

A method for the assessment of bacteria tightness of food-processing equipment

It is essential to ensure that bacterial ingress is prevented during aseptic food-processing operations. Here the procedures for assessing the bacteria tightness of food-processing equipment recommended by the Test Methods subgroup of the European Hygienic Equipment Design Group (EHEDG) are summarized. This paper is the eighth in a series of articles featuring the EHEDG to be published in *Trends in Food Science & Technology*. The EHEDG is an independent consortium formed to develop guidelines and test methods for the safe and hygienic processing of food. The group includes representatives from research institutes, the food industry, equipment manufacturers and government organizations in Europe.*

The Test Methods subgroup of the European Hygienic Equipment Design Group (EHEDG)* is responsible for producing standardized test methods for assessing the hygienic and aseptic capability of food-processing equipment. Methods for assessing in-place cleanability³, in-line pasteurizability⁵ and in-line steam sterilizability⁶ have been approved by the EHEDG and published.

This paper details the recommended test procedure for assessing whether an item of food-processing equipment, intended for aseptic operation, is impermeable to microorganisms. Small motile bacteria penetrate far more easily through microscopic passages than (non-motile) moulds and yeasts. The facultative anaerobic bacterium *Serratia marcescens* (CBS 291.93) is therefore used to test the impermeability of equipment to microorganisms, usually called 'bacteria tightness'. The method is based on a Unilever Research Laboratory procedure⁹ and is designed to determine whether bacteria are able to penetrate from the environment into the product line via the piece of test equipment. The method is suitable for equipment that is already known to be in-line steam sterilizable.

Materials

Indicator microorganism

Serratia marcescens is used as an indicator microorganism to test the bacteria tightness of the equipment.

*Readers requiring further information on the EHEDG are referred to Ref. 1. Details of previously published EHEDG articles are given in Refs 2-8.

The indicator strain is a small, strongly motile, rod-shaped bacterium that is able to penetrate through small holes and crevices, which are very difficult to detect by physical methods.

The indicator microorganism is easily recognized by a strong red pigment, which colours the growth medium (see below) pink on incubation.

Trypticase soy broth (TSB)

The indicator microorganism is cultivated in sterile trypticase soy broth (TSB; 15 g/l trypticase soy; BBL, Beckton Dickinson Microbiology Systems, Cockeysville, MD, USA) at 30°C for 24 hours prior to each experiment. TSB of the same concentration is also pumped through the test apparatus to provide a growth medium for any indicator microorganism able to penetrate the test equipment, and is used to test the antimicrobial properties of gaskets (see below).

Test equipment

Prior to testing, the equipment to be investigated is dismantled and thoroughly degreased (using a solvent such as alcohol), cleaned by hand (using a neutral detergent solution) and, if necessary, descaled (using a 1%, w/w, aqueous acetic acid solution). The dismantled equipment (if relatively small) should then be sterilized in an autoclave at 120°C for 30 minutes before re-assembly under aseptic conditions. Alternatively, the equipment may be reassembled and sterilized in-line by pressurized steam at 120°C for 30 minutes. All construction materials, including all gaskets, must be capable of withstanding the cleaning and sterilization procedures.

Occasionally, gasket materials have antimicrobial properties, which may influence the test results. Therefore, controls should be undertaken in which gaskets are submerged in TSB. A control, without gaskets, should be included. After sterilization in an autoclave at 120°C for 30 minutes, the TSB is inoculated with a freshly prepared culture of *Serratia marcescens* to a concentration of $\sim 10^3$ bacteria/ml; if after incubation at 30°C for 24 hours no red discoloration is observed in the broth with the gaskets, and red discoloration is observed in the broth without the gaskets, then the gasket material must be regarded as unsuitable for this method.

Any equipment with shaft passages must have double seals. The space between the two seals must be flushed with a sterilizing fluid in accordance with the equipment manufacturer's instructions. It must be ascertained that the sterilizing fluid, in the amount leaking into the culture broth, does not adversely affect the growth or motility of *Serratia marcescens*.

Test procedure

Test circuit

An example of a test circuit used for testing bacteria tightness is shown in Fig. 1. An aseptic vessel, fitted with two aseptic flow-through valves (Fig. 2) and containing an appropriate volume of TSB, is sterilized in an autoclave at 120°C for 30 minutes.

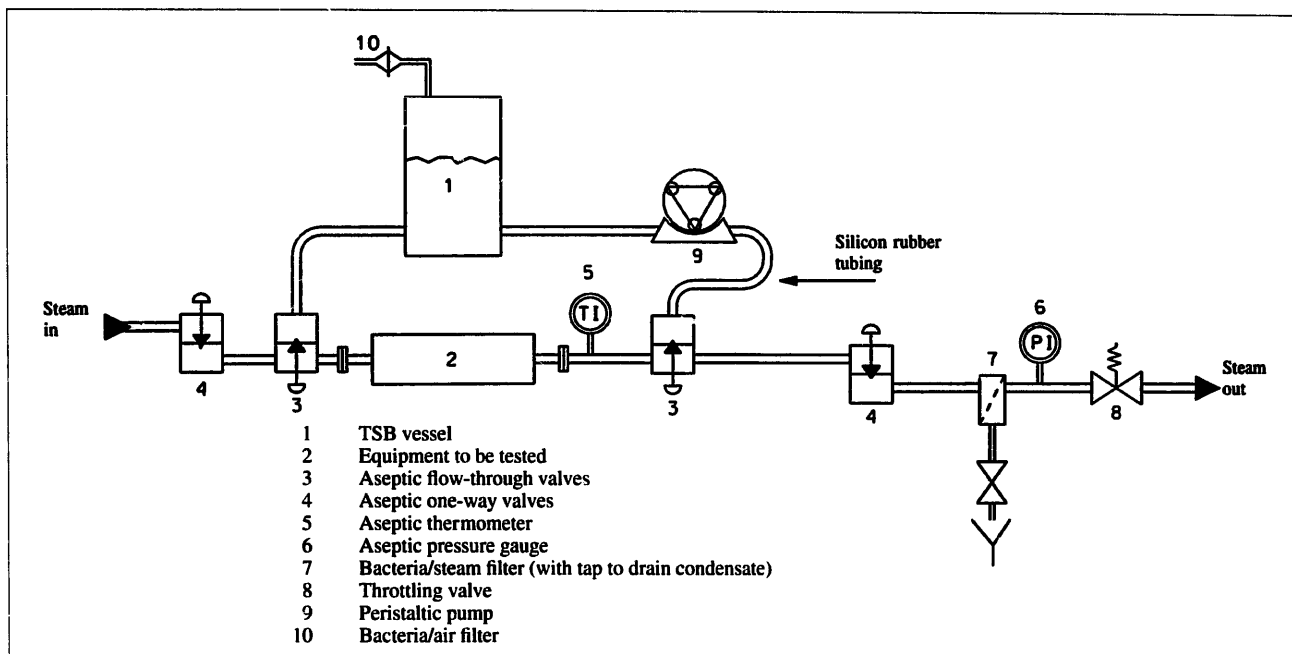


Fig. 1
Test circuit for testing the bacteria tightness of equipment.

Both side connections of the flow-through valves are short-circuited during autoclaving (Fig. 2) and the valves are left in the open position. After autoclaving, the valves are closed and the vessel is incorporated into the test circuit by means of the valves' side connections. Once the test circuit has been assembled, the item of equipment to be evaluated is treated with steam at 120°C for 30 minutes. The steam must be saturated, and the required back pressure (0.2 MPa; 2 bar) absolute, controlled by means of the throttle valve. Temperature and pressure within the system must be in agreement with those expected for saturated steam. If this is not the case, the test is void (the steam may contain gases, such as air). To ensure that no 'cold spots' are formed in the system, care must be taken to ensure that no condensate can accumulate during the steam treatment. When the sterilization procedure is completed, the two one-way valves either side of the flow-through valves are closed. The flow-through valves are then opened, thus effecting an aseptic connection with the TSB vessel. All test circuit components designated 'aseptic' must have been proven to be sterilizable and bacteria tight; otherwise, they may adversely influence the test results. In the case of small equipment, flexible tubing and tube clamps can be used. All components (including any flexible tubing) must be connected such that there are no places where solids or air can be trapped.

Equipment soiling

A freshly prepared culture of the test microorganism (which will contain $\sim 10^9$ microbial cells/ml) is diluted (1 ml in 9 ml) in sterile TSB and spread over all critical and suspect parts of the equipment by means of brushes, syringes, etc. All areas where leakage may occur are treated twice a day, for at least 3 days in succession, or longer if required. Where applicable, the equipment is

operated 10 times after each treatment. To obtain sufficient mixing and to ensure rapid detection of microbial growth, TSB is circulated throughout the test circuit (Fig. 2) for 2 hours every day by means of a peristaltic pump. The flow rate at which the broth is circulated will depend on the volume contained within the system and should be set to give two volume changes within the

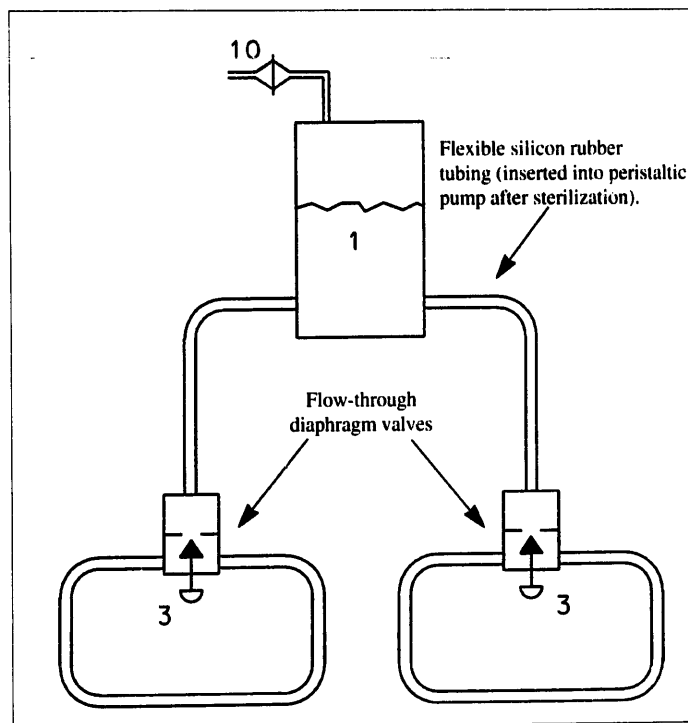


Fig. 2
Trypticase soy broth (TSB) vessel with aseptic flow-through valves during sterilization in an autoclave. Numbered parts refer to those in Fig. 1.

vessel during the 2-hour circulation period each day. The test circuit is kept at ambient temperature (~20–25°C) during the soiling procedure. If the ambient temperature fluctuates outside the stated limits it must be confirmed experimentally that the growth and motility of *Serratia marcescens* are not adversely affected.

Detection of penetrating bacteria

After the soiling procedure the system is kept at ambient temperature (~20–25°C) for 5 more days. The broth is circulated for 2 hours every day at the same flow rate used during the soiling procedure.

Interpretation of results

If the broth still remains clear after the 5-day detection period, the equipment is classified as bacteria tight for the duration of the soiling procedure. If the broth becomes turbid a sample is taken and examined for the presence of *Serratia marcescens* by incubation at 30°C for 2 days; red discolouration of the broth confirms the presence of *Serratia marcescens*.

Discussion

If *Serratia marcescens* is present in the system, the equipment has failed the test and is, therefore, not bacteria tight and hence not suitable for aseptic use. The indicator microorganism is certain to have penetrated from outside the equipment, because the heat resistance of *Serratia marcescens* is so poor that it could not survive the steam treatment of 120°C for 30 minutes.

Tests should be conducted a minimum of three times. If varying results are obtained a thorough examination should be conducted to ascertain whether the tests have been adversely influenced by faults in either the item of test equipment, the test circuit, or the testing conditions and/or analysis. If any faults are discovered, these should be rectified and the tests repeated. If no faults are discovered it can be concluded that the item of test equipment is not bacteria tight and, hence, not suitable for aseptic use.

If an item of equipment is to be assessed for both in-line steam sterilizability and bacteria tightness, the test

for bacteria tightness may be conducted using the same test circuit and batch of broth as the steam sterilizability test⁶, provided that the broth remained clear throughout the 5-day in-line steam sterilizability test.

If a piece of equipment passes the test it cannot be assumed that it will remain bacteria tight in the future unless periodic maintenance is conducted (e.g. timely replacement of seals and gaskets).

This paper summarizes the guidelines and methods recommended by the European Hygienic Equipment Design Group (EHEDG) subgroup on Test Methods. The full report, by A.W. Timperley, J. Axis, A. Grasshoff, C.R. Hodge, J.T. Holah, R. Kirby, J.F. Maingonnat, C. Trägårdh, B.M. Venema-Keur and O. Cerf, is available from: D.A. Timperley, Campden Food and Drink Research Association (CFDRA), Chipping Campden, UK GL55 6LD (tel. +44-386-840319; fax: +44-386-841306).

The EHEDG recommends that laboratories that intend to apply the EHEDG test methods be certified. For details, please contact D.A. Timperley at the above address.

References

- 1 European Hygienic Equipment Design Group (EHEDG) (1992) in *Trends Food Sci. Technol.* 3, 277
- 2 *Microbiologically Safe Pasteurization of Liquid Foods* (1992) in *Trends Food Sci. Technol.* 3, 303–307
- 3 *A Method for Assessing the In-place Cleanability of Food-processing Equipment* (1992) in *Trends Food Sci. Technol.* 3, 325–328
- 4 *Microbiologically Safe Aseptic Packing of Food Products* (1993) in *Trends Food Sci. Technol.* 4, 21–25
- 5 *A Method for the Assessment of In-line Pasteurization of Food-processing Equipment* (1993) in *Trends Food Sci. Technol.* 4, 52–55
- 6 *A Method for the Assessment of In-line Steam Sterilizability of Food-processing Equipment* (1993) in *Trends Food Sci. Technol.* 4, 80–82
- 7 *The Microbiologically Safe Continuous-flow Thermal Sterilization of Liquid Foods* (1992) in *Trends Food Sci. Technol.* 4, 115–121
- 8 *The EC Machinery Directive and Food-processing Equipment* (1993) in *Trends Food Sci. Technol.* 4, 153–154
- 9 Lelieveld, H.L.M. (1985) *J. Soc. Dairy Technol.* 38(1), 14–16

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