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Development of a Method for the Analysis of Microbial Load Reduction Factors on Dishes Cleaned by Hand and by Machine

The aim of this study was to develop a repeatable method for the measurement of microbial load reduction factors after hand and machine dishwashing processes that meet the requirements associated with consumer studies, based on non-pathogen test strains. Microorganisms were isolated from tableware, identified and cultured for this purpose, mixed with soiling agents and lyophilised for storage purposes. Detailed instructions for ambient conditions and disinfection, and preparation and application of soiling agents were defined. The method chosen in this study successfully led to repeatable results. The food matrix of the soiling agents used for the application of test strains shows to have a large impact. This is, therefore, the first repeatable method that enables the comparison of microbial load reduction factors after machine with hand dishwashing. In addition, the method was designed in a way that enables further consumer studies.

Key words: Microbial Load Reduction Factor, microorganisms, disinfection, machine dishwashing process, hand dishwashing process

Entwicklung einer Methode zum Nachweis der Keimzahlreduktion auf handgespültem und maschinengespültem Geschirr. Zielstellung der Arbeit war die Entwicklung einer Methode zum Nachweis der Keimzahlreduktion, die mit guter Wiederholgenauigkeit sowohl beim manuellen als auch beim maschinellen Geschirrspülen eingesetzt und durch die Verwendung nichtpathogener Testkeime auch für Verbraucherstudien angewendet werden kann. Dazu wurden geeignete Keime von verschmutztem Geschirr isoliert, identifiziert, kultiviert, mit bestimmten Lebensmitteln gemischt, gefriergetrocknet und portionsweise eingefroren. In der Durchführung der eigentlichen Versuche wurden diese Lebensmittelverschmutzungen auf Geschirrteilen nach genau festgelegten Methoden aufgebracht und auch die sonstigen Randbedingungen genauestens definiert. Die Ergebnisse der Versuche zeigen bei einer guten Wiederholgenauigkeit den großen Einfluss der Lebensmittelmatrix, in der die Mikroorganismen eingebracht werden. Insgesamt zeigt sich diese Methode als sehr gut geeignet, um die Keimzahlreduzierung an Geschirr unter realitätsnahen Bedingungen bestimmen zu können.

Stichwörter: Keimzahlreduktion, Mikroorganismen, Desinfektion, maschinelles Geschirrspülen, Handgeschirrspülen

1 Introduction

The kitchen is a place of special microbiological interest because food processing and its typical microbiota and the environmental conditions (warm, moist) represent a beneficial habitat for microorganisms [1]. Consequently, the process of dishwashing, defined as the removal of food leftovers on the tableware [2], should be considered from a hygienic point of view. This includes microorganisms which survive the dishwashing process, remain on the tableware's surface and, furthermore, are not visible to the naked eye [3]. The detection of black yeasts in dishwashers [4] and the survival and cross-contamination of food-borne pathogens in the kitchen environment during dishwashing by hand [5] strengthen this aspect.

Although the market saturation of dishwashers is growing, dishwashing by hand is still widespread [6–8]. Therefore, it is necessary to investigate both dishwashing processes.

Several studies emphasize that machine dishwashing reduces the microbial load on tableware more effectively than manual dishwashing by hand [9–12]. Hand dishwashing and drying with a tea-towel could increase the total number of microorganisms up to seven-fold compared to the number before washing [1].

High temperatures are especially considered as most effective for microbial load reduction [1, 13]. Johansson [14] also concluded that the largest influence is due to high temperatures. However, the temperature is only one of the variables influencing the effectiveness of microbial load reduction. In addition to the temperature, the mechanical action, time and chemistry belong to the cleaning factors influencing the cleaning result, represented in Sinner's Circle [15].

Furthermore, it has been shown that several aspects influence the removal efficiency of microorganisms in dishwashing processes: the type of organism and its specific adsorptive strength, the tableware's material, and the surface structure on the tableware. Moreover, it has been shown that the removal efficiency of microorganisms that were mixed with a food matrix is increasing [16]. Mattick [5] investigated the survival of various microorganisms in food debris on soiled dishes, during the washing-up process and subsequent drying, and the potential for transfer of surviving pathogens was also assessed. Their results demonstrate that bacterial pathogens were not always inactivated during washing-up and *Escherichia coli* O157 in particular could withstand subsequent drying on dishes. The risk of transfer from contaminated dishes to a sterile food was low but the contamination of towels and washing-up sponges that may be used to wipe hands and work surfaces, respectively, was of more concern [5]. Therefore, it is of high relevance to understand which kind of consumer relevant dishwashing process delivers which reduction rate of microorganism under which conditions. Lee et al. investigated the sanitization efficacies of commercial manual three-compartment dishwash-

ing processes as a function of washing temperature/time, contaminating organic matter, sanitizing condition, and bacterial type [17]. Although *E. coli* showed better survival when compared with *Listeria innocua* for jellied utensils, there was no significant difference in survival between them for all other washing conditions, reflecting the high variation in the test results for almost all test conditions.

Several studies examining the reduction efficiency of dishwashing processes apply various different methods, such as contact plates [1, 18] or swab rinse/plating out procedures [13, 19]. Radioactive isotopes are also used to detect bacteria [16]. The selection of test strains varied according to the properties, such as heat or chemical tolerance, toxin production, or because they were generally accepted as reference strains [11, 16].

All aforementioned studies suffer from being either not applicable to real-life test scenarios as pathogen test microorganisms are used or not applicable to compare hand and machine dishwashing under the same conditions. Additionally, most studies did not reveal how reproducible they are, in the sense that no repeated tests are done in the same laboratory using the same conditions.

Non-pathogenic test strains are proposed and established in this study to provide a test system also for those laboratories which are not equipped to work under high safety conditions required for handling of risk group 2 microorganisms. Involving consumers as test persons for doing the manual washing up process in a laboratory will be much different and no longer representative to a household process under risk group 2 conditions. Moreover, the test strains used in this study were isolated from soiled dishes and therefore represent authentic isolates adapted to this specific habitat. This is in contrast to most pathogenic test strains which originate from other sources and were adapted to other habitats.

This study focused on the measurement of reduction factors in a comparison of machine and hand dishwashing. Moreover, the method should be applicable for consumer studies in which the non-pathogenicity is a top priority for the selection of test strains and should deliver a good repeatability of the result. Therefore, a new method for preparing equally inoculated test batches of test soils, the lyophilisation process, was applied.

2 Material and Methods

2.1 Isolation and characterization of test strains

Soiled dishes were sampled by contact tryptic soy agar plates (RODAC plates). The agar plates were then incubated for two days at 30 °C. Strains were isolated from these plates on tryptic soy agar at 30 °C and characterized by their cell and colony morphology, Gram reaction, KOH test, aminopetidase test (Bactident, Merck, Darmstadt), and their fatty acid profiles according to Wiertz [20]. Selected isolates were identified by their 16S rRNA gene sequences, as described previously [20]. From these identified isolates, those strains were selected which comply with the following criteria: good growth on trypticase soy agar, formation of distinct and typical colonies on this agar, no pathogenic potential (risk group 1), and high tolerance for freeze-drying. Three strains from three different bacterial phyla were found to comply with these criteria and were used for this study as test strains: *Micrococcus luteus* WS7, *Pseudomonas geniculata* WS40 and *Lysinibacillus macroides* WS26. The strains were deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, with the accession numbers DSM 28269, DSM 28278 and DSM 28270. The 16S rRNA gene sequences of the test strains are deposited at GenBank with the accession numbers KJ452162, KJ452163 and KJ452164.

2.2 Cultivation of test strains

Firstly, cells were transferred from tryptic soy agar (TSA) into 100 ml tryptic soy broth (TSB) and incubated for 48 h at 30 °C. After growth cultures were transferred into centrifuge tubes and were centrifuged for 10 min at 12,900 × *g* (*Eppendorf 5810R,* Hamburg*)*. The cell pellets were re-suspended and washed twice with Ringer solution.

2.3 Food pasteurisation

The food samples intended as a soiling matrix were pasteurised to minimize the microbial background and, simultaneously, to increase the accuracy of the method.

An amount of 300 g of frozen spinach was cooked for 10 min, stirred with a fork and had to reach >80 °C for at least 2 min. It was passed through a grinder with a 2 mm mesh size whilst still hot [21].

An amount of 300 g of fragrant rice was washed with 300 g water, which was poured off using a sieve. The rice was then cooked in an automatic rice cooker and filled up to 800 g with water for pasteurisation [22].

2.4 Batch production

Thereafter, the spinach was mixed with the cell suspension of *Lysinibacillus macroides* WS26, and the rice with the *Micrococcus luteus* WS7 cell suspension, each in the ratio 1:9 (w:w, test strain: food), and each was then stirred with a disinfected fork or spoon for three minutes. The mixture of test strains and food matrix is defined as a "batch". Rice and spinach were used as carrier medium because they represent common soils in dishwashing. Rice especially represents sticky soils due to his high starch content. Spinach in contrary is representing particular soils. Moreover, it was important to choose tableware for the application of the batch which was present in sufficient number and which was suitable for the evaluation by surface swabbing.

The batch was portioned into laboratory vessels, frozen (for at least 24 h) and freeze-dried (96 h; –20 °C; 1 mbar). Lyophilized samples were sealed with parafilm and aluminium foil and were stored under dark, dry conditions at room temperature.

The concentration of test strains was measured, firstly, directly after batch production before freezing, and secondly, after different days of storage. Therefore, each batch was diluted with Ringers solution (1:9, w/w) and homogenized in a paddle blender (stomacher). A decimal dilution series was carried out and 100 µl of each dilution were plated out for colony counting.

2.5 Procedure on testing day

The preparation and application of soiling agents were similar to IEC 60436 [21]. However, some modifications were necessary because this study was targeted to be first applied on Chinese consumers, so it was orientated on Chinese eating and drinking habits. The tableware (see Table 1) used was also oriented on consumer habits regarding what items will have to be cleaned at home by hand or by machine. The full capacity of a dishwasher was the basis of the tableware investigated.

All actions described below refer to test protocol defined for this purpose. Firstly, the lyophilized batch samples were regenerated with boiled water and the other fresh soiling agents were prepared and pasteurized. The soiling agents (see Table 1) were applied onto the tableware with silicone brushes and air-dried for 2 h in controlled ambient conditions (room temperature (23 ± 2) °C; relative humidity (55 ± 5) %). A dishwasher (model: SN55M531TI) from *Bosch and Siemens Household Appliances (BSH,* München*),* developed for the Chinese market, was used for the machine dishwashing. The Eco 50 °C programme was chosen, which reached 63 °C final rinse temperature and took 198 min. The tableware was loaded according to a fixed loading scheme. The dishwasher was cleaned, empty of tableware, after each test run in a dishwashing cycle using the *Magic Daily* programme that reaches 70 °C and the chloric (5 %) *Guardian Plus* detergent from *Ecolab* (Monheim a. Rhein).

The dishwashing by hand was carried out in a double basin sink at a constant water temperature of 35 °C following a fixed protocol (Table 2). This washing order has been chosen to enable repeatable results. Overall, the hand dishwashing of the same load, soiled with the same soling agents took, on average, 30 min.; the tableware was soaked for 20 s in the first sink and the main washing took place in the second sink, using the soft side of sponges. The process was always carried out according to a fixed washing order, by the same person, who wore gloves. Finally, the tableware was drained and air-dried for 1 h. The surface of the sinks were washed and disinfected using the disinfection spray *Meliseptol* from *Braun* (Melsungen) before and after every hand dishwashing cycle.

2.6 Microbiological analyses

A total of 16 pieces of tableware (Table 1) were microbiologically analysed after each dishwashing cycle. A defined area of 49 cm^2 for plates and 36 cm^2 for the bowl was sampled by cotton-wool swabs

The swabs were moved across the surface according to a fixed scheme, where the procedure, movement and pressure of the swab was similar for each sampling. Each swab was transferred into 10 ml Ringer solution and mixed using a vortex for at least 10 s.

The cell suspensions were analysed by the membrane filtration method, which has a detection limit up to 1 cfu per 10 ml. The filter unit was sterilized with denatured ethanol, flamed and rinsed off with sterile water. A membrane filter with a pore size of 0.2 µm and a diameter of 47 mm was placed onto the frit. The sample was put into the funnel and was exhausted until empty. The filter unit was sterilized again with denatured ethanol before its next use.

To prevent blocking of the filter, samples taken from hand dishwashing were diluted before filtering. The degree of dilution depends on the initial microbiological load of the items and their position in the dishwashing sequence.

The membrane filters were transferred on a tryptic soy agar plate and incubated for 72 h at 30 °C. Plates with individual colonies between 0 and 300 were counted after 48 h

Soiling agent	Weight of soiling agents in g item^{-1}	Tableware	Microbiological analysis
Spinach (*Lysinibacillus macroides* WS26)	3.0	Dinner plate (5×)	(5×)
	3.0	Bowl (5×)	
Rice (*Micrococcus luteus* WS7)	1.0	Rice bowl (10×)	(5×)
	0.5	Rice spoon (1×)	
Without soiling agent	0.0	Oval plate (1×)	(1×)
	0.0	Soup bowl 1 (1×)	(1×)
Soya paste	3.0	Wok (1×)	
	1.0	Lunch box (1×)	
	1.0	Cooking knives (2×)	
	1.0	Cooking spoon (2×)	
Flour soup	dip approx. 30 mm into the flour soup	Chopsticks (20×)	
	2.0	Soup bowl (1×)	
	2.0	Soya milk filter (1×)	
Pork soup	1.5	Pot (1×)	
	0.5	Serving spoon (2×)	
	0.2	Soup spoon (10×)	
Egg yolk powder	1.5	Dinner plate (1×)	
	2.0	Bowl (3×)	
Milk powder	10.0	Glasses (5×)	
Green tea	25.0	Mug (5×)	
In total	about 240 g per dishwashing cycle	78 items	12 items

Table 1 Summary of soiling agents and tableware

of incubation. Colonies, which showed a clearly different colony morphology than the test strains, were not taken into account in the evaluation.

Ebner [23] proved that household dishwashers can be a suitable means to disinfect medical equipment. Therefore, the tableware was washed after investigation in a hygienic dishwasher from *Miele* (G7882, Gütersloh), which reached a water temperature of 93 °C for about 10 min. A chloric detergent (5 %) *Guardian Plus* from *Ecolab* (Monheim a. Rhein) was used. Additionally, the tableware was dry-sterilized in a drying chamber for at least 1 h at 170 °C.

2.7 Statistical analysis

A total of five experiments of machine (M1-M5) and five experiments of hand dishwashing (H1–H5) were performed. Five dessert plates, soiled with spinach and *L. macroides* WS26, respectively five rice bowls, soiled with rice and *M. luteus* WS7 were included in each experiment. Therefore, two datasets (Sp; R) could be calculated and a total of four experimental groups existed after one dishwashing cycle: spinach/machine wash (Sp-M), spinach/hand wash (Sp-H), rice/machine wash (R-M), and rice/hand wash (R-H).

The reduction factor was calculated following:

$$R_F = \log_{10}(a) - \log_{10}(b) \quad (1)$$

with:
R_F: as the reduction factor,
a: as the number of cfu per 10 cm^2 per item before dishwashing, and
b: as the number of cfu per 10 cm^2 per item after dishwashing

As experimental data are always influenced by statistical uncertainties, we decided to execute five repetitions for each experimental group to assess the repeatability of the results. The reduction factor for our experiment was calculated as the median of the five items of each experiment. The arithmetic average and standard deviation was calculated using the five median values for each experimental group.

3 Results

The cell suspension of *L. macroides* WS26 and *M. luteus* WS7 had similar initial counts (Table 3). The total cell concentration of *L. macroides* WS26 during lyophilisation and storage decreased by an order of magnitude, whereas the cell concentration of *M. luteus* WS7 remained rather constant both after lyophilisation and after storage. Finally, at the plates with spinach and *L. macroides* WS26 a concentration of 2.3×10^4 cells per 10 cm^2 and at the bowls with rice and *M. luteus* WS7 a concentration of 6.5×10^6 per 10 cm^2 could be applied.

After washing reduction factors were calculated for each item following (1) (Table 4). Comparing the reduction factors for each batch within the machine and hand dishwashing cycles show quite similar values. For rice bowl #4 a tendency of smaller reduction factors for all machine washing cycles may be spotted. This may be associated with the position this bowl has in the dishwasher basket. Also machine cycle #4 shows low reduction factors on almost all items measured. The reason is unknown, but this cycle may be seen as an outlier. Not on all washed and inoculated items microbiological residues could be detected. If no microbiological residues could be detected the reduction factor for these items was assumed to be higher than the highest re-

Nr.	Item	Soiling agent	Description
1	Filter (1×)	Flour	3× in circle upper rim, 3× in circle lower rim
2	Mug (5×)	Tea	4× in circle
3	Wok	Soya	3× in circle at sides, 3× in circle at bottom
4	Dinner plate (1×)	Egg	4× in circle anticlockwise, 4× in circle clockwise
5	Dinner plate (5×)	Spinach	4× O anticlockwise, 4× O clockwise
6	Bowl (3×)	Egg	4× in circle anticlockwise, 4× in circle clockwise
7	Soup bowl (not soiled) (1×)	No soiling agents	3× in circle at rim, 3× in circle at bottom
8	Soup bowl (1×)	Flour	3× in circle at rim, 3× in circle at bottom
9	Bowl (5×)	Rice	5× in circle
10	Rice spoon	Rice	2×↑↓ top to bottom, both sides
12	Pot	Pork	3× in circle at sides, 3× in circle at bottom
13	Bowl (3×)	Spinach	4× in circle anticlockwise, 4× in circle clockwise
14	Oval plate (1×)	No soiling agents	3× ↑↓ top to bottom,
15	Lunch box (1×)	Soya	2× ↑↓ bottom, 2× ↑↓ rim
16	Cooking spoon (2×)	Soya	2×↑↓ top to bottom, both sides
17	Cooking knife (2×)	Soya	2×↑↓ top to bottom, both sides
18	Soup Spoon	Pork	2×↑↓ top to bottom, both sides
19	Serving Spoon	Pork	2×↑↓ top to bottom, both sides
20	Chopsticks (20×)	Flour	2×↑↓ top to bottom, both sides
21	Glasses (5×)	Milk	5× in circle

Table 2 Detailed description of manual dishwashing

duction factor detected for this type of batch (indicated with ">" in Table 4). These values were treated as if they were at the highest level detected for the calculation of the median (Table 5).

We investigated qualitatively whether cross-contamination occurred on two unsoiled items (oval plate and soup bowl) (see Table 1). The items tested positive for cross-contamination in 6 of 10 cases after machine dishwashing. However, cross-contamination could be detected after each hand dishwashing cycle, meaning 10 out of 10 cases.

4 Discussion

The approach of using lyophilised test strains, already combined with the soiling agents shaped up to be very successful, at least for the use of *M. luteus* WS7 in combination with rice. The advantage is that the test strains are easy to store and that the viable counts remained largely constant. Thus ensured that the same initial count of each batch could be applied on each testing day, which is a relative simple way to ensure repeatable microbiological test conditions. Working every day with fresh test strains on freshly prepared soil batches would more likely lead to greater fluctuations and thus hinder a good repeatability. The use of *L. macroides* WS26 together with spinach did not perform as good, as during lyophilisation and storage a loss of at least one order of magnitude of the cell concentration could be detected.

In accordance with previous reports, we also detected cross-contamination during the dishwashing process by the dishwater or sponge on previously heat-sterilized tableware

	Lysinibacillus macroides WS26		*Micrococcus luteus* WS7	
Soiling agent	**Spinach**		**Rice**	
Cell concentration of cultures in cfu ml^{-1}	$1.1 * 10^9$		$1.6 * 10^9$	
Batch fresh (cfu g^{-1})	Not evaluable		$2.1 * 10^8$	
Batch lyophilised				
Number of samples	2	4	2	4
Days of storage	12	97	11	97
Cfu g^{-1}	$2.2 * 10^5$	$2.7 * 10^4$	$9.4 * 10^7$	$8.6 * 10^7$
Arithmetic mean after storage of 1 g batch	$1.2 * 10^5$		$9.0 * 10^7$	
Cell concentration on 10 cm^2 of tableware	Dessert plate with 3.0 g $2.34 * 10^4$		Rice bowl with 1.0 g $6.55 * 10^6$	

Table 3 Cell concentration of *Lysinibacillus macroides* WS26 in spinach, respectively *Micrococcus luteus* WS7 in rice batch during several moments of investigation

	#	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5
Spinach, dinner plates	1	>5.1	5.1	4.8	3.4	5.1	5.1	4.3	4.4	5.1	>5.1
	2	>5.1	>5.1	>5.1	4.0	5.1	>5.1	4.2	5.1	3.9	4.6
	3	5.1	5.1	5.1	4.3	4.4	4.2	4.2	4.2	>5.1	4.6
	4	4.8	>5.1	4.5	3.9	2.6	4.8	4.5	5.1	4.6	5.1
	5	>5.1	>5.1	>5.1	4.2	5.1	4.8	4.3	4.6	4.5	4.6
Rice, rice bowls	1	>7.4	>7.4	>7.4	6.1	>7.4	2.2	1.8	2.1	2.1	1.7
	2	>7.4	6.4	>7.4	5.3	6.7	1.8	1.7	1.9	1.6	1.8
	3	7.4	7.4	7.4	5.5	7.4	2.3	2.7	1.3	2.2	1.7
	4	4.9	5.9	6.6	5.3	5.6	1.6	1.7	2.0	2.5	1.4
	5	7.4	4.9	4.9	6.0	7.4	2.0	0.5	2.2	1.8	1.6

Table 4 Reduction factor of spinach and rice batches after machine (M) and hand (H) washing for all experiments

		M1	M2	M3	M4	M5	H1	H2	H3	H4	H5
Spinachon dinner plates	Median	4.9	5.1	4.8	4.0	5.1	4.8	4.3	4.6	4.5	4.6
	Arithmetic mean ± SD	4.9 ± 0,5					4.6 ± 0,2				
		M1	M2	M3	M4	M5	H1	H2	H3	H4	H5
Rice on rice bowls	Median	7.4	6.2	6.6	5.5	7.0	2.0	1.7	2.0	2.1	1.7
	Arithmetic mean ± SD	6.8 ± 0,8					1.9 ± 0,2				

Table 5 Median of reduction factors, arithmetic mean and standard deviation (SD) of spinach and rice batches after machine (M) and hand (H) washing for all experiments

[1, 11, 14, 19]. Cross-contamination occurred on every item in each hand dishwashing experiment, whereas it was detected less often after machine dishwashing.

The maximum reduction factors achievable of *L. macroides* WS26 (Sp) and *M. luteus* WS7 (R) differ from each other due to different initial counts of the batches (see Table 3). Comparing the arithmetic average of the medians of (Table 5) the reduction factors for spinach soiling, the variation between machine and hand dishwashing is quite low (0.3 RF) and not significant. The difference between the rice soiling is clearly higher (4.9 RF). This difference in the reduction factor by food soil to hand and machine dishwashing may be attributed to differences in the food structure. As reported in Souci [24], rice which has been cooked and, subsequently, dropped off consists of 19% of utilizable carbohydrates, while spinach which has been cooked and dropped off consists of only 0.45% of those carbohydrates. The rice obviously agglutinated and hardened on the tableware during air-drying, whereas the spinach, consisting of 95% water [24], was still very moist and was solvable from the tableware using a low mechanical action. The data suggest (in accordance with Krüger [18]), therefore, that the drying period and the food matrix of soiling agents have a high impact on the removal efficiency of test strains. Therefore, any microbiological reduction assessment comparing machine and hand dishwashing need to carefully select the appropriate, consumer relevant, conditions of which strains are to be used, in which food matrix they are applied, on which items they are applied and how intensive they are dried on those items.

For all tested configurations the standard deviation of the reduction factors within the test series is quite low (Table 5). Considering the fourth experimental group (M4) as an outlier, it would be even lower. While for machine dishwashing the process of cleaning itself has a good repeatability (as it is an automatic process) the low standard deviation shows that also the process of producing the batches of contaminated soiling and the evaluation of the residues have a high level of repeatability. For dishwashing by hand the cleaning process itself is far less standardised. As the standard deviations of the reduction factors of the hand dishwashing process are even lower as for the machine dishwashing, this means that the approach to specify a well-defined procedure of dishwashing by hand was quite successful. Following this conclusion, it seems now to be possible to compare different hand dishwashing procedures regarding their microbiological reduction factors by just defining the way the washing is done in a precise procedure as done in this study. As dishwashing by hand was found to be done very different from consumer to consumer and from country to country [25–28] this will allow to judge these differences also regarding their hygienic aspects. Additionally, the here presented procedure may be used in laboratory studies were "normal" consumers are asked to clean soiled dishes under controlled conditions, like in [25, 27, 28].

In conclusion, the use of lyophilisation of soiling agents with authentic, non-pathogen test strains allows the definition of a soiling process of tableware with a high level of consumer relevance and, together with a well-defined test protocol, enables the repeatable measurement of reduction factors of hand or machine dishwashing under comparable conditions. However, this investigation is only a first step. There are many points which need to be investigated further, like the influence of the food matrix the germs are applied, the tableware the batches are applied at, the cross-contamination, and all in all the test conditions like the program temperature, detergent type and amount. The method presented here provides two fundamentals for answering these questions: The implementation of easy to handle non-pathogenic test strains and the application of lyophilized test material, which allows for a high level of standardization due to the preparation of larger batches with an excellent stability.

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