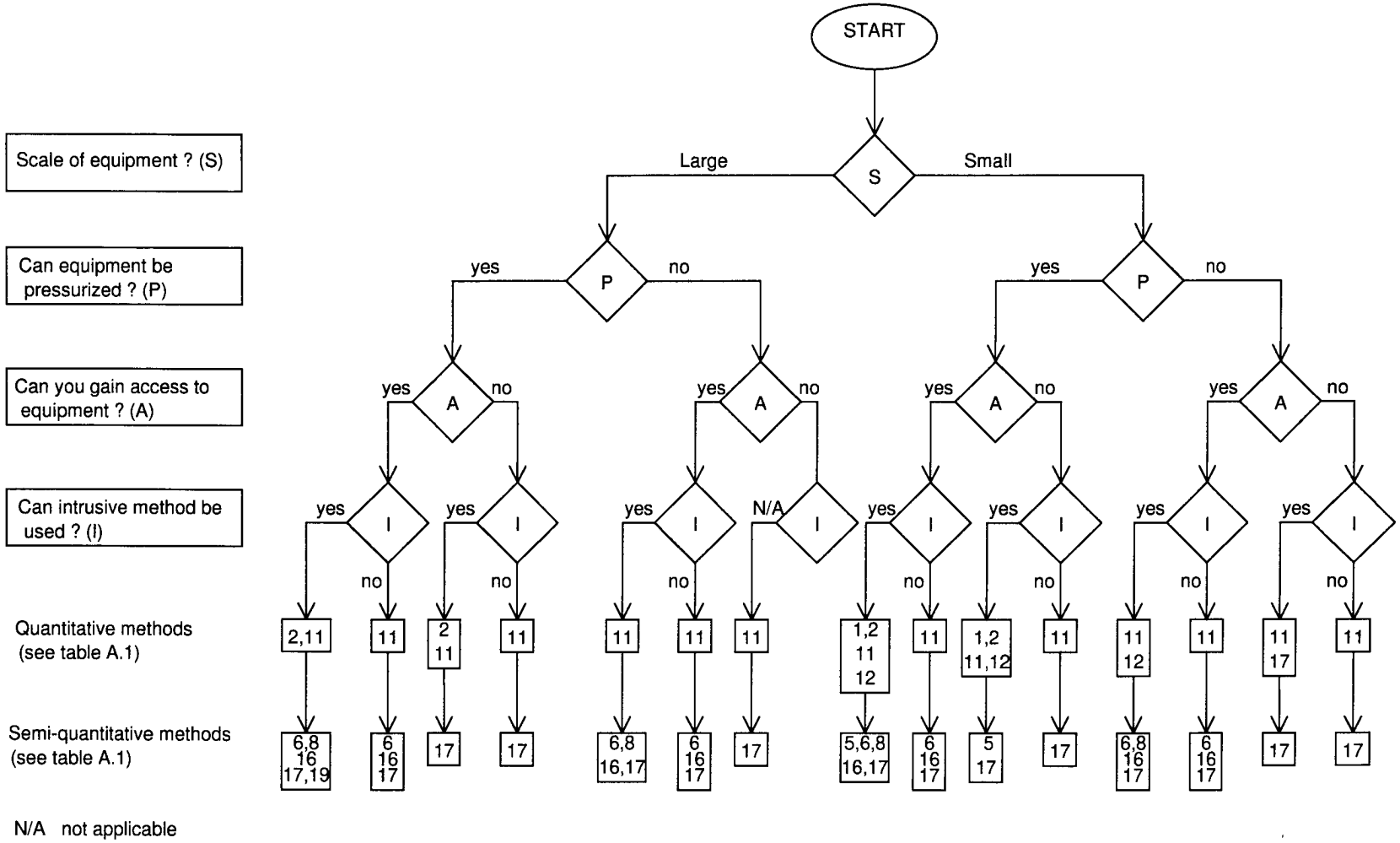


**Figure A.4 — Decision tree for selection of test methods for leaktightness — operational pre-check — rapid and non-rapid results**

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# Annex B (informative)
# Testing procedures for leak rate

## B.1 Correlation between direct test methods and indirect test methods

Correlations between various indirect and direct test methods remain to be established since only a few data from single scientific reports are available. The following correlation is a simple approach:

$$L = \frac{\varphi_m}{C_m}$$

where:

$L$ is the gas- or liquid leak rate of in millilitres per second or grams per second;

$\varphi_m$ is the microorganism leak rate in numbers per second or in grams per second;

$c_m$ is the concentration of microorganisms inside the equipment in numbers per millilitre or in grams per millilitre.

The correlation is based on the assumption of homogenicity, which means first uniform distribution of the microorganism inside the equipment and secondly the same concentration inside the equipment and in the released gas or liquid.

It is well known that in some cases this assumption is not correct and can result in leak rates of microorganisms that are too high if determined by indirect test methods:

— in case of pressure stability testing (gas or air) diffusion of gas through elastomer material will lead to a measured gas leak rate not contributing to leakage of microorganism;

— in case of pressure stability testing (liquid) one can find higher liquid aerosol release than estimated from measured leakage of microorganism (see annex C [6]).

Therefore interpretation of test results always should take into account the specific aspects of the used testing procedures and experimental set-ups. Considering this, the formula give above can be useful to:

— calculate leakage of gas or liquid from counting released microorganism;

— calculate leakage of microorganism from measured leakage of gas or liquid;

— recalculate leak rates determined under specified test conditions to the conditions of equipment in use.

## B.2 Pressure stability testing (gas or air)

### B.2.1 General

Pressure stability testing is a simple and straightforward means of determining leak rates. The method consists of filling the system or equipment under test with gas, pressurizing the system, and recording pressure decrease over a period of time. The sophistication can range from noting the pressure from a pressure gauge to accurate and sensitive pressure transducers linked to automated data recording with microprocessor control. Pressure stability testing can be carried out during a long period of time (several hours, or even a day).

It is essential that equipment is capable of being pressurized. The test is more suited to small equipment volumes for greater accuracy. The test gas should be in thermal equilibrium with the equipment wall otherwise there will be changes of pressure not attributable to leakage. Variations of outside temperature should be followed.

### B.2.2 Example of procedure

This clause gives an example of a pressure stability testing procedure. It should be as follows:

a) check the calibration of the pressure indicator and adjust if necessary;

b) ensure equipment is clean, free from dust, fibres, etc;

c) measure the absolute pressure and temperature of the surroundings;

d) pressurize the equipment to a chosen pressure and isolate pressure line;

e) allow time for gas to come to thermal equilibrium (typically 10 min to 30 min);

NOTE 1 During this waiting period, the pressure can fall.

f) commence the test and note the initial pressure $p_i$ when the gas temperature and ambient temperature are the same;

g) note the final pressure $p_f$ at the end of the test time period $t$;

NOTE 2 The ambient temperature should not change by more than a specified amount, typically 1 °C, over the test period. If temperature changes by more than 1 °C, appropriate adjustments of pressure should be carried out.

NOTE 3 The minimum data required are the test volume, ambient temperature, time period, and the initial and the final pressures. With more pressure/time data, a plot of pressure against time will be approximately a straight line for laminar flow leaks.

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The gas leak rate for the testing conditions is calculated from:

$$L_t = \frac{298 \cdot V}{Tt} (p_i - p_f)$$

where:

$L_t$ is the gas leak rate, in metre to the third power pascals per second;

$V$ is the test volume, in metres to the third power;

$T$ is the ambient temperature of gas, in kelvins;

$t$ is the testing time, in seconds;

$p_i$ is the initial pressure, in pascals;

$p_f$ is the final pressure, in pascals.

The standardized leak rate is calculated from:

$$L_s = L_t \frac{\mu_s(10^{10})}{\mu(p_{in}^2 - p_{out}^2)}$$

where:

$L_s$ is the standardized leak rate, in metre to the third power pascals per second;

$\mu$ is the gas viscosity, in pascal seconds at the temperature of assay;

$\mu_s$ is the gas viscosity at 298 K, in pascal seconds;

$p_{in}$ is the inside gas pressure, in pascals;

$p_{out}$ is the outside gas pressure or ambient pressure, in pascals.

The standardized leak rate is the leak rate that one would have if the pressure inside the equipment was atmospheric and the pressure outside was vacuum. It allows comparison of data from tests carried out at different conditions. The formula for $L_s$ is only valid using absolute pressure values.

Equivalent liquid leak rates at operating conditions can be estimated from gas leak rates at test conditions by:

$$Q = 2L_t \frac{\mu(p_{in} - p_{out})_{liq}}{\mu_{liq}(p_{in}^2 - p_{out}^2)}$$

where:

$Q$ is the estimated volumetric flow rate at operating conditions, in metres to the third power per second;

$\mu_{liq}$ is the liquid viscosity at operating conditions, in pascal seconds;

$(p_{in} - p_{out})_{liq}$ is the difference between inside pressure and outside pressure for liquid flow at operating conditions, in pascals.

An estimate of potential release of microorganisms is obtained by multiplying $Q$ by the microorganism concentration in the feed. This concentration could be defined as a standard concentration for purposes of reporting the test. The formula for $Q$ is only valid using absolute pressure values.

### B.3 Pressure stability testing (liquid)

Pressure stability testing with liquid is an alternative to gas pressurization. The system/equipment under test is filled completely with liquid (commonly water). The system is pressurized and leakage is determined by visual inspection of escaping liquid and/or any pressure loss over time. Although such a test method has been widely deployed, it has attracted criticism and is only suitable for gross leaks. The test is semi-quantitative.

### B.4 Tracer gases

Tracer gases such as Helium or sulfur hexafluoride ($SF_6$), can be used instead of air or water. The reason for their use is often to locate leaks for subsequent repair. Another reason is that many of the test methods are sensitive. The test methods can quantify equipment leakage.

Helium tracer gas is one of the more sensitive test methods. It is used in conjunction with specialist mass spectrometers tuned to detect helium gas.

There are two types of test methods. Tracer probe technique refers to connecting the equipment under test to a test port on the helium detector and evacuating the air to a specified vacuum. Helium is introduced (normally from a gas bottle) via a spray pistol around the test object surface and any leak is detected by helium flow into the detection equipment to yield a leak rate. The alternative detection probe or sniffer probe technique, uses a probe connected to the detection equipment test port. The test object is pressurized with helium or helium/air mixtures and leakage is detected by escape of helium from the test object.

Quantification of overall leak rates can be achieved in various ways. Many of the methods utilize an outer chamber or envelope around the test object.

Halogen detectors based on sensing freons, $SF_6$ etc. are available. The principles for determining leak rates are the same as for helium. These detectors are less sensitive compared with helium detectors but their cost is also less.

Thermal conductivity sensors can be used with a range of test gases provided they have a different thermal conductivity to air. Leak testing is undertaken by pressurizing the equipment with a gas or gas mixture. Leakage of tracer gas is detected by the thermal conductivity imbalance of the tracer gas and the reference air. The most sensitive gases using this technique are hydrogen or helium. Other less sensitive gases are argon, carbon dioxide, refrigerant gas, and neon. Vacuum based methods for using thermal conductivity quantitatively are described in BS 3636 (see annex C [5]).

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### B.5 Sonics and ultrasonics

These test methods are a very fast and convenient means of locating leaks in pressurized systems. Fluid leakage with a high velocity flow generates sound emission by turbulence and cavitation. These sonic disturbances can be transmitted through the medium of the pressurizing fluid, through the containment structure, or through the atmosphere surrounding the leak location. Although audible sonic waves (up to 16 kHz) can be used to identify gross leaks, ultrasonic test methods have two advantages; they can distinguish between the leakage and ambient sound which otherwise can lead to false readings, and ultrasonic waves with a short wavelength are more directional, leading to more accurate leak location.

Detection is either by a microphone or other transducers such as the piezo-ceramic type. Probes either sense from the ambient air or by contact with the surface equipment. Alternatively, coupling materials of solids or liquids are used.

Ultrasonics can only provide semi-quantitative measurement of a leak. It is useful for locating leaks and is non-intrusive compared with bubble formation techniques.

### B.6 Tracer liquids — dyes

Both liquid phase tracers and gas phase tracers can be used for leak detection. Dyed liquid tracers are typically composed of oil or water with a tracer dye to enhance visibility of leakage indications. Fluorescent dyes in water are for example used frequently for detecting microleaks in water or steam systems by inspection with mercury vapour radiation light (365 nm). For detecting leakage points in boilers, pipelines and valves, a useful technique utilizes a special dye system which can be seen by spraying on a solvent developer material. Thus fluorescent tracer liquids which can evaporate as they penetrate small leaks leaving a solid deposit, are redissolved and detected.

### B.7 Bubble point — filters only

There are specialist devices available which measure the integrity of air and liquid filter materials used in pharmaceutical applications. The filter is pre-wetted and then subjected to air pressure gradients. Liquid is held in place by the surface tension.

Two basic test methods can be carried out. One is a pressure hold test where the pressure is held below the bubble point. Reductions in pressure are due to diffusion of air across the filter pore/liquid interface or due to leaks in the system. A maximum permitted pressure reduction due to diffusion is normally provided by the manufacturers of the filter. Higher pressure drop rates would be attributed to leaks. The second is to determine the bubble point which provides an indication of the maximum pore size in the filter.

### B.8 Bubble formation

Leak location by bubble formation is one of the most widely used non-destructive test methods since it is cheap, simple and requires minimum training, with relatively rapid response both to large and small leaks. The well known technique of applying soap solutions to leaking gas pipes to observe bubble formation has been greatly improved by the application of much more sensitive liquid detectors in which bubbles form readily and are easily seen by inspectors.

The principle of bubble formation test is to apply a pressure across the leak with air or a tracer gas and apply the detection liquid in contact with the lower pressure side. Leakage is indicated by the formation of bubbles. It is a qualitative test method.

### B.9 Electronic particle counting

Systems that use electronic methods to monitor, measure or count airborne particles can be used to monitor product molecules and dusts, as well as airborne microorganisms in the biotechnology industry. Such devices, which can be either optical or piezoelectric, do not however, discriminate between viable and non-viable particles. The application to leaktightness measurement requires the test equipment to be located in a particle free environment so that aerosols measured are assumed to be from the test object.

To count single particles, the air sample is drawn through an illuminated chamber which is sufficiently small that it can only hold one particle at a time. As a particle interrupts or scatters the beam it is detected by an opto-electronic method. Details of the illumination (whether white light or laser) and the geometry of the optical system are different for a variety of instruments.

Care should be taken to ascertain whether detected particles have arisen from the challenge process fluid in the test object or elsewhere (such as lubrication oil).

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### B.10 Tracer fluids

Tracer fluids, as alternatives to microorganism suspensions, can be used. The methodologies are similar to the leak rate determination based on capture and enumeration of microorganisms from the aerosol state and the liquid state.

For aerosol determination, a suitable air sampler is required. For liquid emission other than gross leakage, some form of tracer recovery by a washing step with known volumes of solvent can be necessary, to remove the tracer from the adjacent surface and area.

Examples of aerosol determination are sodium chloride (NaCl) or dyes.

A solution of NaCl is electrically conductive and this can be used to determine leakage. For aerosols, an air sampler that collects particles into liquid is recommended. When linked to an in-line conductivity probe, continuous monitoring of aerosol emission can be achieved.

Other test methods such as flame ionizers have been used with salt solutions.

Fluorescent and other dyes, in water or other solvent, are alternative techniques whereby the assay is based on some form of colorimetric technique. Potassium iodide is commonly used for microbiological safety cabinets.

### B.11 Qualitative and quantitative bioaerosol monitoring

Bioaerosol monitoring can be considered as a two step process. The first stage is to collect airborne particles and secondly they have to be detected, identified and enumerated.

Samplers which can be used to collect bioaerosols can be classed into three broad areas: impaction onto solid or semi-solid surfaces, impingement into liquid, and collection by filtration through a porous material such as an appropriate filter. Qualitative aerosol monitoring involves the use of settle plates. This test method relies on gravitational settling, and hence is biased towards the collection of larger particles. Most bioaerosol samplers are quantitative; they sample known volumes of air into a sampler and hence the airborne microorganism concentration can be estimated.

### B.12 Surface swabbing

Surfaces adjacent to the equipment under test can be swabbed, or contact plates applied, to determine the extent of microbial surface contamination. This surface contamination is indicative of a breach of containment of the adjacent equipment.

### B.13 Surface conductivity

As an analogue to swabbing, gross leaks by liquid seepage could be detected by electrically insulated conductivity probes located on the plant surface at potential leakage points.

This is at best a semi-quantitative test method but should provide a rapid response. This test method can be particularly appropriate with biotechnological plants since most of the process fluids are highly electrically conductive.

### B.14 Visual inspection

Gross leaks can be identified visually. Many of the other test methods require an element of visual inspection, e.g. bubble formation and tracer fluids with dyes. Visual inspection is wholly qualitative.

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## Annex C (informative)
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