A method for the assessment of in-line steam sterilizability of food-processing equipment

Food-processing equipment may need to be sterilized before use, and it is important to ensure that the sterilization method applied is effective. Thus, it is necessary to determine under which conditions equipment can be sterilized. Here the procedures to test the in-line steam sterilizability of equipment recommended by the Test Methods subgroup of the European Hygienic Equipment Design Group (EHEDG) are summarized. This paper is the fifth 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. Test methods for assessing in-place cleanability[1] and in-line pasteurizability[4] have been approved by the EHEDG and published; a method for assessing bacteria tightness is currently in draft form (Timperley, A.W. *et al.*, unpublished).

This paper details the recommended test procedure for assessing the suitability of an item of food-processing equipment for in-line steam sterilization. It is advisable to conduct in-place cleanability trials[1] prior to this test in order to verify the equipment's hygienic design. The method is based on a Unilever Research Laboratorium procedure[6], and is designed to indicate whether an item of equipment can be freed internally from viable microorganisms by in-line steam sterilization.

Materials

Indicator microorganisms

A heat-resistant strain of *Bacillus subtilis* (Bac 1-12; LMB 93.57, Laboratory for Microbiology, Delft, The Netherlands) is used as the test organism, and has been chosen for its relatively high D-value (time to reduction of the count of colony-forming units by a factor of 10) of 0.9 minutes at 120°C (Ref. 7). It has a long record of use in assessing the sterilization of food.

The strain is cultivated on trypticase soy agar (TSA; BBL, Becton Dickenson Microbiology Systems, Cockeysville, MD, USA). After incubation at 37°C for 3–5 days, the degree of sporulation is checked microscopically; if it is greater than 10%, the spores are harvested into physiological saline. This suspension is washed twice in physiological saline by centrifugation at 4000*g* for 15 minutes, resuspended in physiological saline, and pasteurized at 80–82°C for 10 minutes. After cooling at 15–25°C, spore suspensions are stored at 5°C until required. Pasteurized spore suspensions may be kept for at least 3 months without losing relevant characteristics. The concentration of spores in the suspension is determined by pour-plating with TSA and incubating at 37°C for 3 days.

Trypticase soy broth (TSB)

Trypticase soy broth (TSB; 15 g/l trypticase soy; BBL, Becton Dickenson Microbiology Systems, Cockeysville, MD, USA) is pumped through the test apparatus to provide a growth medium for any indicator microorganisms surviving on the equipment after the sterilization test procedure.

Steam

The steam should be free from any contaminants that may adversely affect the germination and subsequent growth of *B. subtilis*.

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 the components are relatively small) should then be sterilized in an autoclave at 120°C for 30 minutes. Alternatively, the equipment may be re-assembled and sterilized in-line by steam (120°C for 30 minutes). All construction materials, including gaskets, etc., must be capable of withstanding the cleaning and sterilization procedures. Equipment with shaft passages should be equipped with double seals and provision made for flushing the space between the two seals with a sterilizing fluid.

Occasionally, gasket materials have antimicrobial properties, which may influence the test results. Therefore, controls should be undertaken in which gaskets are submerged in TSB. After sterilization in an autoclave at 120°C for 30 minutes, the TSB is inoculated with the spore suspension to a concentration of ~10^3spores/ml. If no turbidity is observed after incubation at 37°C for 24 hours, the gasket material must be regarded as unsuitable for this test method.

*Readers requiring further information on the EHEDG are referred to Ref. 1. Details of previously published EHEDG articles are given in Refs 2–5.

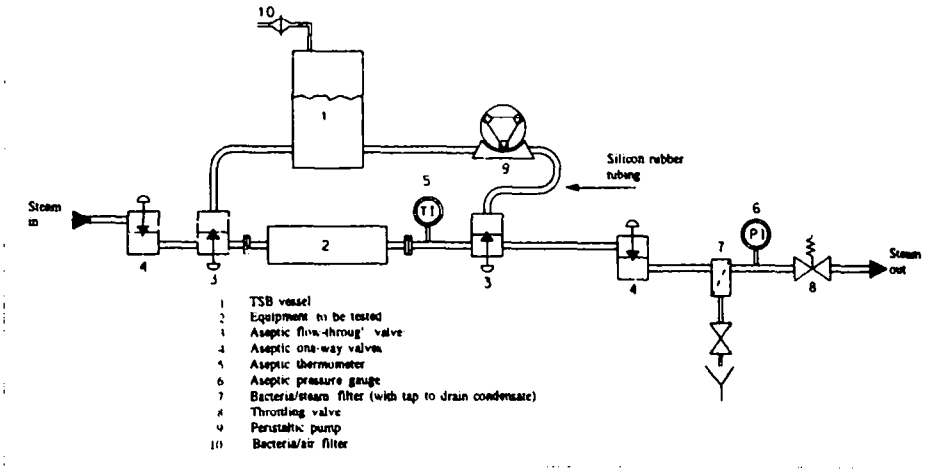

Fig. 1
Test circuit for testing the in-line sterilizability of equipment

Test procedure

Equipment soiling

The spore suspension is diluted with physiological saline to a concentration of ~5×10^7 spores/ml. This diluted spore suspension is used to wet the inner surfaces of the dismantled equipment, including all surfaces that are in contact with each other after reassembly (e.g. gaskets and gasket grooves). The equipment is allowed to dry at 20–25°C (relative humidity ≤65%) for ≥2 hours, after which it is reassembled. If the temperature is lower and/or the humidity is higher than specified, the time allowed for drying may have to be extended. A visual assessment should be made of the coated surfaces, to ensure sufficient drying, prior to reassembly. A sample of the spore suspension is taken and the concentration determined by pour-plating with TSA and incubating at 37°C for 3 days.

Sterilization procedure

An example of a test circuit for conducting in-line steam sterilizability testing 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.

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 side connections of the valves. 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; the required back pressure of 0.2 MPa (2 bar) absolute is controlled by means of the throttle valve. Both 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

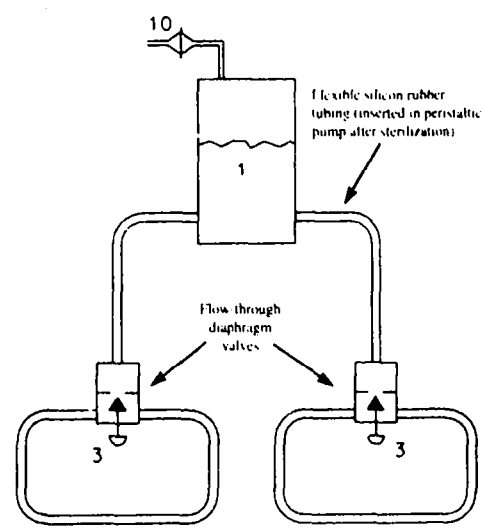

Fig. 2
Trypticase soy broth (TSB) vessel with aseptic flow-through valves during sterilization in an autoclave. Numbered parts refer to those in Fig. 1.

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-size equipment, flexible tubing and tube clamps may be used. All components (including any flexible tubing) must be connected such that there are no places where solids or air can be trapped.

Detection of surviving spores

The TSB is introduced into the test equipment by means of a peristaltic pump. To avoid anaerobic conditions, the broth is circulated for 2 hours every day. 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 vessel during the 2-hour circulation period each day. The test circuit is kept at ambient temperature (~20–25°C) for at least 5 days and, if applicable, the equipment is operated carefully ten times during this period (e.g. valves may be actuated and pumps operated manually). If the ambient temperature fluctuates outside the stated limits, care should be taken that the growth of any surviving spores of *B. subtilis* Bac 1-12 is not adversely affected.

Interpretation of results

If the broth remains clear after 5 days the equipment is classified as in-line steam sterilizable. If the broth becomes turbid, a sample is taken and examined microscopically for the presence of *B. subtilis* Bac 1-12. If there is any doubt as to the identity of the microorganisms a further sample of the broth is pour-plated with TSA and incubated at 37°C for 3–5 days. Colonies of sporulated *B. subtilis* Bac 1-12 are greyish-green.

Discussion

Although the test strain grows readily in TSB at 25°C (turbidity is observed within 24 hours) a relatively long incubation time has been chosen (5 days). The reason for this is that any surviving spores that have been heat damaged will need time to recover. In addition, surviving spores may be trapped or hidden between equipment parts, in which case they have to grow out by multiplication before they reach the circulating broth.

Sterilization at 120°C for 30 minutes is sufficient to kill the spores of *B. subtilis* Bac 1-12 in the concentrations applied, provided there are no cold spots in the system. Turbidity of the broth is, therefore, indicative of the presence of cold spots within the equipment.

Tests should be conducted a minimum of three times, assuming that the three results are the same. If varying results are still obtained after a maximum of five tests 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 obvious faults are discovered and the results still remain variable, it can be concluded that the item of test equipment is unlikely to be suitable for in-line steam sterilization unless modifications to the equipment are made or special measures are taken to avoid such problems in practice.

Equipment that proves not to be sterilizable by the described method may be sterilizable at higher temperatures and/or longer sterilization times. If supporting experimental evidence is available, such equipment may be classified as 'steam sterilizable in-line only with extended sterilization times and/or temperatures higher than 120°C', specifying the applicable times and temperatures.

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, Secretary of the EHEDG, Campden Food and Drink Research Association (CFDRA), Chipping Campden, UK GL55 6LD (tel. +44-386-840319; fax +44-386-841306).

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

References

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3 *A Method for Assessing the In-place Cleanability of Food-processing Equipment* (1992) in *Trends Food Sci. Technol.* 3, pp. 325–328

4 *Microbiologically Safe Aseptic Packing of Food Products* (1993) in *Trends Food Sci. Technol.* 4, pp. 23–25

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