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A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* at 10 and 20 °C

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J.H. TAYLOR, S.J. ROGERS AND J.T. HOLAH. 1999. A number of proprietary disinfectant products (18) used in the food industry were tested for their bactericidal efficacy against *Pseudomonas aeruginosa* and *Escherichia coli* O157:H7 at 20 and 10 °C according to the BS EN 1276 (1997) quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. At 20 °C, 13 products passed at their in-use concentration (under clean and dirty conditions) against *Ps. aeruginosa* and 15 passed against *E. coli* O157:H7. The number of products passing the test at 10 °C was 11 and 14 for *Ps. aeruginosa* and *E. coli* O157:H7, respectively. The products exhibiting reduced efficacy at the lower temperature were amphoterics and quaternary ammonium compounds although some of these types of products were effective at both temperatures. Products that passed against *Ps. aeruginosa* generally also passed against *E. coli* O157:H7. Taking all the results together, only 11 of the total of 18 products achieved a pass result under all the parameters tested. This work demonstrates the need for final verification of disinfectant efficacy by undertaking field trials in the food-processing environment in which the product is intended for use.

INTRODUCTION

Food product contamination may occur from environmental routes such as air, people and surfaces, with the surface route being the most important to control on a day to day basis by the implementation of a sanitation programme (Holah 1995). Disinfection is the final stage in a sanitation programme that is designed to remove product residues and foreign bodies, in addition to reducing the level of micro-organisms to ensure both the safety and quality of food.

In the last few years there have been considerable changes in eating habits in the UK (Anon. 1995) with increased sales of convenience foods such as ready meals, pizza, prepared salads and potted desserts. To optimize product quality and to ensure food safety, the production of these food types is often carried out at chilled temperatures, yet the biocidal efficacy of disinfectants has not been validated at these temperatures.

Low temperature disinfection is often carried out with products which were formulated before the advent of the modern chilled food industry. Certain pathogens, such as *Listeria monocytogenes*, have become significant in chilled environments and, in spite of greater hygiene standards, food factory trouble-shooting audits by the authors have identified problems with *Listeria* spp. surviving on surfaces after cleaning and disinfection. *Listeria* spp. are able to grow at temperatures below 4 °C (Walker *et al.* 1990) and are very tolerant of adverse environmental conditions at low temperatures (Tuncan 1993). Another organism of concern is *Escherichia coli* O157:H7 and outbreaks involving this organism, along with other pathogens, are still increasing (Anon. 1997a) even though we have a good understanding about the environmental route of contamination and the physiology of food pathogens. To date, little, if any, information has been published relating to the resistance of *E. coli* O157:H7 to commercial disinfectants and thus whether it can be controlled by current sanitation programmes.

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There is a range of test methods for evaluating disinfectant efficacy throughout Europe (Reybrouk 1982) and products may have to undergo a range of different tests to satisfy legislative requirements in different countries. Consequently, products available on the market are likely to exhibit various biocidal efficacies because they have been tested under different conditions (level and type of organic load, type of test micro-organism, hard water, etc.) against different methods and to different standards. To take into account these factors and to harmonize testing across Europe, the European Technical Committee CEN/TC216/WG3 has developed suspension tests (bactericidal and fungicidal) specifically for the food, industrial and domestic markets. These tests will be recognized as being appropriate for use within the European Biocide Directive (Anon. 1997b), whereby the efficacy claims of disinfectant products have to be supported by data from recognized test methods.

This paper describes work which was undertaken to investigate the disinfection efficacy of proprietary products used in the food industry. Studies were undertaken to examine the effect of lower temperatures on disinfection efficacy. *Pseudomonas aeruginosa* was used as the test organism because of its known resistance to disinfectant action. Disinfectant efficacy was also tested against *E. coli* O157:H7 to establish whether proprietary products are capable of killing this organism under normal sanitation conditions. Finally, this study enabled a practical evaluation of the new European bactericidal suspension test BS EN 1276 (1997) (Anon. 1997c).

Table 1 Summary of product types and in-use concentration

Product code	Product type	Recommended in-use concentration
1	Quat	1·0%
2	Quat	1·0%
3	Quat	1·0%
4*	Quat/amphoteric	1·0%
5*	Quat/amphoteric	1·0%
6*	Quat/glutaraldehyde	1·0%
7	Amphoteric	1·0%
8*	Amphoteric	1·0%
9	Amphoteric	1·0%
10	Chlorine dioxide	1·0%
11	Potassium hydroxide/sodium hypochlorite	5·0%
12	Sodium hypochlorite	500 ppm
13	Peracetic acid/hydrogen peroxide	0·3%
14	Iodophor	1·0%
15	Biguanide	1·0%
16*	Biguanide/quat	1·0%
17	Sodium dichloroisocyanate	333 pm
18	Acid detergent/sanitizer	1·0%

* These disinfectants have been specifically formulated and marketed for use in the UK chilled food industry 'high care' areas operating at low temperatures.
Quat, Quaternary ammonium compound.

MATERIALS AND METHODS

Commercially available products (18) were chosen to represent a range of products with respect to price and product type (encompassing both oxidative and non-oxidative biocides) and markets throughout Europe. The product types included in the study are shown in Table 1.

Products were tested according to the BS EN 1276 (1997) quantitative suspension test for the evaluation of the bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas against *E. coli* O157:H7 (CCFRA reference code CRA 4497) and *Ps. aeruginosa* (NCIMB 10421) at two different temperatures (20 °C is the standard temperature in the test method and 10 °C an additional test temperature which also reflects the temperature encountered in chilled food-processing environments) using temperature-controlled water-baths (10 and 20 °C). The products were tested at three concentrations, including their recommended in-use concentration (usually 1% for both liquids (v/v) and solids (w/v), unless specified, see Table 1), in both clean and dirty conditions.

An 8·0-ml sample of disinfectant diluted in water of standard hardness (300 mg kg^{-1} $CaCO_3$) at concentrations of 0·63% (v/v), 1·25% (v/v) and 2·5% (v/v) to give final test concentrations of 0·5%, 1·0% and 2% was added to 1·0 ml bovine albumen (Fraction V; BDH, Poole, UK) serum at concentrations of 0·3% (w/v) and 0·03% (w/v) (to represent dirty and clean conditions, respectively) to which 1·0 ml of the bacterial suspension ($1·5–5·0 \times 10^8$ cfu ml^{-1}, grown in high nutrient conditions) had been added. After a contact time of 5 min, 1·0 ml of the test mixture was pipetted into 8·0 ml neutralizer (comprising polysorbate 80 3·0% (v/v), saponin 3·0% (w/v) and lecithin 0·3% (w/v)) and 1·0 ml deionized water. After 5 min neutralization time, duplicate 1·0-ml volumes were pour plated with tryptone soya agar and incubated at 37 °C for 48 h prior to counting.

Three validation (control) procedures were undertaken in parallel for each disinfection test on all occasions as follows.

Validation A. The test was undertaken with the addition of 8·0 ml water of standard hardness in place of the disinfectant to ensure that there was no biocidal action of the other experimental parameters.

Validation B. Neutralizer (8·0 ml) and 1·0 ml water were added to the bacterial suspension and then plated out to ensure that the neutralizer had no disinfectant activity.

Validation C. Bacterial suspension (1·0 ml) was added to neu-

tralized disinfectant to ensure that the disinfectant had been neutralized.

Full details of the disinfection and validation procedures are described in the test method (Anon. 1997c). For safety reasons, all *E. coli* O157:H7 testing was carried out in a Class II safety cabinet.

The reduction in viability was calculated by subtracting the log of the viable count after disinfection (Na) from the log of the initial count in the test chamber ($N \times 10^{-1}$). To pass the test, products must achieve a five log reduction in viable counts. In this study, tests were undertaken twice and, where necessary, a third decisive test was carried out with the overall performance of products assessed at their in-use concentration.

RESULTS

Pseudomonas aeruginosa at 20 °C

The results in Table 2 show that 13 of the 18 products tested in this study achieved pass results at 20 °C for both clean and dirty conditions against *Ps. aeruginosa*. The products that failed under both dirty and clean conditions were an amphoteric (no. 7), chlorine dioxide (no. 10), a biguanide (no. 15) and an acid detergent/sanitizer (no. 18). A quaternary ammonium product (no. 1) passed under clean conditions but failed (on two occasions) under dirty conditions.

Escherichia coli O157:H7 at 20 °C

The results in Table 2 show that 15 of the 18 products passed for both clean and dirty conditions at 20 °C. Products which passed against *Ps. aeruginosa* also passed against *E. coli* O157:H7 but products failing against *Ps. aeruginosa* did not necessarily fail against *E. coli* O157:H7. The biguanide (no. 15) failed under dirty conditions but passed under clean conditions for *E. coli* O157:H7. The quaternary ammonium product (no. 1), which failed under dirty conditions against *Ps. aeruginosa*, passed against *E. coli* O157:H7. Chlorine dioxide (no. 10) and the acid detergent/sanitizer (no. 18) failed under both clean and dirty conditions.

Pseudomonas aeruginosa at 10 °C

The results in Table 3 show that 11 products achieved a pass under both clean and dirty conditions against *Ps. aeruginosa*. The products that failed were two quaternary ammonium products (nos 1 and 2), two amphoterics (nos 7 and 8), chlorine dioxide (no. 10), a biguanide (no. 15) and an acid detergent/sanitizer (no. 18). Of these, two products (an amphoteric (no. 8) and a quaternary ammonium (no. 2)) showed a temperature effect (i.e. passed at 20 °C but failed at 10 °C).

Escherichia coli O157:H7 at 10 °C

The results in Table 3 show that 14 products passed under clean and dirty conditions and that products passing against *Ps. aeruginosa* passed against *E. coli* O157:H7.

The products that failed were an amphoteric (no. 7), chlorine dioxide (no. 10), a biguanide (no. 15) and an acid detergent/sanitizer (no. 18). Only one product (amphoteric (no. 7)) showed a temperature effect (i.e. passing at 20 °C but failing at 10 °C) for *E. coli* O157:H7.

DISCUSSION

Evaluation of disinfection efficacy

Only 11 of the 18 products gave a pass result at the recommended in-use concentration against the two organisms for both temperatures on at least two occasions for both clean and dirty conditions. Work by Jacquet and Reynaud (1994) also showed that, of eight disinfectants tested (at recommended in-use concentration) using the French Standard AFNOR NFT 721701989 at 20 °C, only two products achieved a five log reduction in viable counts against the test organisms (*L. monocytogenes, Staphylococcus aureus* and *Enterococcus faecium* strains). These results show that the efficacy of some products may be compromised by certain conditions, such as low temperature and organic residues, which may be encountered in food-processing environments.

With factories operating at chilled temperatures (10 °C) several chemical suppliers have formulated their disinfectants specifically to be used in these environments; these are marked with an asterisk in Table 1 (other products are generally formulated for use at ambient temperatures). As expected, all five of these products passed the tests for both dirty and clean conditions at this temperature and at 20 °C. Such formulation of products would mask temperature effects and may explain why there were only two types of product giving a clear difference in activity with temperature.

Other workers have found that temperature has a marked effect on biocidal efficacy. Tuncan (1993) found that a quaternary ammonium and an iodophor can be ineffective against some *Listeria* spp. at cold temperatures (ranging from 2 to 15 °C), whilst being effective at 25 °C. However, it was found that efficacy was improved by increasing the exposure time or concentration. The concentrations used for the quaternary ammonium were up to 200 ppm (0·02%) and for the iodophor 50 ppm (0·005%), which are much lower than those used in this study. Tuncan (1993) also found that the various species of *Listeria* demonstrated different sensitivities to the disinfectants. Gelinas *et al.* (1984) studied temperature effects

Table 2 Results at 20 °C

Product type	Product code	Clean/ dirty	*Pseudomonas aeruginosa* Disinfectant in-use concentration			*Escherichia coli* Disinfectant in-use concentration		
			× 0·5	× 1·0	× 2·0	× 0·5	× 1·0	× 2·0
Amphoteric	7	Clean	F	F	P	P	P	P
		Clean	F	F	P	F	P	P
		Dirty	F	F	P	P	P	P
		Dirty	F	F	F	F	F	P
		Clean				P	P	P
		Dirty				P	P	P
Amphoteric	8	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	F	P	P
		Dirty	P	P	P	P	P	P
Amphoteric	9	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	F	P	P	P	P	P
Chlorine dioxide	10	Clean	F	F	P	F	F	P
		Clean	F	F	P	F	F	P
		Dirty	F	F	P	F	F	P
		Dirty	F	F	P	F	F	P
Potassium hydroxide/sodium hypochlorite	11	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
Sodium hypochlorite	12	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	P	P	P	F	P	P
Peracetic acid/hydrogen peroxide	13	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	P	P	P	P	P	P
Iodophor	14	Clean	P	P	P	P	P	P
		Clean	P	P	P	F	P	P
		Dirty	P	P	P	F	P	P
		Dirty	P	P	P	F	P	P
Biguanide	15	Clean	F	F	P	P	P	P
		Clean	F	F	P	F	P	P
		Dirty	F	F	F	F	F	F
		Dirty	F	F	P	F	F	F
Biguanide/quat	16	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
Sodium dichloroisocyanate	17	Clean	P	P	P	P	P	P
		Clean	F	P	P	P	P	P
		Dirty	F	P	P	F	P	P
		Dirty	F	P	P	P	P	P
Acid detergent/sanitizer	18	Clean	F	F	F	F	F	F
		Clean	F	F	F	F	F	F
		Dirty	F	F	F	F	F	F
		Dirty	F	F	F	F	F	F

P (pass), 5-log reduction or greater in viable counts; F (fail), less than 5-log reduction in viable counts; Quat, quaternary ammonium compound.

Table 3 Results at 10 °C

Product type	Product code	Clean/ dirty	*Pseudomonas aeruginosa* Disinfectant in-use concentration			*Escherichia coli* Disinfectant in-use concentration		
			× 0·5	× 1·0	× 2·0	× 0·5	× 1·0	× 2·0
Quat	1	Clean	F	F	P	P	P	P
		Clean	F	F	P	P	P	P
		Dirty	F	F	P	F	P	P
		Dirty	F	F	P	F	P	P
Quat	2	Clean	F	F	F	P	P	P
		Clean	F	F	F	P	P	P
		Dirty	F	F	F	F	P	P
		Dirty	F	F	F	F	P	P
Quat	3	Clean	F	P	P	P	P	P
		Clean	F	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	F	P	P	F	P	P
Quat/amphoteric	4	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	F	P	P	F	P	P
Quat/amphoteric	5	Clean	P	P	P	P	P	P
		Clean	F	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	F	P	P	F	P	P
Quat/glutaraldehyde	6	Clean	F	P	P	P	P	P
		Clean	F	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	F	P	P	P	P	P
Amphoteric	7	Clean	F	F	F	F	F	P
		Clean	F	F	F	F	F	P
		Dirty	F	F	F	F	F	P
		Dirty	F	F	F	F	F	F
Amophoteric	8	Clean	F	F	P	P	P	P
		Clean	F	F	P	P	P	P
		Dirty	F	F	P	P	P	P
		Dirty	F	F	P	P	P	P
Amphoteric	9	Clean	P	P	P	P	P	P
		Clean	F	P	P	F	P	P
		Dirty	P	P	P	P	P	P
		Dirty	F	P	P	F	P	P
Chlorine dioxide	10	Clean	F	F	P	F	F	P
		Clean	F	F	P	F	F	F
		Dirty	F	F	F	F	F	F
		Dirty	F	F	F	F	F	F
Potassium hydroxide/sodium hypochlorite	11	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
Sodium hypochlorite	12	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P

Table 3 *Continued*.

Product type	Product code	Clean/ dirty	*Pseudomonas aeruginosa* Disinfectant in-use concentration × 0·5	× 1·0	× 2·0	*Escherichia coli* Disinfectant in-use concentration × 0·5	× 1·0	× 2·0
Peracetic acid/hydrogen peroxide	13	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
		Dirty	P	P	P	P	P	P
Iodophor	14	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	P	P	P	F	F	P
		Dirty	P	P	P	F	P	P
		Clean				P	P	P
		Dirty				F	P	P
Biguanide	15	Clean	F	F	P	F	F	P
		Clean	F	F	P	F	F	F
		Dirty	F	F	F	F	F	F
		Dirty	F	F	P	F	F	F
Biguanide/quat	16	Clean	F	F	F	P	P	P
		Clean	P	P	P	P	P	P
		Clean	F	P	P	F	P	P
		Dirty	F	F	F	P	P	P
		Dirty	F	P	P			
		Dirty	F	P	P			
Sodium dichloroisocyanate	17	Clean	P	P	P	P	P	P
		Clean	P	P	P	P	P	P
		Dirty	F	P	P	P	P	P
		Dirty	F	P	P	P	P	P
Acid detergent/sanitizer	18	Clean	F	F	F	F	F	F
		Clean	F	F	F	F	F	F
		Dirty	F	F	F	F	F	F
		Dirty	F	F	F	F	F	F

P (pass), 5-log reduction or greater in viable counts; F (fail), less than 5-log reduction in viable counts; Quat, quaternary ammonium compound.

on eight products and found that the efficacy was greatly influenced by temperature (4–50 °C). Glutaraldehyde, chlorhexidine and amphoteric products were particularly affected, whilst sodium hypochlorite was found to be least affected by temperature. The results were expressed as minimum concentrations obtained by the Association of Official Analytical Chemists (Anon. 1980) use-dilution method against *Ps. aeruginosa*. It is difficult to make direct comparisons because different methods were used and there are no data at 10 °C. They noted a synergistic effect with glutaraldehyde and a quaternary ammonium and indeed in this study a five log reduction in viable counts was obtained for a product combining glutaraldehyde and a quaternary ammonium (for the tests undertaken).

It is not clear why some disinfectant products exhibit temperature effects, although it may be related to the reduced temperatures lowering the metabolic activity of the test micro-organisms or potentially the onset of the cold shock response which may enhance resistance to disinfection. These topics are discussed in a comprehensive review article by Berry and Foegeding (1997) on cold temperature adaptation and growth of micro-organisms.

The 5-min contact time in the disinfectant test was chosen because it is representative of the time taken for disinfectant to run off equipment surfaces after application. In practice, however, the effectiveness of products may be enhanced by prolonged contact times, e.g. the use of soak ranks or repeat applications. The presence of organic residues after cleaning

may be reduced by more thorough cleaning. The food industry normally requires products to pass under dirty conditions, as poor hygienic design of equipment may result in crevices and dead areas that may protect food soil from cleaning and there are occasions when equipment has not been adequately cleaned.

The relative strengths and weaknesses of laboratory disinfectant tests have recently been reviewed by Holah *et al.* (1998) and ways in which they can be improved to more closely simulate in-use conditions in the future are discussed. In the short term, and especially for high-risk chilled food-processing, final assurance of product disinfectant performance can only be validated by undertaking studies in a food-processing environment using a comprehensive microbiological verification programme with environmental sampling.

The results for *E. coli* O157:H7 show that, under suspension test conditions, it is not a particularly resistant organism. Where a five log reduction in viable counts was achieved with *Ps. aeruginosa* it was also achieved against *E. coli* 0157:H7. This work shows that the control of *E. coli* O157: H7 in the factory environment should be effective with a thoroughly designed sanitation programme incorporating an appropriate disinfectant.

Evaluation of BS EN 1276 (1997)

The nature of this work with the number of tests undertaken has allowed an opportunity to evaluate the test method itself, particularly in terms of practical ease. The concept of the test is simple, i.e. the addition of 8·0 ml disinfectant to 1·0 ml bacteria and 1·0 ml interfering substance for a 5-min contact time followed by 5 min neutralization prior to pour plating to enumerate the number of viable micro-organisms remaining. However, there are a number of issues which make this test complex.

The test procedure requires an initial inoculum of 1·5–5·0 $\times$ 10^8 cfu ml^{-1} which may be measured by a variety of means as specified in the test method, including the use of a spectrophotometer or nephelometer. This laboratory performs this by using a spectrophotometer to measure the optical density (O.D.) and adjusting the microbial suspension concentration to obtain the required O.D. as determined from a calibration of O.D. against total viable count (TVC) for each organism. In early trials using this method, only a 70% success rate was achieved (i.e. although the O.D. was set according to the calibrations, the actual TVC was not as expected). With an ongoing improvement in calibration data and with daily testing being undertaken by trained operators, achieving the correct inoculum level became less of a problem.

In practice, however, the actual level of the starting inoculum has to be between 2·0 and 3·0 $\times$ 10^8 cfu ml^{-1} because the standard method dictates that the three validation tests must recover viable cells in the range 2·0–3·0 $\times$ 10^3 cfu ml^{-1}.

The contact time in the test procedure is 5 min $\pm$ 10 s and, for economic reasons, laboratories may undertake the test for one organism at three concentrations of disinfectant under both clean and dirty conditions (six reactions) along with the three validation controls (one-test reaction for validation B and two-test reactions each for validations A and C). This means that one test procedure with validations involves 11 reactions, which have to be manipulated in a sequence allowing for the contact times and the 10 s tolerance. If the procedure is staggered with too large intervals then all the 11 reactions cannot be carried out in time. This sequence of manipulations, undertaken to maximize time output, requires training to ensure competency.

It is not clear at this stage how repeatable and reproducible this suspension test is. Repeatability studies have been undertaken on previous suspension tests on which BS EN 1276 (1997) is based (Bloomfield *et al.* 1991, 1995; Holah 1995) but it is not known whether this test is more or less repeatable. To ascertain this, a European Committee for Standardisation (CEN)-funded European ring trial is currently being undertaken through the auspices of CEN/TC 216 which hopes to report the results at a later date.

In conclusion, *E. coli* O157 does not seem to be any more difficult to kill using food industry disinfectants than *Ps. aeruginosa*. Products that passed at 10 °C also passed at 20 °C and only three products showed a temperature effect. However, of the six parameters examined (two organisms, two temperatures and two soil levels) only 11 of 18 products achieved the required 5 log reductions. This work demonstrates the importance of undertaking laboratory disinfectant tests under appropriate simulations of in-use conditions and of considering final verification with a field trial in the food-processing environment by the user. The BS EN 1276 (1997) quantitative bacterial suspension test is not difficult to undertake but it does require training and experience to become fully proficient and should be a useful method to harmonize disinfectant testing across Europe.

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