

Report number 130598

Validation of the in-place cleanability of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H according to the EHEDG test procedure.

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SUMMARY

At the request of K-PATENTS, Elannontie 5, Fin-01510 Vantaa, Finland the in-place cleanability of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H was assessed according to the test procedure of the European Hygienic Equipment Design Group (EHEDG) [ref 1].

The test results show that K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H including the Sanitary Gasket 2.5", is cleanable in-place at least as well as the reference pipe. The tests were conducted three times on one test object of one type. The results of the tests were comparable with each other.

The results obtained are representative of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H in the size range of 1.5-2.5".

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1 INTRODUCTION

At the request of K-PATENTS, Elannontie 5, Fin-01510 Vantaa, Finland the in-place cleanability of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H was assessed according to the test procedure of the European Hygienic Equipment Design Group (EHEDG) [ref. 1].

2 DESCRIPTION OF THE TEST OBJECT

Name of test object	: K-PATENTS Sanitary Refractometer and Flow Cell
Type	: PR-03-A62-HSS (sensor) and EFC-15-SI-H (cell)
Diameter of the inlet port	: 1.5"
Materials of construction	: Stainless steel
Type of Seal	: Sanitary Gasket 2.5"
Material of Seal	: EPDM
BI numbers	: 130598

3 TIME SCHEDULE

The test objects arrived at the Biotechnological Institute, Holbergsvej 10, 6000 Kolding in May 1998 and were registered under BI number 130598. The investigation was carried out in May 1998.

4 MATERIALS and METHODS

Before conducting the test programme all elastomeric components have been checked against the test strain for antimicrobial properties. Prior to testing, the test object and the reference pipe (having a 0.5 μ Ra internal roughness) were dismantled, thoroughly cleaned and degreased by hand and steam-sterilized in-line or autoclaved at 121°C for 30 minutes.

The test object and reference pipe were reassembled with an auxiliary pipe at each end and soiled under 5 bar (gauge) pressure with a soured milk solution with spores of the test strain *Bacillus stearothermophilus* var. *calidolactis* (NIZO C953), mixed to give a final concentration of approx. 10^5 spores per cm^3 in the milk. The air pressure of 5 bar was applied 3 times to the closed assembly and held at pressure for 2 minutes on each occasion. Whilst under pressure, any movable parts of the test object were operated a total of ten times. After draining and drying by flushing with dry filtered air at a velocity of 1.0 m/s (for 2 to 4 hours) until an exterior relative humidity of $\leq 0.5\% \pm 0.3\%$ was achieved, the test object was cleaned in-place in an in-place cleaning test rig (see Appendix B) by:

1. Rinsing with cold water for 1 minute
2. Circulating a 1% (w/v) detergent solution at $63^\circ\text{C} \pm 2^\circ\text{C}$ for 10 minutes
3. Rinsing with cold water for 1 minute

For stages 1, 2 and 3 the mean velocity of flow in the reference pipe was 1.5 m/s. At the end of both rinsing procedures samples of the outflowing water were taken and two 5 ml portions of each were pour-plated with modified Shapton and Hindes agar (MSHA).

After cleaning, the inner surface of the test object and reference pipe were covered with molten MSHA. After the agar had fully solidified the test object and reference pipe were placed in an incubator at 58°C for 24 hours.

After incubation the test object and reference pipe were examined for the presence of yellow discoloration in the agar. The degree of discoloration in the agar taken from the test object was compared to the degree of discoloration in the agar taken from the reference pipe.

A detailed description of the test procedure including a drawing of the test rig is enclosed in Appendix B.

5 RESULTS

The test was conducted three times at one test object of one type. The results of the independent tests were comparable with each other. The gasket of the test object showed no antimicrobial properties. In Table 1 the average yellow discoloration of the test object and reference pipe is summarized.

Table 1 Survey of the test results of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H

Test object	Average discoloration (%)
K-PATENTS Sanitary Refractometer and Flow Cell	
1 Refractometer	0
2 Flow Cell	0
3 Gasket	0
Reference pipe	5

The test results show that K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H including the Sanitary Gasket 2.5" is cleanable in-place at least as well as the straight reference pipe.

6 CONCLUSIONS

The test results show that K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H including the Sanitary Gasket 2.5" is cleanable in-place at least as well as the reference pipe.

The results obtained are representative of K-PATENTS Sanitary Refractometer PR-03-A62-HSS and Flow Cell EFC-15-SI-H in the size range of 1.5-2.5".

7 RECORDS

The test objects are stored under BI number 130598.

Original data sheets, protocols and the final report will be filed in the archives of BI for 5 years after completion of the study.

8 REFERENCES

- 1 A method for the assessment of in-place cleanability of food processing equipment, European Hygienic Equipment Design Group, Doc. 2, December 1992.

9 AUTHENTICATION

We, the undersigned, herewith declare that the tests reported here were carried out according to the agreed protocols, that this report contains an accurate description of the results obtained and that the results relate only to the tested objects.

Date: May 1998

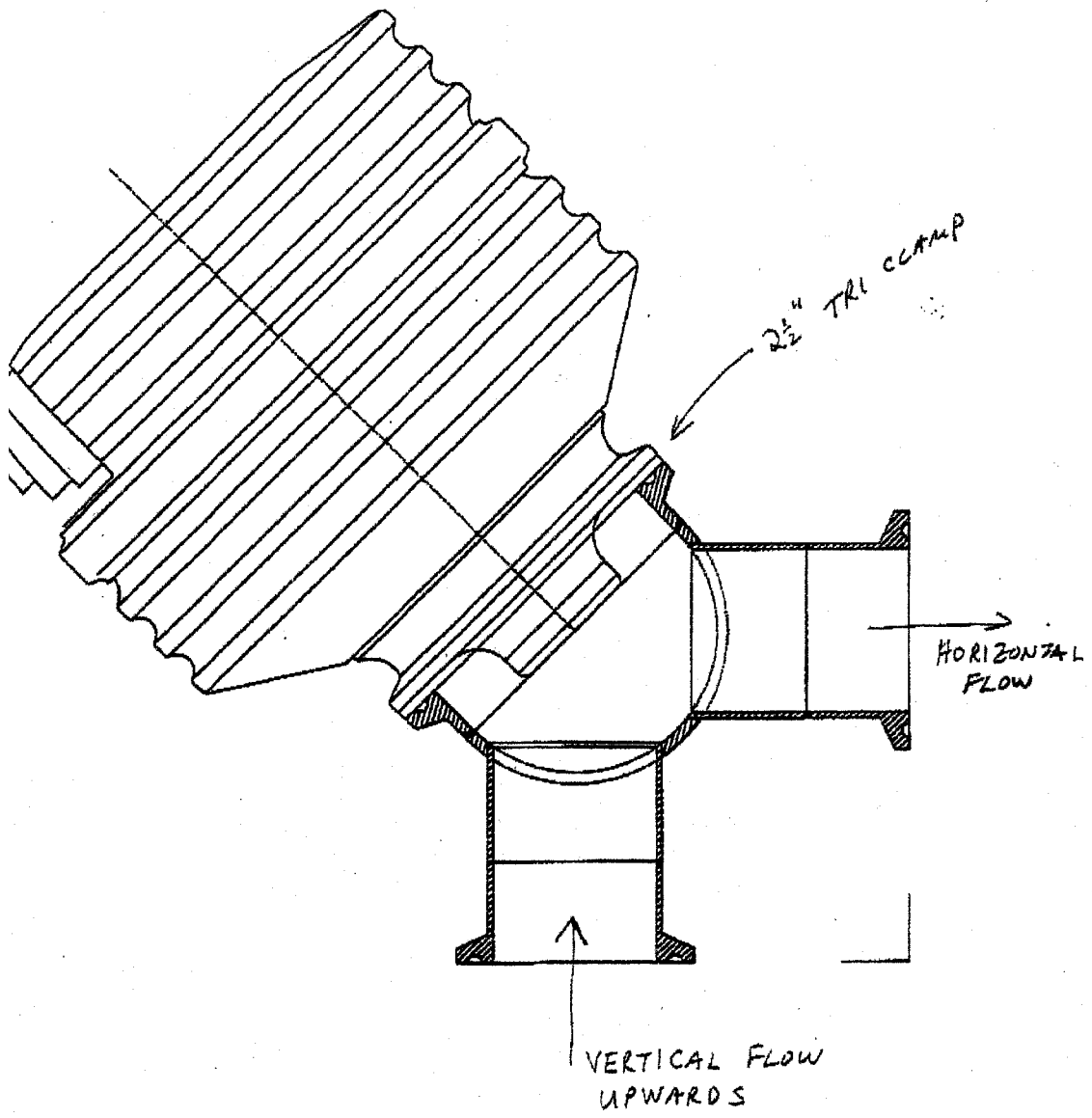
MICHAEL DAHL

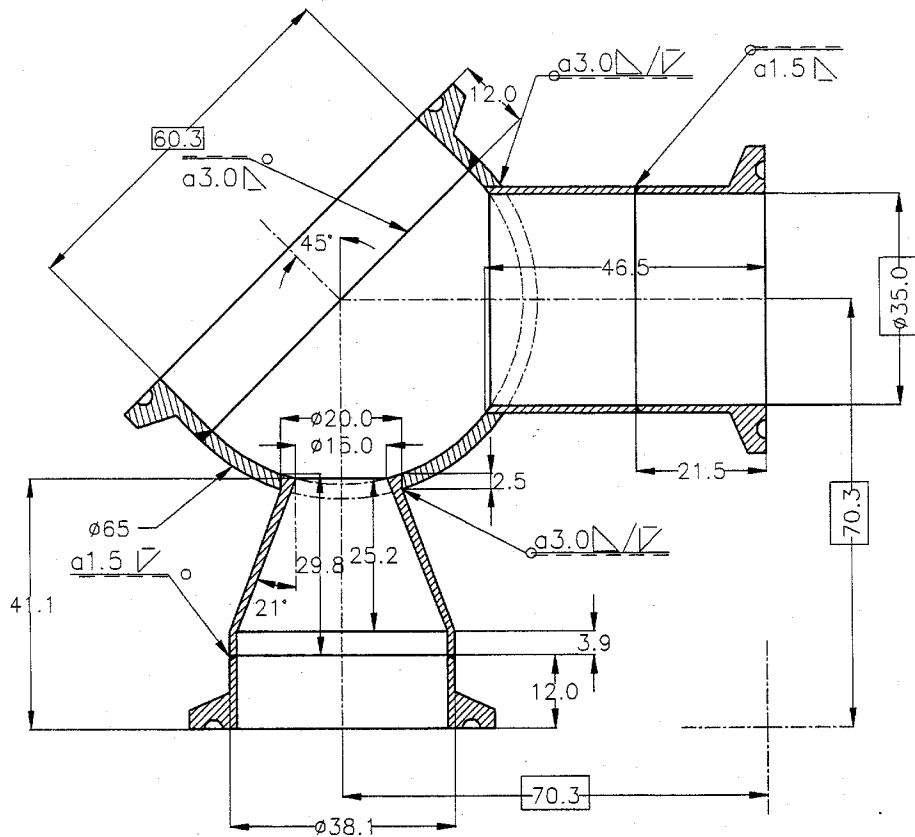
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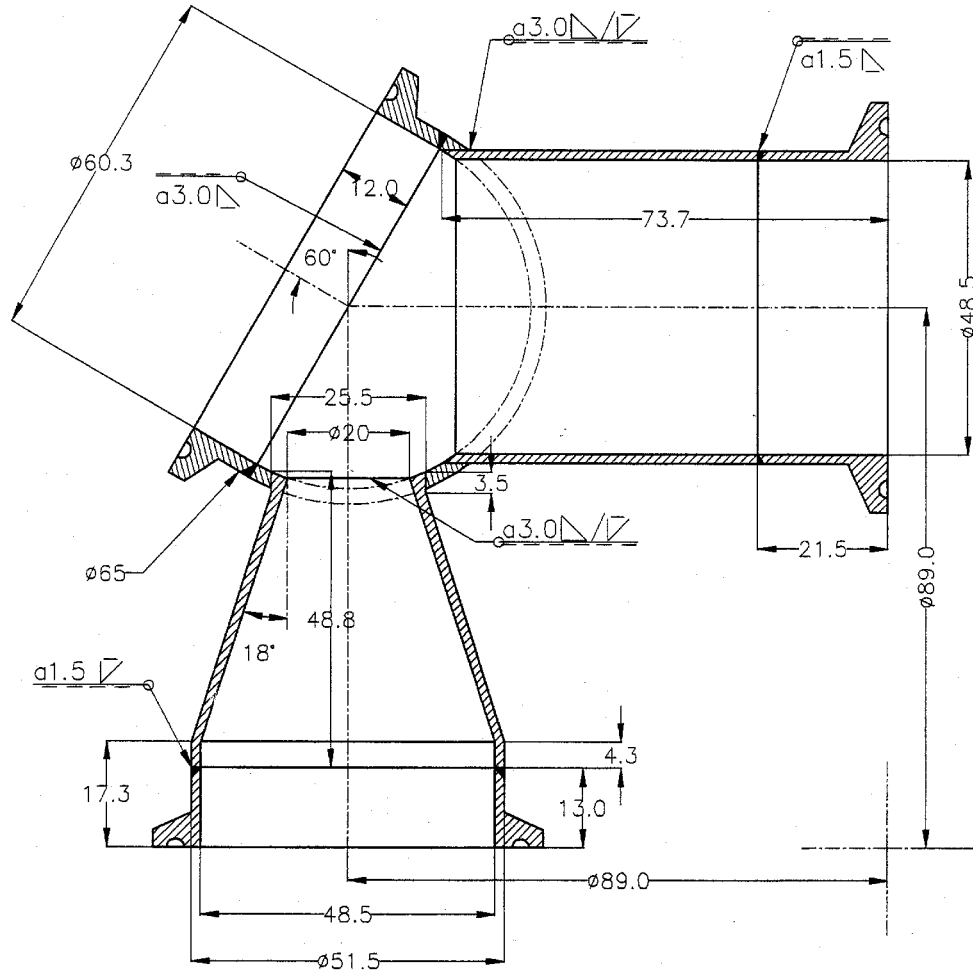
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APPENDIX A Detailed information on K-PATENTS Sanitary Refractometer and Flow Cell

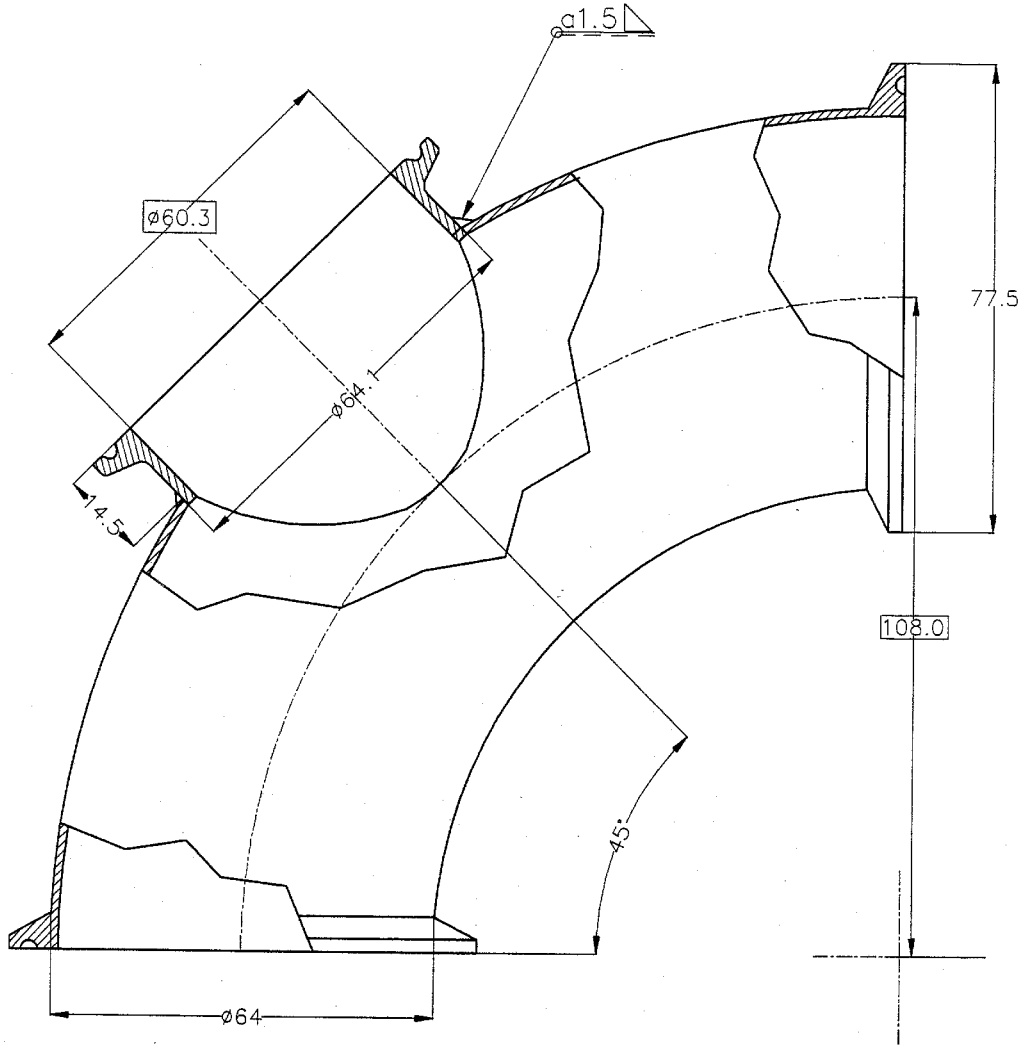




Itemref	Quantity	Title/Name, designation, material, dimension etc			Article No./Reference	
	1	1 kpl 2.5", 2 kpl 1.5" hitsausrennags			6204	
Designed by		Checked by	Approved by - date	Filename	Date	Scale
HS					31.3.1998	1:1
Owner				Title/Name		
Janesko Oy				EFC-15-RI-H		
				Drawing number	Edition	Sheet
				222		



Item ref	Quantity	Title/Name, designation, material, dimension etc			Article No./Reference	
	1	3 kpl 2.5" hitsausrengas			6205	
Designed by	Checked by	Approved by - date	Filename	Date	Scale	
HS				31.3.1998.	1:1	
Owner			Title/Name			
Janesko Oy			EFC-20-RI-H			
			Drawing number	Edition	Sheet	
			223			



enref	Quantity	Title/Name, designation, material, dimension etc			Article No./Reference	
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signed by	Checked by	Approved by - date	Filename	Date	Scale	
HS				20.1.1998	1:1	
war			Title/Name			
Janesko Oy			EFC-25-BI-H			
			Drawing number	Edition	Sheet	
			224			

APPENDIX B EHEDG in-place cleanability test procedure

To facilitate the design, testing and maintenance of hygienic food-processing equipment, it is important to be able to assess the relative cleanability of various components of the equipment using standardized test procedures that have been developed from a sound scientific basis. This paper summarizes the procedures recommended by the Test Methods subgroup of the European Hygienic Equipment Design Group (EHEDG). This paper is the second 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.*

With the increased public awareness surrounding food hygiene, food manufacturers' desires to improve product safety, and impending European Community legislation on the hygienic design of food machinery¹, the European Hygienic Equipment Design Group (EHEDG) aims to address these issues from a scientifically and technologically sound basis. A number of countries have national standards and/or directives applicable to food machinery, but there are relatively few international standards; those that exist are predominantly dairy based²⁻⁹, are too general, and are based on 'experience' rather than scientific data. In the USA, a number of guidelines in the form of third-party approval schemes have been developed for the dairy industry (the 3-A standards) and for food service equipment (the National Sanitation Foundation; NSF). Unfortunately, however, the 3-A standards have no benchmark of cleanability (see Definitions) or test regimes to establish cleanability, and the NSF standards are not applicable to the hygienic design of general food processing equipment.

This article describes a standard test procedure for assessing cleanability that has been developed by the EHEDG subgroup on Test Methods. The procedure is based on an adaption¹⁰ of a method described by Galesloot *et al.*¹¹, and is designed to indicate areas of poor hygienic design of equipment in which product or microorganisms are protected from the cleaning process. It can also be used to compare the in-place cleanability of different equipment designs. The method is based on comparing (in the laboratory) the cleanability of a test item with that of a straight piece of pipe. The degree of cleanliness is based on the removal of a 'soured milk soil' containing bacteria, and is assessed by evaluating the number of bacteria remaining after

A method for assessing the in-place cleanability of food-processing equipment

cleaning with a mild detergent. The level of cleaning used is designed to leave some soil in the reference pipe to facilitate comparisons.

The test is intended, therefore, as a basic screening test for hygienic equipment design, and is not indicative of performance in industrial cleaning situations. However, the comparative performance of different pieces of equipment in the laboratory test is likely to be relative to performance in practice. Due to limitations of scale and/or design, the test methodology may be unsuitable for some equipment types. The EHEDG intends to address the performance of equipment and production lines in practice in future test procedures.

Required materials

Microorganisms

A thermophilic test strain is used in this technique as it allows the manipulation of test equipment in non-sterile conditions such that any contamination of the equipment with organisms other than the test strain is unlikely to develop at the thermophilic growth temperatures used in the test procedure. *Bacillus stearothermophilus* var. *calidolactis* (NIZO C953) was chosen as the test strain as it is fast growing, has spores that are resistant to the detergent solutions used in the test procedure, and produces well-defined colour reactions in the growth medium used.

Definitions*

Cleanability: Suitability to be freed from soil.

Comparative cleanability: Cleanability of equipment relative to a reference.

In-place cleanability: Suitability to be cleaned without dismantling.

Soil: Any undesired matter including product residues, whether or not containing undesired microorganisms.

Sterilization: Removal or destruction of microorganisms, including all relevant bacterial spores (those able to contaminate, multiply or survive in the product and harmful to the consumer or to product quality).

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

*Readers requiring further information on the EHEDG are referred to *Trends in Food Science & Technology*, Vol. 3(11), p. 277.

The strain is cultivated on nutrient agar containing 0.082 g/l MnSO₄. After 2–3 days' incubation at 58°C, the degree of sporulation is checked; if it is >10%, the spores are harvested into physiological saline. The suspension is washed twice in physiological saline by centrifugation at 4000g for 15 minutes and pasteurized in 7 ml portions at 95°C for 5 minutes. After cooling at 15–25°C, spores are stored at 5°C until required. It is preferable that the spore suspension is left for 1–2 weeks prior to use, and its concentration should be checked periodically by pour-plating with (modified) Shapton and Hindes agar¹² (SHA) and incubation for 24 hours at 58°C.

Soured milk soil

A 'soured milk soil' is prepared by adding a mesophilic acidified or soured milk starter culture to a suitable volume (dependent on the volume of the section of equipment being tested) of commercial sterile skim milk and incubating at 30°C for 24 hours. The starter culture is added as a commercial freeze-dried culture (e.g. Rhone-Poulenc's 'Culture MM100', or other MM series, consisting of *Streptococcus lactis* subsp. *lactis*, *S. lactis* subsp. *cremoris* and *S. lactis* var. *diacetylactis*, available from Marschall-Eurozyme UK, Stockport, UK) to a level of ~0.1 g/l. The starter culture should be maintained, sealed, at 4°C. A quantity of *B. stearothermophilus* spore suspension is then added to the milk to produce a final concentration of ~10⁵ spores per millilitre.

Detergent solution

A mild detergent solution of standardized formulation is required. The components should have no sporicidal effects at the concentrations used.

Test equipment

Prior to testing, the equipment to be investigated and the appropriate reference pipe section and ancillary fittings are 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 disinfection should be undertaken using a suitable biocide (e.g. 1000 ppm hypochlorite for 20 minutes), followed by rinsing with sterile distilled water.

Test procedure

Equipment soiling

The equipment to be tested is coupled to a reference section of pipe of known internal surface roughness (0.5 µm roughness average) according to ISO 468:1982 (Ref. 13), with a short auxiliary length of pipe at each end to form a test section (Fig. 1). The reference pipe, auxiliary pipe and inlet to the test item should all be of the same internal diameter. (In some cases, particularly with centrifugal pumps that are not self priming, the

diameter of the inlet port can be larger than the outlet port and, consequently, larger than the majority of piping used within a system. In such cases the reference pipe may be chosen according to the dimension of the outlet pipe, though this must be indicated in the test report.) Suitable couplings should be used (e.g. to ISO 2853:1976)⁴, and all internal joint surfaces should be flush. The test section is then filled with the soured milk soil and closed at either end. The test section should be pressurized three times to 5 bar (or to a higher pressure if required) using a closure plate with a suitable air union, and held at this pressure for 2 minutes on each occasion. While under pressure, any movable parts are operated to simulate in-use conditions (e.g. valves are opened and closed). A total of ten operations should be undertaken within the three periods of pressurization.

The soured milk is then drained and the test section dried by flushing with dry filtered air. This generally takes 2–4 hours, depending on the airflow through the test item. A sample of the drained milk is diluted to 10⁻², 10⁻³ and 10⁻⁴, then 1 ml portions are pour-plated with SHA to determine the initial spore concentration. This is done at this stage, rather than prior to filling the test section, to allow for any dilution of the milk soil with water retained after autoclaving.

Cleaning

The soiled test section is mounted in a purpose-built test apparatus (Fig. 2), without removing the auxiliary pipes. To ensure a standardized flow of cleaning solution through the test item, a straight length of pipe with a diameter five times that of the reference pipe should be inserted before and after the test section. The following cleaning procedure is initiated:

- rinse with cold water (10–15°C) for 1 minute;
- circulate a 1.0% (w/v) detergent solution at 63°C ± 2°C for 10 minutes;
- rinse with cold water (10–15°C) for 1 minute.

For all pipe sizes, cleaning solutions should be circulated at a mean flow velocity of 1.5 m/s within the reference pipe. Samples of the two rinse waters are taken at the outlet of the equipment as close as possible to the end of drainage, and 5 ml portions are pour-plated with SHA. Approximately 3–30 spores per 10 ml should be recorded from the pre-rinse (indicating the removal of some soil), while no spores should be recovered from the final rinse (all free spores should have been removed by this rinse). No spores in the pre-rinse, or the presence of spores in the final rinse indicate unsatisfactory test procedures, which may lead to spurious results.

Detection of residual soil

After cleaning, the test section is removed from the test apparatus, and the internal surface of the reference pipe and the relevant surfaces of the test equipment are covered with molten SHA to a depth of at least 5 mm: the reference pipe is partially filled with SHA, cooled on the outside with water, and rotated until the agar just

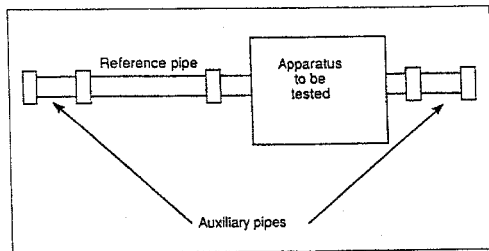


Fig. 1
Configuration of the reference pipe and test item section.

begins to solidify. To facilitate this, the reference pipe is aseptically sealed at each end by a sterile, transparent, polycarbonate disc, one of which has a 4 mm hole in the middle to prevent anaerobic conditions during incubation. After the agar has fully solidified, the reference pipe and test equipment are placed in an incubator at 58°C for 24 hours. If it is not possible to cover the internal surfaces in this manner, the equipment may be dismantled and covered with a thin layer of agar, although precautions should be taken to prevent the agar drying out during incubation – for example, by placing the equipment in a sealed plastic bag.

Assessment and interpretation

After incubation, the test equipment and reference pipe are examined for the presence of yellow discolourations and/or colonies in the SHA (which is normally purple, as it contains the dye Bromocresol purple). This may be facilitated by carefully removing

the agar by gentle agitation or by flushing with water. If yellow zones are visible, an estimation of the total size of the yellow area is made. Experience has shown that a relatively small yellow area (covering ~5–30% of the pipe surface) for the reference pipe indicates a normal cleaning procedure. If the yellow area in the reference pipe covers >30% of the surface, interpretation of results is difficult and the test should be repeated. If colonies are visible, calculation of the quantity of residual soil can be made by comparing the colony count with the initial concentration of spores in the soured milk.

Fundamental to this test methodology is comparison of the test item with the reference pipe, given that the degree of cleaning has resulted in the yellow area accounting for 5–30% of the agar within the reference pipe. This degree of soil retention serves as a control for variability, both within and between laboratories. It is recognized, however, that due to differences in the materials used (e.g. milk source, water hardness, and use of an acidified or soured milk starter culture), test conditions will vary both within and between laboratories. Where changes in materials result in a greater or lesser retention of soil, the cleaning procedure should be modified to rectify this situation. It is permissible to alter the degree of soil drying, the rinse and/or cleaning times, the concentration of the detergent or the detergent solution temperature. The mean flow velocity of 1.5 m/s in the reference pipe must, however, be retained.

In general, and assuming that there is a relatively small yellow area in the reference pipe, three results are possible for the test item, as detailed below.

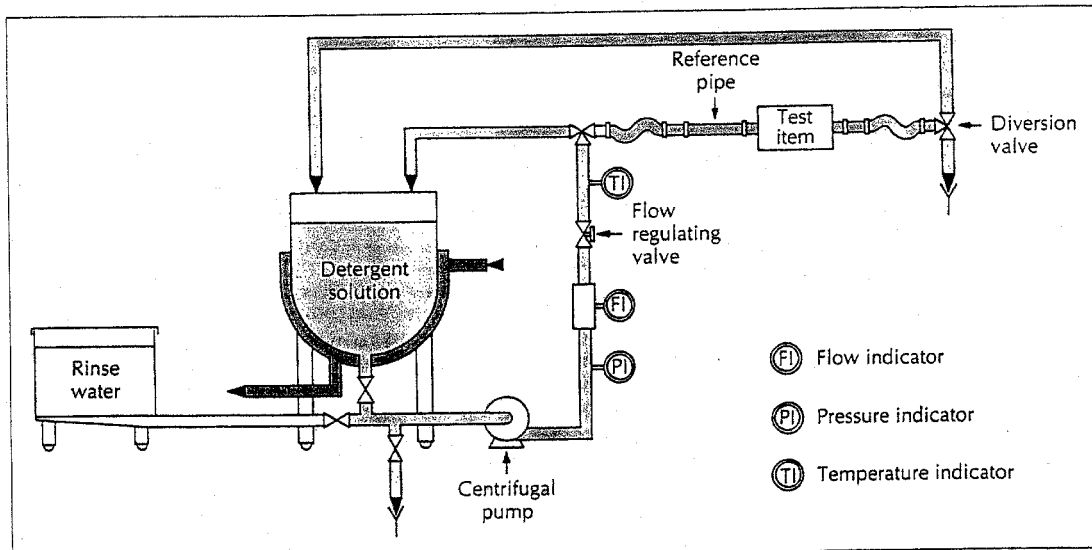


Fig. 2
Test apparatus to assess the in-place cleanability of equipment parts ('test items'). Dark grey, heating/cooling solution; medium grey, detergent solution; light grey, rinse water.

Presence of milk residues

If visible milk residues are present when the test item is observed/dismantled prior to the application of SHA, there are either serious hygienic design faults inherent within the test item or there has been a fault with the test methodology. It is not necessary to undertake any microbiological examination, as spores are clearly present. Microbiological analysis of the reference pipe is, however, required. The test should be repeated; if milk residues are found again in the test item (in the same area), no further tests are required, and the need for design changes should be stressed to the manufacturer of the test item. If a fault is identified in the test methodology, this should be rectified and the test repeated.

Presence of yellow zones and/or colonies

Alternatively, yellow zones may be present in the test item. To investigate whether the yellow zones are related to the degree of cleaning (as is likely the case if they are randomly distributed), or whether they are indicative of poor hygienic design, the test procedure should be repeated up to a maximum of five times. Presence of retained soil in the same area of the test equipment on three separate occasions indicates areas that are difficult to clean, and hence areas in which improvements in hygienic design should be considered.

The cleanability of the test equipment can be compared to that of the reference pipe by assessing the relative percentage areas of yellow zone. If the percentage area of yellow zone in the test item is similar to that of the reference pipe, the degrees of cleanability of the test item and reference pipe are similar. If the percentage area of yellow zone is less or greater in the test item than in the reference pipe, the test item is, correspondingly, more or less cleanable. In comparative equipment trials, the relative area of yellow zone remaining in each of the pieces of equipment tested can be used to measure their relative cleanabilities. When undertaking comparative equipment trials, the age and condition of the equipment tested should be recorded.

No yellow zones/colonies present

In some cases, it is possible that no yellow zones will appear in the test item. If this condition is found on three successive occasions, no further test repeats are required and the test item can be described as 'particularly cleanable'. If, on subsequent tests, yellow zones are found in the test item, further repeats should be undertaken to establish whether areas of poor hygienic design are apparent. There have been occasional indications that some gasket materials may have antibacterial properties, such that spores present on their surfaces are prevented from germinating and/or growing in SHA. Areas of poor hygienic design may, in such cases, not be apparent as yellow zones. If this is suspected in a test result, controls can be undertaken in which gaskets are inoculated with a spore culture (containing $\sim 10^8$ spores per millilitre), covered with SHA, and incubated at

58°C for 24 hours. If yellow zones do not become apparent in the agar, the gaskets may have antimicrobial properties and test results should be treated with caution.

Conclusions

The procedure described above provides a recommended test method for assessing cleanability that can be used as a basis for comparing or verifying the hygienic design of new and existing food-processing equipment. Furthermore, the use of such a standardized procedure should facilitate assessment of the comparative cleanabilities of different items of equipment assessed at different times or in different laboratories.

While the method 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.

This paper summarizes the guidelines and methods recommended by the European Hygienic Equipment Design Group (EHEDG) subgroup on Test Methods. The full report, by J.T. Holah, B.M. Venema-Keur, C. Tragardh, H. Illi, M. Lalande 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).

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