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Hygienic Performance of Commercial Dishwashers with Water-Change System – An Experimental Study

Commercial dishwashers are used in community facilities such as hospitals, refectories, nursing homes or food services. In this area, kitchen and food hygiene is of great importance to ensure consumer health. The aim of this study was to verify the hygienic performance of commercial dishwashers with a water-change system by use of bioindicators. This test method is described in DIN SPEC 10534. *Micrococcus luteus* was used as the test germ instead of *Enterococcus faecium* as it imposes no pathogenic risk. *Micrococcus luteus* was identified in initial studies as an appropriate test germ for hygiene studies. Cleaning and rinsing temperatures and holding time of the cleaning program were varied. High rinsing temperature with a constant cleaning temperature and high cleaning temperatures with a constant rinsing temperature led to a reduction of the viable cell count. Increasing the cleaning temperature resulted in a higher decrease of the viable cell count than increasing the rinsing temperature.

Key words: Reduction factor, viable cell count, micrococcus luteus, bioindicators, hygienic risk, DIN SPEC 10534

Hygieneleistung gewerblicher Geschirrspülmaschinen mit Wasserwechselsystem – eine experimentelle Studie. Gewerbliche Geschirrspülmaschinen werden in Seniorenheim-, Kantinen-, Kita- und Krankenhausküchen sowie in weiteren Institutionen, wo Lebensmittel verarbeitet und serviert werden, eingesetzt. In solchen Institutionen spielen Küchen- und Lebensmittelhygiene eine große Rolle, um die Gesundheit des Verbrauchers zu gewährleisten. Diese Arbeit befasst sich mit der Überprüfung der Hygieneleistung von gewerblichen Geschirrspülern mit Wasserwechselsystem mit Hilfe von Bioindikatoren. Die Methode ist in der DIN SPEC 10534 beschrieben. Abweichend zu den Vorgaben dieser Methode, wurde der als Testkeim vorgesehene Mikroorganismus *Enterococcus faecium* durch den apathogenen Keim *Micrococcus luteus* ersetzt. Dieser hatte sich in vorangegangenen Untersuchungen als geeigneter Testkeim herausgestellt. Das verwendete Reinigungsprogramm wurde durch Herabsetzung und Erhöhung der Reinigungs- und Nachspültemperaturen, sowie durch Verlängerung der Haltezeit variiert. Die Ergebnisse zeigen, dass sowohl eine Erhöhung der Reinigungstemperatur bei konstanter Nachspültemperatur, als auch eine Erhöhung der Nachspültemperatur bei konstanter Reinigungstemperatur zu einer Steigerung der Keimzahlreduktion führte. Es ist erkennbar, dass eine Erhöhung der Reinigungstemperatur eine niedrigere Keimzahl zur Folge hatte, als die entsprechende Erhöhung der Klarspültemperatur.

Stichwörter: Reduktionsfaktor, Keimzahl, Micrococcus luteus, Bioindikatoren, Hygienisches Risiko, DIN SPEC 10534

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1 Introduction

Food-contamination can occur at any point in the production process. Foodborne infections caused by improperly cooked or mishandled food at home or in food services or markets are considered to be very relevant [1–3]. In fact, it appears that cleaning work, which reduces the amount of microorganisms on kitchen surfaces and kitchenware, is of great importance to lessen those infections [1].

The cleaning of cutlery- and crockery also contributes to kitchen hygiene. The German Federal Institute for Risk Assessment (BfR) advises households to clean dishes directly after use and recommends pre-rinsing them if they are heavily soiled. If dishwashers are used, the temperature of the program selected should not fall below 60 °C. Furthermore, the interior, primarily the filter and the door gasket, has to be cleaned regularly [4].

The fact that door gaskets are often contaminated, especially by fungus, has been discovered by Zalar et al. [5] and confirmed by Döğen et al. [6].

In contrast to households, commercial dishwashers have to be checked regularly by technical personnel authorized by the manufacturer. It is important for maintaining operational and functional safety and for upholding warranty claims [7]. This will assure hygienically clean dishes in public areas.

Studies have already been conducted that tested isolated bacterial strains within manual and automatic dishwashing experiments in the domestic area [8, 9]. There are different standard guidelines regarding commercial dishwashers, such as the standards of the German Institute for Standardization DIN 10510, DIN 10511, DIN 10512 and the DIN 10522, which describe type-specific methods of testing [10–14].

Additionally, the draft DIN SPEC 10534 "Food hygiene – Commercial dishwashing – Hygiene requirements" sums up the standards existing already and gives an overview of commercial dishwashing requirements regarding hygiene. [15] This pre-standard was published not only in German, but also in English for a Europe-wide application. The DIN SPEC 10534 describes the cleaning capacity tests of tank-dishwashers. The washed dishes, for instance, have to be checked visually and with microbiological testing methods. The microbiological testing can be performed by using bioindicators or contact plate samples. However, a description of the testing of water-change dishwashers does not yet exist [10].

It was therefore the aim of this study to check whether the potential pathogen germ *Enterococcus faecium (E. faecium)* [10, 16] of DIN SPEC 10534 can be replaced by an apathogenic germ with similar sensitivity to the time and temperature conditions in commercial dishwasher [15]. Additionally it should be investigated whether commercial water-change

dishwashers can be assessed regarding their hygiene performance in a way similar to that described in DIN SPEC 10534.

2 Experimental Procedure

2.1 Initial selection of test strains

The initial step of this study was to investigate the possibility of substituting *Enterococcus faecium (E. faecium)*, a bacterium of risk group 2 which is normally used for cleaning studies, for an appropriate bacterial strain of risk group 1 [10, 16]. Therefore, four apathogenic bacterial strains were tested: *Micrococcus luteus (M. luteus), Lysinibacillus macroides (L. macroides)* [8], *Pseudomonas fluorescens (P. fluorescens)* [17], *and Geobacillus atrophaeus (G. atrophaeus)*.

Prior to this study, an *M. luteus* (DSM 28269*) and an L. macrolides strain* (DSM 28270) were isolated from a culture that had been sampled by soy agar contact plates (RODAC plates) from soiled dishes [5]. *P. fluorescens* (DSM 50090) is used in a hygiene standard for washing machines and was chosen because of its fluorescent properties [17]. *G. atrophaeus* (DSM 675) is a test strain which was easy to count and showed heat stability properties.

2.2 Preparation of the test soiling medium

According to DIN SPEC 10534, the test soiling medium was made from of 0.6% bovine albumin (*Carl Roth* 8076.2), 1.0% mucin (*Carl Roth* 8494.1), 3.0% maize starch (*Carl Roth* 4701.1) and 95.4% sterile, deionized water (DI water). At first, mucin and albumin were dissolved by stirring at 50–60 °C. Separately, a maize starch-solution was prepared with boiling water. After cooling to room temperature, both solutions were mixed under sterile conditions.

2.3 Cell cultivation

Firstly, cells of these stock cultures on tryptic soy agar (TSA, *Carl Roth* X937.2) were transferred to 100 ml tryptic soy broth (TSB, *Carl Roth* X938.1) and were incubated by shaking for 24 h at 30 °C. Secondly, 2 ml of the incubated culture was transferred into 200 ml fresh TSB and again stored at 30 °C in a shaking incubator for 24 h.

The cells were subsequently harvested and pelleted in a centrifuge (*Hettich Universal 320*, Tutlingen) at 3000 rpm for 10 min. The cells were then re-suspended in 10 ml sterile, physiological saline solution (NaCl, *AppliChem* 7647–14–5). This washing step was repeated twice and the cells were finally re-suspended in 2 ml NaCl.

Thereafter the cell count was determined as colony forming units per milliliter (cfu/ml). A cell count between $1 \cdot 10^9$ and $1 \cdot 10^{10}$ cfu/ml is needed to achieve a cell count between $1 \cdot 10^8$ and $1 \cdot 10^9$ cfu/ml in the test soiling cell solution.

2.4 Bioindicators

The germ carriers (Fig. 1) are made of stainless steel, with an 80 grit longitudinally ground surface on one side. They have one drill hole to the left and one to the right of the contamination area for fastening them onto holders [7].

The germ carriers were cleaned, autoclaved and dried before being used for soiling. Thereafter, the cell suspension was mixed with the test soiling medium in a 1:10 ratio. The ground contamination area of each germ carrier was subsequently soiled with 100 µl of test soiling cell solution which had to dry on the surfaces for at least 5 h.

According to DIN SPEC 10534 the viable cell count on the bioindicators should be more than 1×10^7 cfu/ml. To ensure the cell count required, reference bioindicators of all strains were examined. Bioindicators were placed into covered test tubes with 10 ml NaCl. The bioindicator tubes were vortexed thoroughly until the residual soiling was completely dissolved. The NaCl solutions of reference bioindicators were analyzed by determination of the viable cell count (see 2.5).

2.5 Test procedure

2.5.1 Undercounter- one- tank dishwasher

A commercial undercounter one-tank dishwasher (*Miele G 8066*, Bielefeld) was used for the initinal step of the investigation. Eighteen plates were loaded into the rack. Different from DIN SPEC 1054 10 instead of 18 plates had holders for the bioindicators. The latter were arranged in an alternating order to cover all positions in the rack. Nine different combinations of temperature and holding time (HT) in the cleaning process were tested (Table 1). Each combination was tested three times successively and repeated three times on different days.

Four cleaning cycles were carried out before starting the test runs to get a constant temperature. Subsequently, the bioindicators were placed on holders, 52.5 g of cleaning detergent (*REGSM, Dr. Weigert*, Hamburg) was dosed directly into the tank and the first cleaning cycle was started. Rinse aid (*KEGSM, Dr. Weigert*, Hamburg) was dosed automatically and set at 2%. The door was left open for 2 min after each cleaning cycle to replace the bioindicators and the cleaning detergent (8.7 g) was replaced. The procedure was repeated twice.

The inside of the dishwasher, plates, and holders were cleaned with chlorine tablets (*Neodisher, Dr. Weigert,* Hamburg) after each test series. When the cleaning cycle was finished, the tank was emptied and refilled with fresh water. Two more cleaning cycles were carried out to ensure the removal of all residues of chlorine. The tank was also emptied and refilled between these two cycles.

2.5.2 Water-change dishwasher

The "*Kurz*" [Short] program was used for the experiments with the commercial dishwasher with a water-change sys-

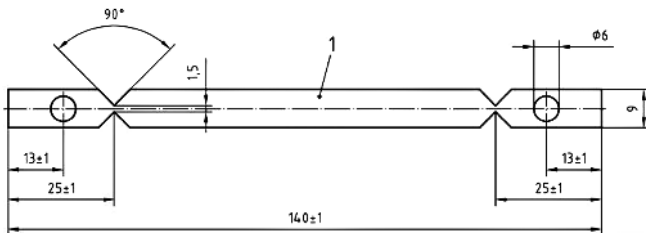

Figure 1 Test germ carriers according to DIN SPEC 10534

Temperature/°C	Time/s		
45	60	120	180
55	60	120	180
65	60	120	180

Table 1 Settings of test runs for undercounter-one-tank dishwasher

tem (*Miele PG 8056,* Bielefeld). The HT and the combination of the cleaning and the rinsing temperature (RT) were modified in every test according to Table 2.

The first seven tests kept a cleaning temperature (CT) of 30 °C, whilst the RT were adjusted to a temperature between 45 °C and 65 °C.

The other seven tests were performed at a CT between 45 °C and 65 °C, whereas the RT stayed at 30 °C.

Eighteen plates were loaded into the plate rack, according to a fixed loading scheme, for each test, whilst six of them had holders, onto which bioindicators were placed (Fig. 2).

After starting the program, 22 ml of cleaning agent (*KEGSM, Dr. Weigert,* Hamburg) were added at the dispensing temperature (Table 3). An amount of 2.3 ml of rinse aid (*REGSM, Dr. Weigert,* Hamburg) was used accordingly for the final rinse

The inside of the dishwasher was cleaned with chlorine tablets (*Neodisher, Dr. Weigert,* Hamburg) after sampling.

Each experiment was carried out three times, starting with a temperature of around 30 °C. Moreover, for each testing day, one bioindicator was kept at room temperature as positive control.

2.6 Microbiological analysis

After carrying out the test, the bioindicators were taken from their holders with sterile tweezers and placed into covered test tubes with 10 ml NaCl. The bioindicator tubes were vortexed thoroughly until the residual soiling was completely dissolved.

The NaCl solutions of the washed bioindicators and the positive controls were analyzed by determination of the viable cell count (C). Consequently, a dilution series was performed and the samples were applied to TSA plates. After 48 h of incubation at 30 °C, the viable cell count was determined by counting the colonies on each plate and calculating the weighted arithmetic average.

$$C_{gew.} = \frac{\Sigma C}{(n_1 \cdot 1) + (n_2 \cdot 0,1)} \cdot d \cdot 10 \qquad (1)$$

$C_{gew.}$ = weighted arithmetic average

ΣC = sum of viable cell count of all TSA plates, used for calculation

n_1 = count of TSA plates with the lowest evaluable dilution stage

n_2 = count of TSA plates of the next higher dilution stage

d = dilution factor of the lowest evaluable dilution stage

Additionally, standard deviation of C were determined for each test run.

2.7 Statistical analysis

2.7.1 Calculation of reduction factor

In order to evaluate a reduction factor, the weighted arithmetic average $C_{gew.}$was calculated for each sample. Since

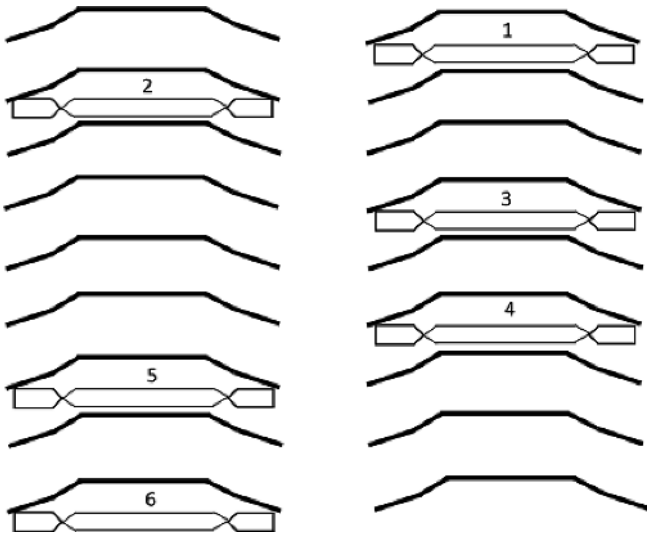

Figure 2 Number and position of bioindicators

Test run	Cleaning parameters		Rinsing parameters	
	Temperature/°C	Holding time/s	Temperature/°C	Holding time/s
1	30	60	45	60
2		90		90
3		120		120
4		60	55	60
5		90		90
6		120		120
7		60	65	60
8	45	60	30	60
9		90		90
10		120		120
11	55	60		60
12		90		90
13		120		120
14	65	60		60

Table 2 Modification of cleaning and rinsing temperature and holding time

cleaning temperature/°C	dispensing temperature/°C (cleaning agent)
30	30
45	30
55	40
65	50
rinsing temperature/°C	**dispensing temperature/°C (rinse aid)**
30	30
45	40
55	50
65	60

Table 3 Dispensing temperatures

each experiment was performed three times, an arithmetic average was calculated and used as Y:

$$R_F = \log_{10} \cdot X - \log_{10} \cdot Y \quad (2)$$

R_F = the reduction factor
X = count of cfu on positive control plate
Y = count of cfu on washed bioindicator plate

2.8 Calculation of significant differences

The t-test was used to evaluate the significant difference of test sequences with the undercounter-one-tank dishwasher. This method is used for nonparametric tests.

3 Results

3.1 Analysis of reference bioindicators

After the reference bioindicators of each test strain had been evaluated, the expected and the analyzed cell count were compared (Fig. 3). Whereas the difference for *M. luteus* was just one order of magnitude, the other test germs showed differences of three or four orders.

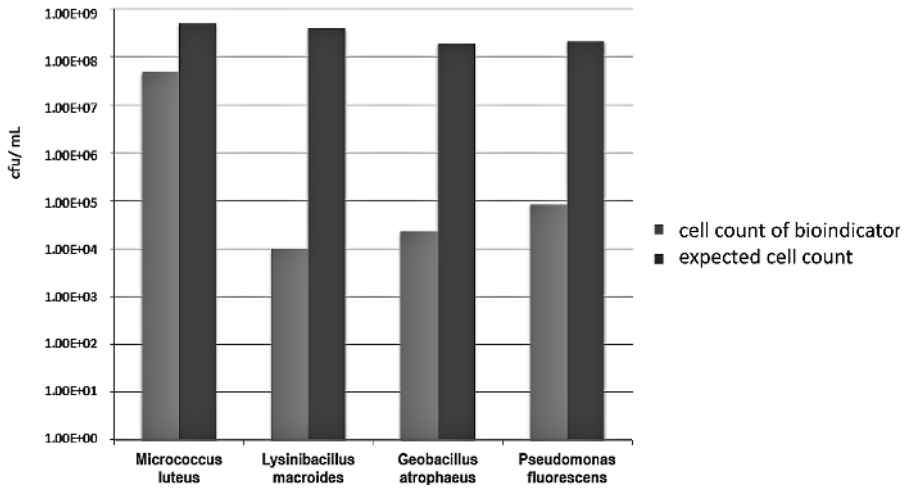

Figure 3 Evaluation of reference bioindicators

3.2 Results of test runs with the undercounter-one-tank dishwasher

Regarding the examination of reference bioindicators only *M. luteus* could reach the required cell count on the bioindicators. Consequently, test runs were carried out solely with *M. luteus*. These test series investigate whether *M. luteus* is an appropriate test strain to replace *E. faecium* for hygiene studies. A t-test was carried out for each test sequence in addition to the reduction factor and standard deviation (Fig. 4). Results over all test runs showed that higher temperatures and longer HT led to a stronger reducing effect. Significant differences could be discovered. The t-test indicated that an increase of temperature of 10 K had a significant effect on test runs with the same HT. However, a rise of HT of 60 s had only a significant influence at 65 °C.

3.3 Hygienic performance of water-change dishwashers

In test runs one to seven, parameters were set to a constant CT about 30 °C. The RT varied between 45 °C and 65 °C and the HT between 60 and 120 s. An RT of 45 °C and a HT about 60 s led to a decline of the viable cell count. An increase in HT with the same RT had no higher reducing effect. A similar reduction of cells to that at 45 °C was conceded at an RT of 55 °C and HT of 60 s. A longer HT at same RT showed a decrease in the viable cell count. The highest reduction was achieved at a CT of 30 °C and an RT of 65 °C (Fig. 5).

The RT was set to 30 °C, CT varied between 45 and 65 °C and HT between 60 and 120 s in the second part of test runs. A CT of 45 °C and an HT about 60 s led to a reduction of the viable cell count. The variation of HT did not result in a stronger decrease of cells. The increase of the CT to 55 °C showed a greater decline of microorganisms. Varying the HT had no enhancing effect on the reduction of the cell count. A similar decline to a CT of 55 °C of microorganisms showed the test run with a CT of 65 °C (Fig. 6).

3.4 Reduction factor of test runs according to DIN SPEC 10534

In addition to the hygienic performance, the reduction factor of each bioindicator per test run was calculated (Table 4). A reduction factor of (3.8 ± 0.9) $\log_{10}$-stages was determined

in the test run with a CT of 30 °C, an RT of 45 °C, and an HT of 60 s. Test run two showed similar results to test run one. In test run four, only one bioindicator reached a reduction of ≥5 $\log_{10}$-stages and in test run five, 50 % of bioindicators showed a reduction of ≥5 $\log_{10}$-stages. In test runs six and seven, a reduction of ≥5 $\log_{10}$-stages was determined for all bioindicators. Test runs with an RT of 65 °C and an HT of 90 and 120 s were omitted as it is assumed that all bioindicators would fit the requirements.

In test run eight, only one bioindicator indicated the reduction of ≥5 $\log_{10}$-stages. The other bioindicators achieved a reduction of (4.05 ± 0.65) $\log_{10}$-stages. In test run nine, 50 % of the bioindicators reached a reduction ≥5 $\log_{10}$-stages. In test runs 11 to 14 each bioindicator showed a reduction ≥5 $\log_{10}$-stages. Analogous to test runs with an RT of 65 °C, increasing the HT was not conducted for test runs with a CT of 65 °C.

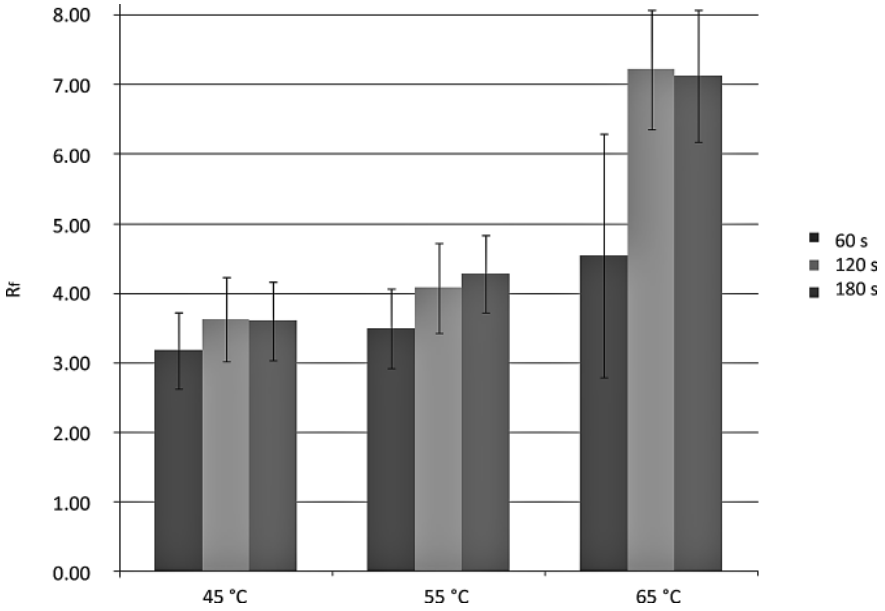

Figure 4 Results of test runs with *M. luteus*

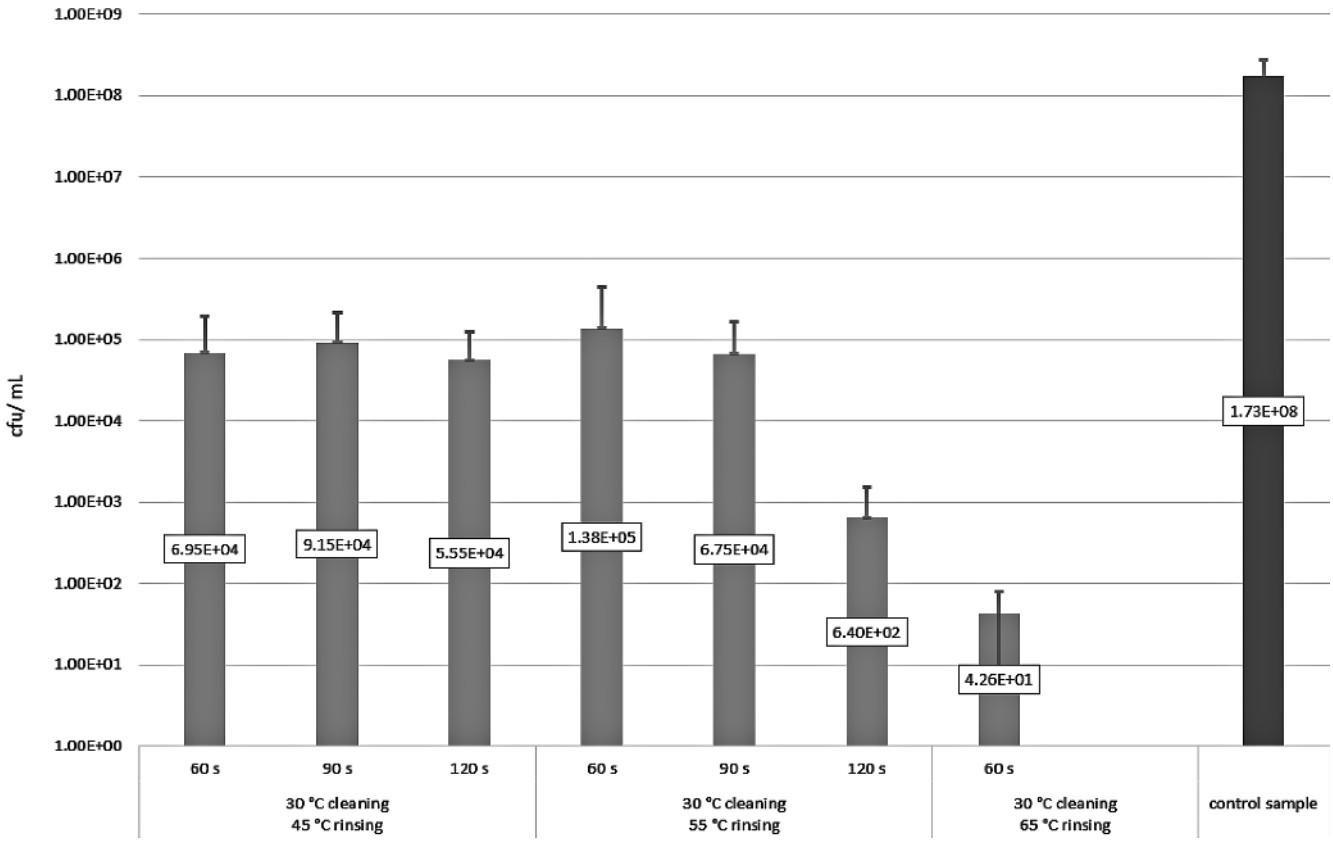

Figure 5 Comparison of test runs with constant cleaning temperature (test runs 1, 2, 3, 4, 5, 6 and 7)

It was determined over all test runs that the bioindicators in the front of the rack (4, 5 and 6) often showed a stronger reduction of the cell count than the bioindicators in the back (1, 2 and 3).

4 Discussion

The initial investigation of this study identified *M. luteus* as an appropriate test strain to replace *E. faecium* in hygiene studies, especially in studies with undercounter-one-tank dishwashers. The other test strains *L. macroides, P. fluorescens, and G. atrophaeus* could not reach the required cell count on bioindicators. The following test runs with an undercounter-one-tank dishwasher and *M. luteus* as the test strain showed a significant difference when increasing the temperature about 10 K at the same HT. With respect to this outcome in further investigations *M. luteus* was used as a test germ for the following experiments.

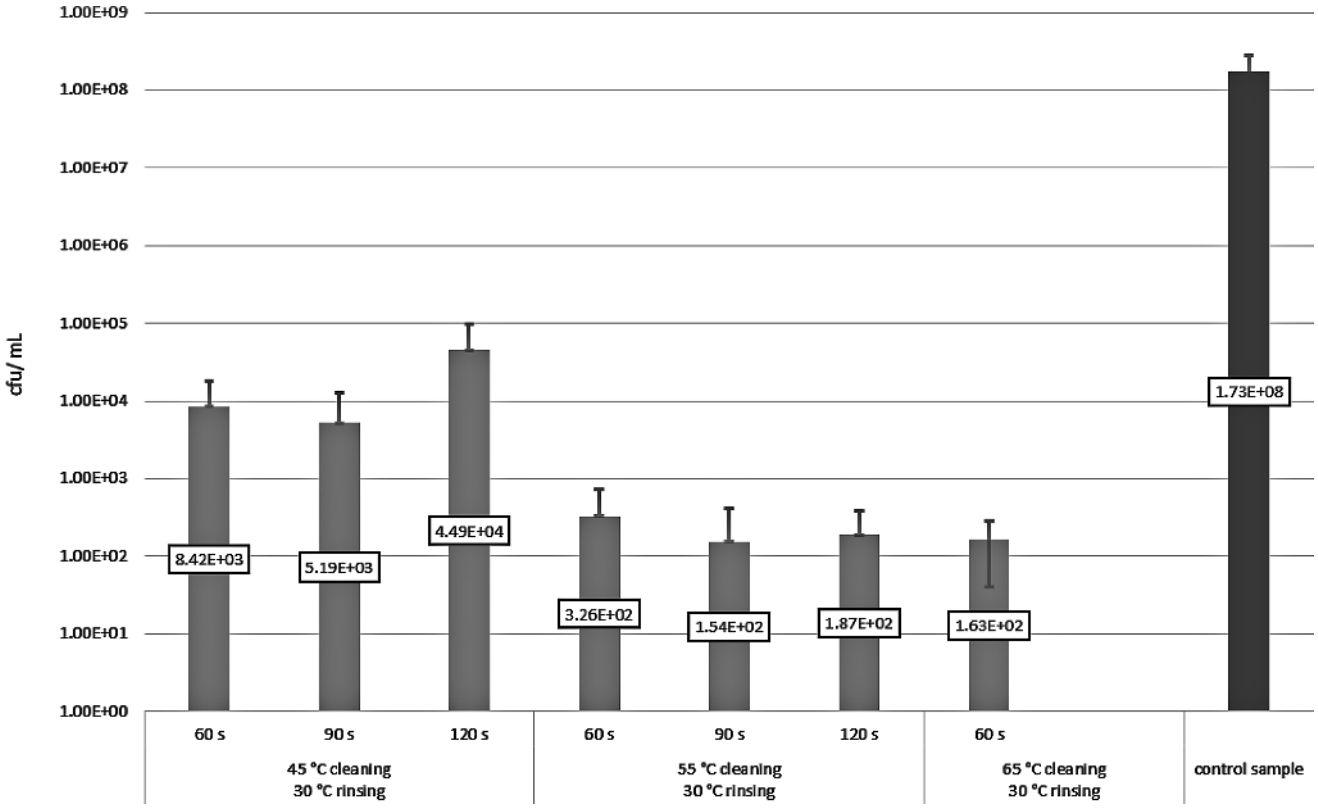

Figure 6 Comparison of test runs with constant rinsing temperature (test runs 8, 9, 10, 11, 12, 13 and 14)

test run	bioindicator 1	bioindicator 2	bioindicator 3	bioindicator 4	bioindicator 5	bioindicator 6
	Reduction factor $\log_{10}$-stages					
1	2.90	4.10	4.40	4.40	4.40	4.70
2	3.20	2.90	4.50	4.60	4.50	4.50
3	3.40	3.10	4.20	4.30	4.20	4.70
4	2.50	4.30	4.80	4.70	**5.10**	4.80
5	3.00	3.20	4.70	**5.00**	**5.10**	**5.10**
6*	**5.00**	**5.90**	**6.20**	**5.60**	**6.20**	**6.50**
7*	**6.60**	**6.60**	**6.30**	**7.20**	**8.30**	**7.20**
8	3.40	3.50	4.20	4.70	**5.00**	4.70
9	3.40	3.60	**5.00**	**5.70**	4.90	**7.70**
10	2.60	2.60	3.50	4.10	4.00	4.10
11*	**6.00**	**6.10**	**6.10**	**5.90**	**5.10**	**5.70**
12*	**5.90**	**7.10**	**5.10**	**6.20**	**6.80**	**6.60**
13*	**5.40**	**7.90**	**7.90**	**5.30**	**7.90**	**5.40**
14*	**6.80**	**5.90**	**5.80**	**8.30**	**6.10**	**6.10**

Table 4 Reduction factor of bioindicators per test run (* 90 % of bioindicators reached reduction of 5 $\log_{10}$-stages)

According to the results obtained with the water-change dishwasher, it can be shown that an increase of temperature and HT led to a decrease in the viable cell count. Harpel et al. [18] came to similar results in 1994 in an experimental study with *E. faecium*. In their study, they used a CT from 50 °C to 60 °C and an RT of 63 °C. They came to the conclusion that a combination of temperature and HT constitutes an important part of cleaning results [18].

Comparing test runs with RT and CT at 45 °C, a higher CT caused lower cell counts than the same RT. The same outcomes were obtained in test runs with a CT and RT of 55 °C. The test run with an RT of 65 °C led to a reduction 0.8 $\log_{10}$-stages higher than the test run with a CT of 65 °C.

According to Sinner [19], a combination of temperature, mechanics, chemistry and time led to the best cleaning results. If one of the four factors is decreased one of the others has to be increased to achieve the same results [19].

A reduction of test germs during the dishwashing process is caused by a combination of moist heat and chemical influence. The antimicrobial effect of moist heat is mostly based on a denaturation of transport, and the structural and catalytic proteins of cells. Subsequently, they lose their ability to multiply. Chemicals penetrate into the cells and react with inner cell substances which are essential for the survival of the cells. The surfactants in detergents stop the oxygen exchange with the environment and lead to an inactivation of the cell [20].

Furthermore, the pH-value can also have an effect on cell growth [21, 22]. The pH-values vary between the cleaning detergent (pH 14) and the rinsing aid (pH 2). The antimicrobial effect of the detergent increases with higher temperature and longer HT [21]. It is possible that the combination of the temperature of 65 °C, the ingredients and pH-value of the rinse aid has a higher antimicrobial effect than the combination of the same temperature and the cleaning detergent.

Another reason for the high variations in some experimental series could be attributed to the technical characteristics of spray arms. The spray arms of dishwashers circulate according to the different number and arrangement of nozzles. Water is distributed over the wash ware with the help of circulation [23].

When water hits bioindicators at specific positions a higher reduction of germs is reached. The cell count of bioindicators which were placed in front of the basket showed a higher reduction of cell count then the ones at the back. It might be possible that plates from the front cover the plates in the back part and therefore, bioindicators in the back are less accessible to the water.

According to DIN SPEC 10534 a commercial dishwasher fulfils the hygienic requirements if a reduction factor of 5 $\log_{10}$-stages is determined in 90 % of bioindicators tested. For the other 10 % a reduction of 4 $\log_{10}$-stages is established [15].

Regarding the results of the reduction factor, an adequate hygiene performance was indicated in test runs with an RT of 55 °C and HT of 120 s, and an RT of 65 °C and HT of 60 s. Furthermore, test runs with a CT of 55 °C and 65 °C each with an HT showed an appropriate hygiene performance.

To ensure hygienic safety of wash ware in commercial institutions, considering these results, temperatures ≥55 °C and an HT of 60 s in the cleaning process should be achieved during dishwashing. As in commercial dishwashing temperatures are usually set between 55 °C and 65 °C [21], therefore, no higher risk of infection diseases can be assumed from the wash ware.

5 Conclusion

This study showed the applicability of DIN SPEC 10534 to dishwashers with a water-change system, especially using a short program. Furthermore, it has been proven that a risk group 1 test germ (*M. luteus*) can be used to test the hygienic performance of commercial dishwashers. It could be recognized that hygienic performance changes, if temperature and HT of the cleaning and rinsing process were varied. The requirements of DIN SPEC 10534 could be implemented in 6 of 14 test runs.

Whether there are limits of the appliance or the method when using other cleaning programs with different profiles have to be investigated due to using only one program with various temperatures and HT in this study.

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