Code of Practice

Testing Hygienic Filling Machines of VDMA Class V (Aseptic Filling Machines):
External Sterilization of Packaging Materials

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This code of practice is the English translation of a publication drawn up by the VDMA Working Party for “Interface Problems in Aseptic Plants”.

Suggestions concerning the contents of the code of practice may be sent to the Verband Deutscher Maschinen- und Anlagenbau e.V. (VDMA), Fachabteilung Verpackungsmaschinen, Lyoner Straße 18, 60528 Frankfurt/M. (Fax: 0069/6603-1211); nuv@vdma.org.
A list of publications of the working party on the subject of near-sterile and aseptic filling can be requested from the above address. This list and all publications are also available for downloading from the internet free of charge. (WWW.VDMA.ORG/PACKTECH, heading: Technik-Aseptik).
1. Introduction

In some filling machines of VDMA category V it is a requirement of the system that, in the case of unsterile packaging materials introduced into the interior of the machine (usually bottles or tubs as well as closures), even the outer surfaces not in contact with the product have to be wholly or partially sterilized. The purpose of this measure is to reduce the microbial load borne by the packaging materials to be filled. The requirements on the sterilization performance of this equipment is usually lower than those in sterilizing equipment for sterilizing areas of the packaging material in contact with product and are specified as a function of the system.

The subject matter of this code of practice is the checking of the degree of effectiveness of such devices for exterior sterilization of packaging materials. This involves what is known as a "count reduction test" which includes the artificial inoculation of the exterior surfaces not in contact with product of the packaging materials to be filled.1

The starting point of the test is the successful completion of the operation for cleaning and sterilizing the machine. After the containers which have been artificially inoculated on the outside have been introduced into the filling machine, the disinfection program for the exterior sterilization of packaging materials is started. When it is finished the number of surviving microorganisms is determined.

The choice of test microorganism suitable for the sterilization process in question is particularly important. The selection criterion is the resistance of the microorganisms and their spores to the sterilizing medium. Accordingly, this code of practice specifies test microorganisms regarded as suitable for checking sterilization methods which had been introduced for class V machines (aseptic filling machines) at the time the code of practice went to press.

The sterilization performance of a class V machine depends on numerous machine parameters such as, inter alia, the concentration and temperature of the hydrogen peroxide, the moisture content and temperature of the steam and the duration of exposure to the sterilizing agent. These boundary conditions have to be specified prior to testing. The degree of effectiveness stated in the test report always relates to the boundary conditions established in advance. Accordingly, these must be recorded in the test report.

Extensive specialist knowledge is required for carrying out the test methods described here. The test methods should, therefore, be undertaken only by institutes, laboratories or machine manufacturers that are well acquainted with them.2 It is recommended that the manufacturer of the machine to be tested be involved. It should be noted at this point that as a general principle only specialist microbiology staff should be entrusted with the procurement, storage and handling of the highly concentrated microbiological suspensions of test microorganisms needed for the test methods.

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1 Other codes of practice for checking hygienic filling machines of VDMA class V are published in the same series on the subject of sterilizing packaging materials and sterilizing the sterile zone of the machine interior.

2 A reference list of independent institutes familiar with the test methods can be requested from the publisher of the code of practice.
2 Definition of terms

**Hygienic filling machines of VDMA class V (aseptic filling machines)**

Filling machines operating in aseptic manner (aseptic plants) are packaging machines which fill a sterile product (e.g. food) without recontamination into a sterile pack, the latter usually having been sterilized in the machine. To achieve this, high demands are imposed on the effectiveness of the devices for sterilizing the packaging materials, the interior of the machine and the parts conveying product (see VDMA Food Processing Machinery and Packaging Machinery Publications 2006/No. 11). Thus, in packaging sterilization a count reduction of test microorganisms suitable for the sterilization method in question of at least 4 logs is considered necessary.

Aseptic filling machines are typically employed in the filling of low acid products (pH ≥ 4.6) which should be shelf-stable without refrigeration for a relatively long period.

**Inoculation**

Artificial application of a germ carrier with test microorganisms.

**Sterile zone in the machine interior**

That region in the interior of an aseptic filling machine which after completion of sterilization must be kept free of germs in order to prevent recontamination of the sterile product during filling.

**Test microorganism**

Test microorganisms are used to check the performance of sterilization devices. They should exhibit a high and as far as possible defined resistance to the sterilizing method being investigated. The should also be easy to detect and present no hazard to health. The description of a test microorganism should contain the following characteristics: name, D value, precise designation of strain (ATTC No. or DSM No.) and batch number (in the case of ready-made spore suspensions).

3 Specification of test microorganisms for checking devices for external sterilization of packaging material

3.1 Sterilization by means of hydrogen peroxide

It is customary to use the spores of Bacillus subtilis SA 22 (identical to NCA 72-52 and DSMZ 4181)\(^3\) or other strains of Bacillus subtilis resistant to hydrogen peroxide comparable to Bacillus subtilis SA 22.\(^4\)

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\(^3\) Instructions for preparing the spore suspension may be found in Appendix I of this code of practice.

Sources of supply of ready-made suspensions of spores of this strain include:

- The National Food Laboratory, Inc.
  Process Research & Microbiology Division
  6363 Clark Avenue
  Dublin California 94568-3097
  Fax: 510-833-8795

- BAG - Biologische Analyse system GmbH
  Amtsgerichtstraße 1-5
  35423 Lich
  Fax: 06404/3087

- Merck KGaA
  Frankfurter Straße 250
  64283 Darmstadt
  Fax: 06151/722000

(Bacillus subtilis (BGA) spore suspension for the growth inhibitor test, Merck Art. No. 1.10649)

Since the method of preparing the suspension of the spores has an effect on their resistance characteristics the production specification or the source of supply of the spore suspension should be noted in the test report. A check on the resistance of the spores to the sterilizing agent to be investigated is recommended.
3.2 Sterilization by means of steam and dry heat

It is customary to use spores of the strain Bacillus stearothermophilus NCA 1518 (identical to DSM 5934)\(^4\). Clostridium sporogenes PA (SC-218) is also employed for sterilization by means of steam.

3.3 Sterilization by means of peracetic acid

It is customary to use the spores of Bacillus subtilis SA 22 (identical to NCA 72-52 and DSMZ 4181)\(^5\) or other strains of Bacillus subtilis resistant to hydrogen peroxide comparable to Bacillus subtilis SA 22.

3.4 Media for suspending spores

Ethanolic solution (70 %) or distilled water\(^7\). Other additives are to be specified in the test report.

4. Test method

4.1 General procedure

In this test method the exterior surfaces of the packaging materials to be sterilized are inoculated outside the filling machine with a suspension of spores of the test microorganism and loaded into the machine. After passing through the external sterilization section the packaging materials are removed, the area to be checked is subjected to microbiological analysis and the mean logarithmic count reduction rate is determined. This is referred to as a count reduction test.

4.2 Test method

i) Prior to testing it has to be established jointly with the manufacturer of the machine which points on the outer surfaces of the packaging are important for exterior sterilization and where these points are to be inoculated.

ii) Supply of at least 25 packaging units which for the test have been inoculated under the test conditions described above.

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\(^4\) Wunderlich et al. (2006) are describing a test procedure to check the resistance of spore suspensions to hydrogen peroxide. Spore suspensions tested for resistance to hydrogen peroxide can be obtained from the IVV in Freising:
Fraunhofer Institut für Verfahrenstechnik und Verpackung IVV
Giggenhauser Str. 35
D-85354 Freising
Fax: (+49 8161) 491-666

\(^5\) Sources of supply of ready-made suspensions of spores include:
The National Food Laboratory, Inc.
Process Research & Microbiology Division
6363 Clark Avenue
Dublin California 94568-3097
Fax: 510-833-8795

BAG - Biologische Analysensystem GmbH
Amtsgerichtstraße 1-5
35423 Lich
Fax: 06404/3087

Since the resistance of the spores to moist heat may vary from batch to batch the D\(_{121}\) value and the method of calculating it should be stated in the test report. (For description of biological indicators of resistance to moist heat see EN ISO 14161.)

\(^6\) For sources of supply and instructions to prepare spore suspensions see the foot notes in paragraph 3.1.

\(^7\) The test microorganisms should as far as possible be applied in distilled water or 70 % alcohol since use of buffers or common salt solution may give rise to high salt concentrations in the course of drying and any protective layers formed as a result may give a false indication of killing rates.
same conditions by the spraying method. Of these, 5 units are needed for determining the amount of spores applied.
In supplying these units care has to be taken that the dried microorganism suspension is not rubbed off during transport. In order to take account of the possibility of abrasion during transport, the containers for determining the amount of spores applied should be selected after the containers have arrived at the filling machine.

iii) Introduction of the inoculated containers into the filling machine. If the machine contains more than one sterilization section for exterior sterilization, the packaging units introduced are to be uniformly distributed over the sterilization sections. However, at least 2 packaging units should pass through each sterilization section.

iv) Sterilization of the packaging under production conditions.

v) Sterile removal of the sterilized containers.

vi) Rinsing of the outside of the sterilized containers with common salt/peptone or Ringer’s solution (as soon as possible after removal).

vii) Determination of the final microbial count. In cases of growth, test for possible contamination with extraneous microorganisms.

viii) Calculation of the mean logarithmic count reduction rate in accordance with VDMA (2002), Section 5.2, for each disinfection section.

5. Test report

Information to be recorded in the test report with reference to this code of practice includes:

- Name of institute conducting the test
- Brief description of the aseptic plant tested (manufacturer, exact model name, type of packaging sterilization device, type of exterior packaging sterilization, number of sterilization sections)
- Settings for the machine parameters relevant to sterilization in agreement with the machine manufacturer
- Establishing the parts of the outer surfaces of the packaging materials of importance for exterior sterilization
- Required count reduction performance of the aseptic plant (if not otherwise agreed: a mean logarithmic count reduction of ≥ 3)
- Nature and concentration of the sterilizing agent
- Precise name of the test microorganism; statement of values from resistance testing, if available
- Description of spore suspension (concentration, production specification or source of supply)
- Documentation of the distribution of the spore suspension on the inoculated containers
- Total number of inoculated containers placed in the filling machine
- Documentation of the sequence of operations for the exterior sterilization of packaging materials
- Statement of test results (mean logarithmic count reduction achieved for each sterilization section investigated)
- If need be, any deviations from the test specification
- Date of test runs
- Test staff involved

8 At variance with this specification the drip method may be agreed. With regard to the application methods see VDMA (2002), sections 4.1 and 4.2.
9 A review of the critical process parameters prior to running the test is recommended (e.g. concentration of the hydrogen peroxide, temperatures)
10 Since this necessarily requires intervention in the sterile region of the filling machine, this is a critical step in carrying out the test. Accordingly, the possibility of contamination with extraneous microorganisms must, as a rule, be considered in the course of evaluation.
6. **Use of the code of practice for checking nonaseptic filling machines**

The test specifications set out in this code of practice may also be applied with appropriate modifications for checking sterilization equipment in hygienic filling machines that do not meet the strict requirements of class V machines.\(^\text{11}\) In doing so the test microorganism has to be matched to the sterilization method and, if need be, to the field of application.

7. **References**

D.T. Bernard et al.: Validation of Aseptic Processing and Packaging, Food Technology, December 1990, pp. 119-122

VDMA FV NuV, Frankfurt 2000
VDMA Food Processing Machinery and Packaging Machinery Publications 2000/No. 3
Checklist "Quality Assurance and Maintenance" for aseptic filling machines for the food industry, obtainable as downloadable file from www.vdma.org/packtech

VDMA FV NuV, Frankfurt 2000
VDMA Food Processing Machinery and Packaging Machinery Publications 2000/No. 2 (2\(^{\text{nd}}\) edition 2006)
Hygienic Filling Machines for Liquid and Viscous Foods - Classification and Typical Fields of Application, obtainable as downloadable file from www.vdma.org/packtech

VDMA FV NuV, Frankfurt 2002
VDMA Food Processing Machinery and Packaging Machinery Publications 2002/No. 6
Code of Practice - Testing the Effectiveness of Aseptic Plants Fitted with Packaging Sterilization Devices, obtainable as downloadable file from www.vdma.org/packtech

VDMA FV NuV, Frankfurt 2006
VDMA Food Processing Machinery and Packaging Machinery Publications 2006/No. 11
Aseptic Packaging Machines for the Food Industry - Minimum Requirements and Basic Conditions for Intended Operation, obtainable as downloadable file from www.vdma.org/packtech

Substitutes VDMA 8742

In: Verpackungsrandschau Nr. 2/2006, TWB, S. 57-60

8. **Selected standards**

EN ISO 14161
Sterilization of health care products - Biological indicators - Guidance for the selection, use and interpretation of results (ISO 14161:2000)

EN 1650
Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 2)

DIN 55405, Part 3
Packaging; terminology; packages (in German)

\(^\text{11}\) A classification of hygienic filling machines for the food industry is presented in the VDMA publication “Hygienic Filling Machines for Liquid and Viscous Foods – Classification and Typical Fields of Application” (VDMA FV NuV 2000, 2\(^{\text{nd}}\) ed. 2006)
Appendix I
Culture conditions for the test strain Bacillus subtilis SA 22 and preparation of the spore suspension

Bacillus subtilis SA 22 is first of all precultured in tryptone soya broth for 24 hours at 30 °C before samples of 0.1 ml each of the preculture are transferred by means of Drigalski spatula onto plate count agar and incubated for 5 days at 30 °C.

The Petri dishes in which the test microorganism has grown are each flooded with 3 ml of sterile 0.9 % saline solution after which the bacterial spores are scraped off carefully using a Drigalski spatula.

The spores harvested from several Petri dishes are combined and centrifuged at 10,000 g for approximately 20 minutes. The supernatant liquid is then decanted off and the sedimented spores are resuspended in sterile 0.14 M Sørensen phosphate buffer (K$_2$HPO$_4$/KH$_2$PO$_4$, pH 7). After this centrifuging and resuspension are carried out twice in the same way until finally the spore sediment is finely suspended in sterile phosphate buffer. The spore suspension obtained in this way is heated for 20 minutes at 80 °C in order to kill off vegetative unspored cells (pasteurization). The resultant spore suspension may be kept at 4 °C for about 3 months. The spore count should range from $10^8$ to $10^9$/ml.