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

Food-processing equipment that cannot be or does not need to be sterilized may need to be pasteurized, and it is important to test the hygienic characteristics of such equipment to ensure that it can be pasteurized effectively. Here the procedures recommended by the Test Methods subgroup of the European Hygienic Equipment Design Group (EHEDG) are summarized. This paper is the fourth 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.*

Food-processing equipment may need to be pasteurized or sterilized (see Definitions); it would, therefore, be useful for equipment to meet both requirements. However, easy-to-clean equipment may contain construction materials that cannot withstand treatment with steam at 120°C because of insufficient resistance to either the temperature or the pressure required. Such equipment may nevertheless be very suitable for hygienic processing in applications where sterility is not essential, for example in the production of pasteurized products or of milk products such as yoghurt or buttermilk that prevent the growth of sporeforming bacteria as a result of fermentation by lactic acid bacteria. Alternatively, it may be desirable to avoid unnecessary sterilization (e.g. to save energy); however, sterilizable equipment is not necessarily also pasteurizable. Therefore, a method has been developed to determine whether such equipment can be pasteurized by circulation with hot water to reduce the number of relevant vegetative microorganisms and fungal spores to below detection levels.

*Readers requiring further information on the EHEDG are referred to Ref. 1. Details of previously published EHEDG articles are given in Refs 2–4.

Required materials

Indicator microorganisms

The survival rate of heat-resistant ascospores of the fungus *Neosartorya fischeri* var. *glabra* (CBS 11155) is used as an indicator of the efficacy of the test pasteurization procedure.

Before *N. fischeri* was accepted as a suitable test strain, several heat-resistance experiments were carried out with different spore suspensions. Rates of destruction of the spores at various temperatures (measured as 'D-values') are given in Table 1. As can be seen, there is a range in the measured D-values at certain temperatures. For this reason, it is advisable to check the heat resistance of stored spore suspensions regularly. A suitable procedure is to pasteurize a sample of the suspension at 80°C for 30 minutes just before use. If, during this time, the level of viable spores decreases by less than a factor of ten, the spores can be used for the pasteurization experiments.

Definitions*

Hygienic equipment Class I: Equipment that can be cleaned in-place and freed from relevant microorganisms without dismantling.

Hygienic equipment Class II: Equipment that is cleanable after dismantling and that can be freed from relevant microorganisms by steam **sterilization** or **pasteurization** after reassembly.

Pasteurization: Thermal destruction of vegetative microorganisms, excluding thermoresistant bacterial spores. Clean equipment is **pasteurizable** if it can be freed from such microorganisms by treatment with hot potable water of up to 95°C for 20 minutes; alternative conditions can be used depending on local circumstances. (In the dairy industry, usually refers to the destruction of pathogenic and some spoilage microorganisms.)

Sterilization: Removal or destruction of microorganisms, including all relevant bacterial spores. Clean equipment is steam/hot-water **sterilizable** if it can be freed from relevant microorganisms by treatment with saturated steam/water at 120°C for 30 minutes (alternative conditions can be used depending on local circumstances).

* These definitions have been drawn up by the EHEDG in an attempt to prevent confusion regarding terminology relevant to hygienic processing.

Table 1. Rate of destruction of *Neosartorya fischeri* ascospores at various temperatures

Temperature (°C)	D-value (minutes)[a]
80	>30
83	4–6
85	1.8–4.3
87	0.3–1.5
90	<0.2

[a] Time to reduction of the count of colony-forming units by a factor of 10

The fungus is cultivated on oatmeal agar ('Difco 0052', Difco Laboratories, Detroit, MI, USA) at 37°C for ~14 days. The culture is examined microscopically for the presence of the characteristic ascospores, then the surfaces of the agar plates are scraped with a sterile spatula and the biomass is suspended in physiological saline. The main part of the mycelium is removed by filtration, and the suspension of spores is pasteurized at 80–81°C for 10 minutes. Pasteurized spore suspensions are cooled then stored at –18°C. In this way, only the relatively heat-resistant ascospores survive. These may be kept for a year without losing relevant characteristics.

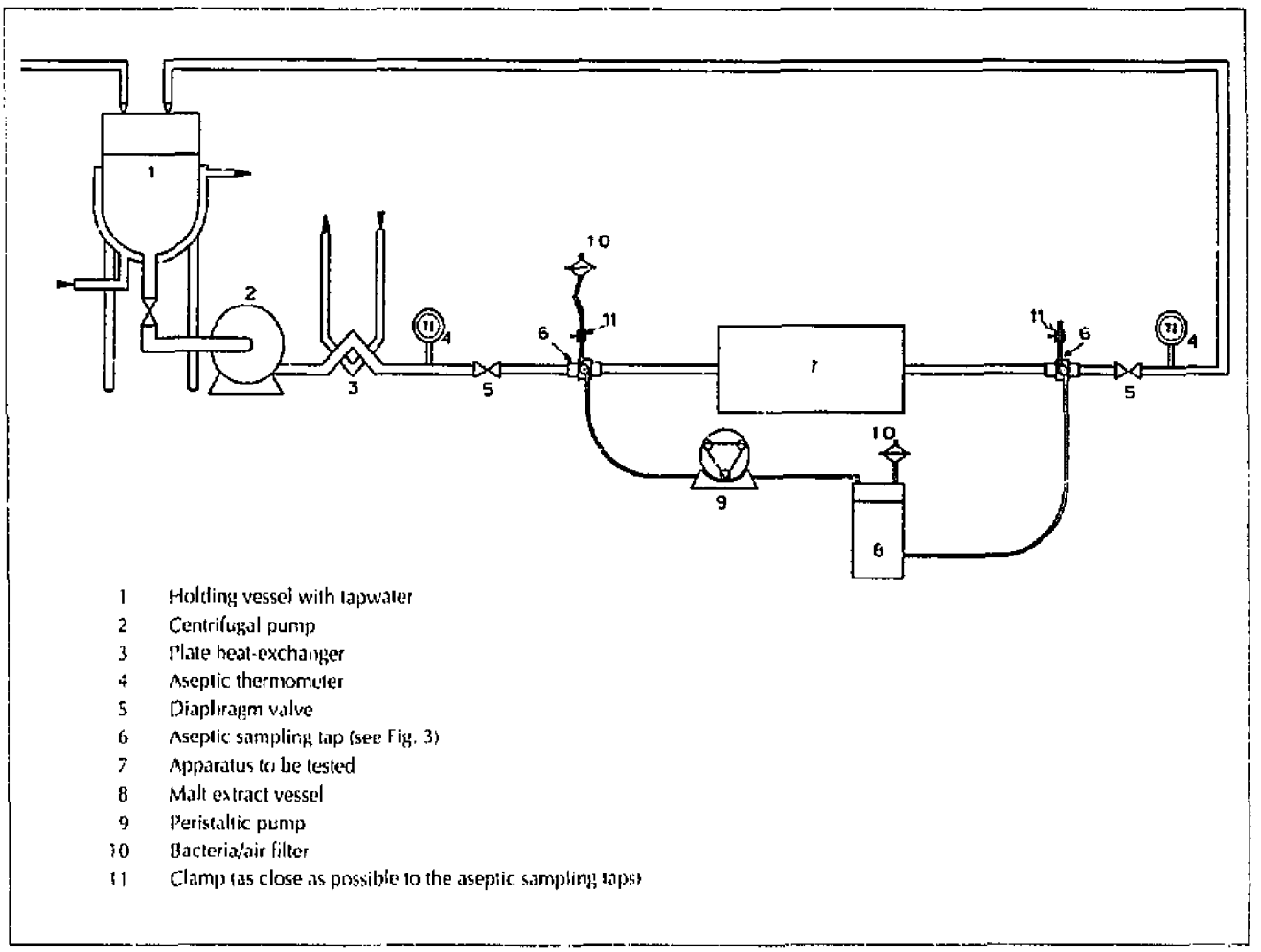
Malt extract

Malt extract ('Oxoid L39', Oxoid Ltd, Basingstoke, UK) is flushed through the test apparatus to provide a growth medium for any indicator ascospores remaining on the equipment after the pasteurization test procedure. The antibiotic oxytetracycline (Gist Brocades NV, Delft, The Netherlands) is added to prevent the growth of bacteria, which may interfere with detection of the indicator microorganism; the ascospores themselves are resistant to oxytetracycline.

As part of the test circuit (between the two diaphragm valves; see Fig. 1 and 'Pasteurization procedure', below) contains a certain quantity of water, which cannot be drained easily, care must be taken to ensure that the malt extract vessel contains a quantity of double-strength medium to compensate for the water present in the circuit. In this way, the desired end concentrations of malt extract solution (20 g/l) and oxytetracycline solution (100 mg/l) are obtained by mixing the contents of the circuit and the malt extract vessel (via the peristaltic pump; see below).

Test equipment

Prior to testing, the equipment to be investigated is dismantled and thoroughly cleaned, degreased and descaled. The dismantled equipment (if relatively small) should then be sterilized in an autoclave at 120°C for 30 minutes or, alternatively, the equipment can be reassembled and sterilized in-line by steam for 30 minutes. If the construction materials of the test item are not compatible with autoclaving, chemical decontamination should be undertaken using a suitable biocide (e.g. 1000 ppm hypochlorite for 20 minutes, followed by rinsing with sterile distilled water).



1 Holding vessel with tapwater
2 Centrifugal pump
3 Plate heat-exchanger
4 Aseptic thermometer
5 Diaphragm valve
6 Aseptic sampling tap (see Fig. 3)
7 Apparatus to be tested
8 Malt extract vessel
9 Peristaltic pump
10 Bacteria/air filter
11 Clamp (as close as possible to the aseptic sampling taps)

Fig. 1
Test circuit for pasteurizability test.

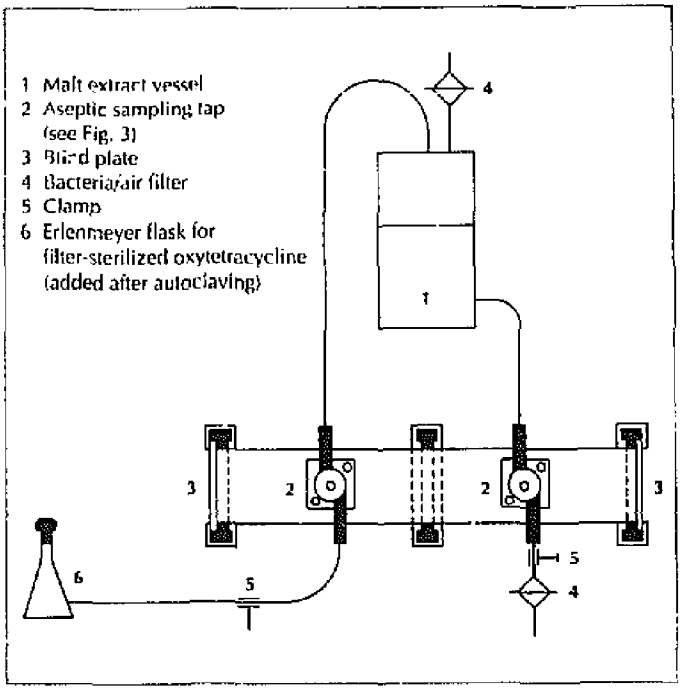


Fig. 2

Malt extract vessel arrangement during autoclaving.

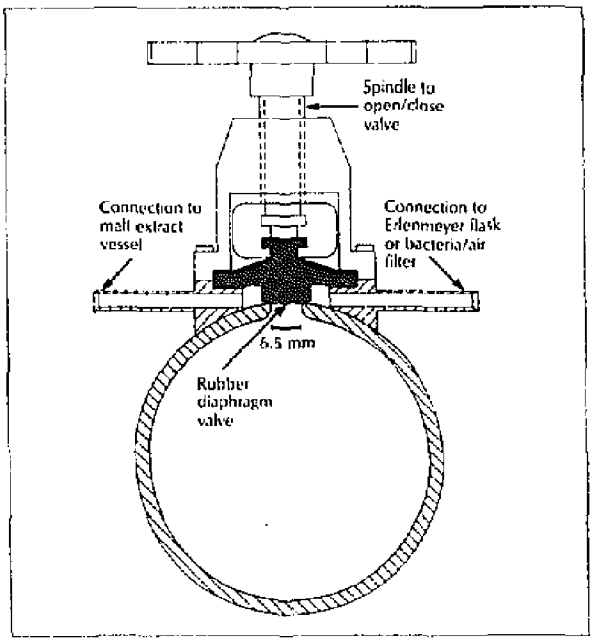


Fig. 3

Aseptic sampling tap on a short pipe, shown in cross section. A rubber diaphragm valve (black), constructed integrally with the sampling tube connecting the malt extract vessel and the Erlenmeyer flask or bacteria/air filter (see Fig. 2), is controlled by the spindle, which opens/closes a 6.5mm diameter hole in the pipe, releasing sample into the sampling tube. The housing is of stainless steel. Such a tap, also incorporating two additional valves (not shown) to permit decontamination of the valve chamber and sampling tube, is commercially available ('Type 55' from Het Stempel BV, Zwijndrecht, The Netherlands).

Test procedure

Equipment soiling

The spore suspension is diluted with physiological saline to a concentration of ~5 × 10^7 spores/ml. The inner surface of the apparatus to be investigated, including all parts in contact with each other after reassembly (e.g. gaskets and gasket grooves), is wetted with the diluted spore suspension using a small brush (autoclaved at 120°C for 30 minutes before use). The equipment is allowed to dry at ~20°C and a relative humidity of 55–65% for two hours, after which it is reassembled. A sample of the diluted spore suspension is taken to determine the spore concentration by plating on malt agar and incubating at 37°C for 2 days.

Pasteurization procedure

An example of a circuit for carrying out the pasteurization test is shown in Fig. 1.

An aseptic vessel containing double-strength malt extract is sterilized in an autoclave at 120°C for 30 minutes, using the arrangement shown in Fig. 2. This vessel is connected to the test circuit via two aseptic sampling taps (see Fig. 3) on short pieces of pipe. To avoid the growth of spore-forming bacteria that may still be present after the pasteurization procedure, filter-sterilized oxytetracycline is added by means of the Erlenmeyer flask (see Fig. 2) to a concentration of 200 mg/l after sterilization of the malt extract and cooling to ambient temperature. Subsequently, the aseptic sampling taps are closed.

The contaminated apparatus is mounted in the test circuit as shown in Fig. 1. When the circulation system is complete, tap water is circulated via a centrifugal pump and a plate heat exchanger. The temperature is measured at the outlet of the tested equipment and is kept at 90 ± 0.5°C. The water is circulated at a rate of 25 l/min for 30 minutes via a holding tank with a capacity of 100 l.

After this pasteurization treatment, the two diaphragm valves are closed. The system is quickly cooled to ambient temperature by flushing the outside with cold water, and both clamps are closed and both aseptic sampling taps are opened. Then malt extract (single strength) is circulated through the apparatus by means of a peristaltic pump. In the meantime, a sample is taken from the holding vessel and cooled quickly. To determine the level of surviving ascospores in the circulation water, samples of this water are plated onto malt agar containing oxytetracycline and incubated at 37°C for 3–4 days.

Detection of surviving ascospores

As *N. fischeri is* aerobic, the malt extract is circulated through the equipment continuously. To maintain sufficiently aerobic conditions in the test circuit, the flow rate, f (the capacity of the peristaltic pump) must obey the equation $f = c \times V$, where f is in ml/min, c is 0.02 min^{-1} and V is the volume of culture broth (in ml).

The malt extract is circulated and the test arrangement is kept at a temperature of 23–25°C for at least 14 days, because germination and growth of the mould are rather

slow. Where applicable, the equipment is operated several times during the incubation period.

If ascospores are present in the equipment after the pasteurization treatment, the circulated malt extract first becomes slightly turbid, then excessive growth of white mycelium occurs on the surface of the medium in the vessel and on the vessel wall. If there is any doubt about the identity of this growing organism, plating a sample onto oatmeal agar or malt agar is an easy way to confirm the presence of *N. fischeri*.

Three test replicates are undertaken. If no mould growth is observed in any of the three tests, it is concluded that the equipment can be pasteurized with hot water of 90°C within 30 minutes.

Discussion and conclusions

It should be emphasized that sterilizable equipment is not necessarily also pasteurizable. In the case of hot-water decontamination, the influence of 'cold spots' is much greater than with steam sterilization; with steam, heat transfer is much better. Thus, the presence of 'dead legs' will influence the choice of decontamination procedure. If decontamination is done with hot water, then if air is entrapped (e.g. in dead legs pointing upwards), a significant surface area might be exposed to a relatively low water activity, and the temperature might be significantly lower than intended. In the case of a dead leg pointing downwards, there is no risk that air will be entrapped. However, in the case of steam sterilization, condensate may become trapped, while if using hot water, the water in the dead leg will become stagnant; in either case the heat treatment may prove insufficient. The ability to test whether or not a particular piece of equipment can be efficiently decontaminated by pasteurization is thus useful for determining whether more aggressive decontamination methods or redesign of the equipment are required.

While the test method described here has been shown to be reproducible, workers new to the required techniques may require a degree of familiarization! Feedback to the authors (see general address below) is most welcome. The authors may also be contacted in case of difficulty in selecting suitable equipment.

This paper summarizes the guidelines and methods recommended by the European Hygienic Equipment Design Group (EHEDG) subgroup on Test Methods. The full report, by B.M. Venema-Keur, J. Axis, A. Grasshoff, C.R. Hodge, J.T. Holah, R. Kirby, J-F. Maingonnat, C. Trägårdh 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 will certify laboratories that intend to apply the EHEDG test methods. 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