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Evaluation of sanitizers

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SUMMARY

A description is given of experiments on surface sanitizing which were carried out under standardized conditions of soiling, sanitizing and surface count estimation. The number of bacteria remaining or surviving on the surface of wood and polyethene after cleaning and sanitizing has been estimated using a shake-rinse technique. The results that were reproducible showed again the very bad cleanability of wood by normal cleaning techniques. Results were fairly good on non porous surfaces such as hard rubber, polyethene and Plexiglas.

Under practical circumstances growth on cleaned surfaces overnight has to be taken into consideration.

ZUSAMMENFASSUNG

Es sind Experimente beschrieben über die Prüfung von Desinfektionsmitteln und kombinierten Reinigung-Desinfektionsmitteln an Oberflächen. Verschmutzung, Reinigung, Desinfektion und Oberflächenkeimgehaltbestimmung waren standardisiert. Der Oberflächenkeimgehalt wurde bestimmt mit einer Schüttelmethode. Die Versuchsergebnisse waren gut reproduzierbar. Harte nicht-poröse Materialen wie Polyethen, hartes Rubber und Plexiglas konnten gut gereinigt und entkeimt werden. Holz war slecht zu reinigen und konnte mit chemischen Desinfektionsmitteln nicht gut entkeimt werden.

Die Entwicklung von Bakterien auf gereinigten und entkeimten Oberflächen kann u.U. von grosser Bedeutung sein.

1. INTRODUCTION

The laboratory evaluation of disinfectants and sanitizers has a long history showing a very great number of tests applied. The first tests were more empirical, the later ones showed some understanding of the action of sanitizers.

Since disinfectants are generally used to destroy all types of vegetative bacteria and sanitizers to reduce the bacterial count to safe levels, the word sanitizer will be used in this paper. Moreover sanitizing also includes a condition of cleanliness.

The tests used may be divided in two types, viz. the suspension tests and the carrier or surface tests.

The suspension type test is especially useful for screening and characterizing purposes since temperature, pH, water hardness and concentration of sanitizer and soil can be easily varied.

Soil is defined as organic material present on cleaned or uncleaned surfaces, which material interferes with the bactericidal action of sanitizers. In meat factories soil comprises meat, fat, blood and soluble material from these items.

A special type of suspension test is the capacity test in which bacteria together with soil are repeatedly added to a sanitizing solution. After a short exposure period a loopful of the mixture is subcultured following each addition. This test shows clearly the soil carrying capacity of a sanitizer. The carrier or surface test is more in accordance with practical conditions since in practice mostly surfaces have to be treated. However, a standardization of a surface test is not easy especially as soiling and cleaning have to be standardized too.

Some modern standardized suspension tests are the "germicidal and detergent sanitizer" test of the A.O.A.C. (1, p. 70), the British Standard Method (3) and the Standard of the I.D.F. (10).

Capacity tests show the adverse effect of soil on the bactericidal activity of sanitizers more clearly than ordinary suspension tests. The capacity test has been standardized by the A.O.A.C. as the "available chlorine germicidal equivalent concentration" test (1, p. 66) and by the I.D.F. (9).

Suspension and capacity tests should be carried out according to one of the standards mentioned.

Recent surface tests for the evaluation of sanitizers were reviewed by Davis (5). The English test method for dairy detergentsanitizers is described by Cousins (4) while its variant, the Lisboa tube test, is discussed by Mitchell (12).

Experiments on the bacterial cleanliness in meat factories with regard to cleaning and sanitizing were published by Kelch and Palm (11), Gehring (7), Hansen (8), Ølgaard (13) and Bartels et al. (2). However, these experiments in meat factories were not carried out to evaluate sanitizers or detergent sanitizers.

In the experiments described further we tried to standardize a laboratory surface test using small blocks of wood and other materials. We studied the effect of the material and of the (detergent) sanitizer on the surface count of cleaned and sanitized materials.

2. SURFACE TEST WITH (DETERGENT) SANITIZERS USING BLOCKS

2.1. Material and methods

We used blocks (7 x 4 x 1 cm) of steamed beechwood - both small cross sections covered with an Epikote paint - and of hard polyethene. Moreover some experiments were carried out with blocks of hard rubber and Plexiglas. Both large surfaces of the blocks were carved many times to simulate practical conditions.

The blocks were soiled by immersion in a fluid meat-fat suspension at 20 °C for one hour. To this suspension bacterial cultures of <u>Escherichia coli</u>, <u>Staphylococcus aureus</u> or <u>Streptococcus faecalis</u> had been added which outnumbered the flora present. After soiling the blocks were cleaned first by a 0.5 1 water rinse to remove the bulk of the adhering soil, then by soaking for 2.5 min. in each of two successive detergent or detergent sanitizer solutions - to decrease the carrying over of bacteria - and scrubbing manually for 5 sec. in each solution. After a short consecutive rinse with tap water the blocks were transferred to screwcap bottles containing 100 or 150 ml of a cold (4 °C) solution containing thiosulphate and Tween 80 to inactivate small amounts of available chlorine or quaternary transferred, and shaken mechanically for one hour. This shaking period was chosen because a longer period did not largely increase the number of bacteria estimated.

Some of the blocks treated in the detergent solutions received a short treatment in a sanitizing solution for 5 min. before being shaken.

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After shaking the bacterial content of the shaking solution was estimated using a plate count medium and a selective medium. The number of bacteria removed from the surface of the cleaned or sanitized blocks by shaking was calculated.

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The cleaning solution contained 0.3 % T-pol, the detergent sanitizer solution 0.5 % of a commercial compound containing a non-ionic detergent and a quaternary ammonium compound. The sanitizing solution contained 0.3 % chloramine-T.

The temperature of cleaning and sanitizing solutions was 20 $^{\circ}C_{\circ}$ 2.2. <u>Results</u>

Some results of some T-pol cleaned blocks are given in Table 1. The figures mentioned are means of duplicate experiments. From the duplicate values the dispersion index D^2 was calculated (6) and mentioned in the table. For duplicate experiments the probability of D^2 exceeding 5.0 is 0.025, therefore the reproducibility of the experiments appeared to be quite good.

Plate count	bacterial species	number of bacteria on cleaned (T-pol) blocks		
Solling medium		wood	polyethene	
1.3x106	E. coli	1.2x10 ⁵ (2.4) ^x	1.0x10 ²	
2.5x106	S. aureus	2.0x105 (2.7)	1.5x10 ³	
1.1x107	Str. faecalis	4.0x10 ⁵ (3.2)	2.8x10 ³ (1.6)	

Table 1. Cleanability of wood and polyethene

x) Figures in brackets are D²-values

The cleanability of blocks of hard polyethene was good, that of wood very bad. Similar good results were obtained with hard rubber and Plexiglas blocks.

Table 2 shows the relative effect of a simple cleaning compound, with or without an additional sanitizing treatment and of a combined detergent-sanitizer. The D^2 -values are also given. They show a good reproducibility for the sanitizing experiment too.

The results of the tests with the detergent sanitizer were only slightly better than those with the T-pol alone when the treatments were applied to wood. The separate cleaning and sanitizing showed the lowest bacterial counts.

Using polythene the differences were small and because of the low counts no conclusion can be drawn.

Bacterial species	Material	T-pol cleaning	T-pol cleaning and chloramine T sanitizing	Detergent sanitizing
E. coli	wood	1.2x10 ⁵	5.6x10 ³ (0.1) ^x	4.9x10 ⁴ (0.7)
	polyethene	1.0x10 ²	< 1.0x10 ²	< 1.0x10 ²
S. aureus	wood	2.0x10 ⁵	1.5x10 ³ (2.7)	8.5x10 ⁴ (0.5)
	polyethene	1.5x10 ³	< 1.5x10 ³	< 1.5x10 ³
Str. faecalis	wood	4.0x105	1.4x10 ⁴ (0.2)	2.8x10 ⁵ (2.6)
	polyethene	2.8x10 ³	< 1.5x10 ²	1.5x10 ²

Table 2. Effect of detergent sanitizer, separate cleaning and sanitizing on blocks of wood and polyethene

x Figures in brackets are D²-values

However, in practice cleaning and sanitizing are generally carried out at the end of the working day and we are interested not only in the cleanliness immediately after the sanitizing but also in the situation on the following morning. The possibility of growth on cleaned surfaces is wellknown.

In Table 3 growth on cleaned and sanitized surfaces is compared. Growth on wood appeared to be very rapid under the favourable consitions of the experiment. On polyethene the growth was less, without doubt as a result of the better cleanability of polyethene. Moreover the growth on sanitized surfaces was less than on surfaces that had only be cleaned, which suggests a bacteriostatic action of small sanitizer concentration. The same could be observed in experiments with other bacteria.

Table 3. Growth of bacteria on cleaned/sanitized surfaces during storage of the blocks for 24 hours at 23-25 °C, rel. humidity <u>+</u> 90 %. E. coli bacteria added.

Material	Treatment	Number of bacteria on the surface just after the after additional		
Wood	T-pol	2.0x105	1.3x109	
	T-pol + chloramine T	8.0x103	1.8x108	
	Det. sanitizer	5.0x104	4.5x108	
Polyethene	T-pol	4.5x10 ²	2.5x10 ⁵	
	T-pol + chloramine T	<1.5x10 ²	1.6x104	
	Det. sanitizer	4.5x10 ²	3.0x103	

Other hard and non-porous materials as hard rubber and Plexiglas showed the same results as polyethene.

3. CONCLUSIONS

For the evaluation of sanitizers and detergent-sanitizers both suspension tests and surface tests are useful. It is possible to use standardized suspension tests since several of these tests have been described. From the experiments described it appears possible to standardize a surface test too. Using a good standardized soiling, cleaning, sanitizing and surface count estimation technique, reproducible results can be obtained. Growth after cleaning and sanitizing must be taken into consideration.

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4. REFERENCES

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