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PLATFORM FOR PROFESSIONAL CLEANING

HYGIENE OF REFILLABLE SPRAY BOTTLES II

Research into the hygienic effect of a WIP/RIVM treatment in simulated use and in institutional cleaning practice.

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SUMMARY

In the past, questions have been posed regarding the hygienic condition of spray bottles used in institutional cleaning for cleaning surfaces (Bilkert M., 2016). Since no research data was available for the Dutch situation at the time regarding the hygienic condition of spray bottles, Dutch the Cleaning Research Association (VSR) decided to conduct research in this area.

It led to the study *Hygiene of Refillable Spray Bottles* (Terpstra & Kessel, 2018). The aim of the study was to explore and determine whether spray bottles in the institutional cleaning sector are microbially contaminated, and if so, pose a hygiene risk. Additionally, if a microbial contamination does exist, to determine whether the organisms are freely found in the residual liquid in the spray bottles (free germs) or also in any biofilm (bound germs). And finally, to determine whether an existing contamination can be eliminated with a single hygienic treatment using a disinfectant (active chlorine). The study revealed that the liquid in refillable spray bottles used in institutional practice may be microbially contaminated. Germs were found in 33 of the 55 spray bottles examined. The degree of contamination ranged from 3.0 LOG CFU up to 9.0 LOG CFU per spray bottle. Furthermore, it appeared that the spray bottles contained both free germs and bound germs. The numbers of bound germs were in the same order of magnitude as the numbers of free (unbound) germs. The results further showed that a single hygienic treatment of contaminated spray bottles does not result in uncontaminated spray bottles.

The aim of the present study is to investigate to what extent the hygiene of spray bottles in institutional cleaning practice improves with the application of a daily hygienic treatment in compliance with the guidelines of the Dutch Working Party on Infection Prevention (WIP) and the Dutch National Institute for Public Health and the Environment (RIVM). To achieve this, a laboratory simulation study and a field study were conducted.
In the laboratory study, the effect of the hygienic treatment was investigated in a set-up where spray bottles were exposed to an infected cleaning agent for 6 hours every day for a period of 14 and 28 days. Half of the spray bottles in the study were treated hygienically (daily) after exposure in accordance with the WIP/RIVM guidelines; the other half received no hygienic treatment. A neutral daily cleaner, a neutral interior cleaner and an alkaline sanitary cleaner were used as cleaning agents.

The spray bottles that were exposed to contaminated sanitary cleaner and treated hygienically remained uncontaminated. In all other spray bottles, contamination was found after 14 and 28 days. The degree of contamination (total plate count) of the hygienically treated spray bottles is roughly 3.5 decimal places lower than that of the untreated spray bottles.

In the field study, unused new spray bottles were issued at 7 Dutch healthcare institutions. The cleaning staff were requested to use the spray bottles in their normal daily routine.

In addition to this, the spray bottles had to be treated hygienically at the end of every working day in compliance with the hygiene guidelines of the Dutch Working Party on Infection Prevention (WIP) and the Dutch National Institute for Public Health and the Environment (RIVM). After a period varying from 11 to 52 days, the spray bottles were collected for hygienic examination. In 3 of the 7 institutions, no contamination was found in any of the spray bottles used. Contamination to a greater or lesser degree was found in the spray bottles from the other 4 institutions. The infection rate for the contaminated bottles ranged from 3.2 to 7.0 LOG CFU. Comparison of this result with previous research provides indications that the average and maximum degree of contamination is reduced by a hygienic treatment in compliance with the WIP and RIVM.

CONTENT

CHAPTER 1 INTRODUCTION

1.1 History and Background of the study

A senior inspector from the Healthcare Inspectorate (IGZ) voiced her doubts during an interview with the media (Bilkert M., 2016) about the hygienic quality of spray bottles used in institutional cleaning when cleaning surfaces.

In an interview she says the following:
"In the past, items such as liquid cleaning agents were supplied in large tanks. Now products are much more compact or even individually packed. You still see that concentrated products are diluted on site. The dilution is filled in reusable spray bottles. There is usually no 'watertight' method for cleaning and drying the spray bottles. Because what about the hose and the spray nozzle on this type of spray bottle? This has not been researched; I can't prove it, but in the end a humid environment is a breeding ground for microorganisms."

Because there was a need for more information on this subject and as far as the Cleaning Research Association (VSR) is aware, no research had been conducted into this phenomenon for the Dutch situation, the VSR carried out a study (Terpstra & Kessel, 2018). The aim of the study was to explore and determine whether and to what degree refillable spray bottles in the institutional cleaning sector are microbially contaminated and as such pose a hygiene risk. And, if a microbial contamination is present, to determine whether the organisms are free in the residual liquid in the spray bottles (free germs) or also in any present biofilm (bound germs). And finally, to investigate whether the contamination can be eliminated by a single treatment of the contaminated spray bottles with a disinfectant (250 ppm active chlorine).

The study revealed that the liquid that is dosed with refillable spray bottles used in institutional practice may be microbially contaminated. Of the 50 spray bottles investigated in the study, germs were found in 33 spray bottles. Furthermore, it was found that the spray bottles contained both free germs (in biofilm) and bound germs. The numbers of bound germs found were in the same order of magnitude as the numbers of free (unbound) germs.
It also emerged that contaminated spray bottles, after a single hygienic treatment with active chlorine (250 ppm, 5 minutes) in compliance with the hygiene guidelines of the Dutch Working Party on Infection Prevention (WIP) and the Dutch National Institute for Public Health and the Environment (RIVM) (Appendix 7.1), were not yet germ-free. The numbers of germs were lower than in the untreated spray bottles. This implies that germs in an already formed biofilm cannot be eradicated with a single hygienic treatment in compliance with the WIP/RIVM guidelines.

It is not known whether and to what extent a biofilm with bound germs is formed in institutional use where the spray bottles are hygienically treated daily in compliance with the WIP/RIVM guidelines.

1.2 Purpose of the study

The purpose of the present study is to investigate to what extent the hygiene of spray bottles in institutional cleaning practice improves with the use of a daily hygienic treatment in compliance with the WIP/RIVM guidelines

1.2.1 Research question

The aim of the research has been operationalised in answering the following two research questions:

1. Does a clean refillable spray bottle remain uncontaminated (free from bound germs) if during simulated laboratory use it is contaminated daily over long periods of time and is also treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?
2. Does a clean refillable spray bottle remain uncontaminated (free from bound germs) over long periods of time if during institutional use it is treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?

CHAPTER 2
DESIGN AND IMPLEMENTATION

2.1 Introduction

The VSR research *Hygiene of Refillable Spray Bottles I*. (Terpstra & Kessel, 2018) has shown that spray bottles used in practice can be contaminated with free germs that can easily be rinsed away and bound germs that cannot be removed by 'normal' rinsing and even with a single hygienic treatment using active chlorine in compliance with the RIVM/WIP guidelines cannot be eliminated.

Because a single hygienic treatment has a limited hygienic effect, the current research is investigating whether a daily hygienic treatment (in compliance with guidelines drawn up by the WIP and RIVM) can lead to a germ-free spray bottle. This will be investigated in two different ways. A study in the laboratory (Laboratory simulation) and a study conducted in daily institutional cleaning practice (field study).

2.2 Hygiene for simulated use in the laboratory

The laboratory simulation focuses on answering the research question: Does a clean refillable spray bottle remain uncontaminated (free from bound germs) if during simulated laboratory use it is contaminated daily over long periods of time and is also treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?

2.2.1 Research design of laboratory simulation

In the laboratory, clean spray bottles are initially exposed for a fixed period to conditions that can be compared with daily institutional use in terms of exposure and hygienic treatment. This part of the study is set up in such a way that the laboratory simulates conditions that represent a worst-case scenario with regard to contamination and an ideal scenario with regard to daily hygiene treatment; both compared to normal institutional cleaning practices.

The study is started with 3 different types of unused, uncontaminated spray bottles. The spray bottles are filled daily in the morning with artificially contaminated detergent (3 different types). At the end of each day, half of the bottles are emptied, and the other half emptied and additionally treated hygienically. The bottles are then kept empty until the next morning. After 14 days, half of the spray bottles in the laboratory are examined for bound germs. After 28 days, the other half of the spray bottles are examined in the same manner.

2.2.2 Execution of laboratory simulation

Simulation use and contamination.
The method for this part is schematically shown in Figure 1.

The trial is started with three different types of unused clean spray bottles; 4 bottles of each type. And 3 different cleaning agents corresponding with the spray bottles; a daily cleaner (A), an interior cleaner (B) and a sanitary cleaner (C).

In the morning, the spray bottles are filled with a working solution of the cleaning agent to which a quantity of micro-organisms has been added. The composition and creation of the various working solutions of the cleaning agents is specified under the paragraph Materials and resources. After the bottles are filled, they are stored in a climate chamber at 22°C.

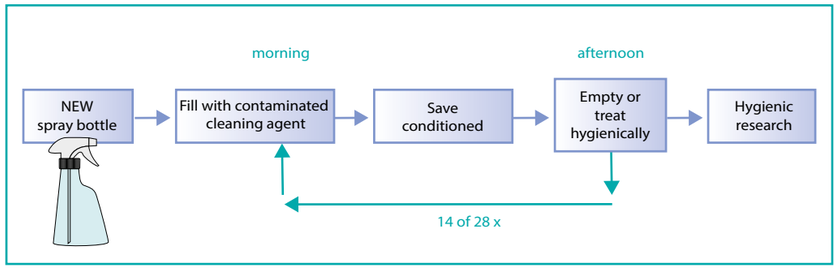
After exactly 6 hours, half of the spray bottles, including the spray nozzle, are emptied and then kept inverted on a sterile tissue at 22 °C until the next morning. The other half of the spray bottles, are also, precisely after 6 hours, hygienically treated according to the method stated in appendix 7.1 and also stored inverted on a sterile tissue at 22°C until the next morning.

The order of the hygienic treatment of the spray bottles is rotated by cleaning agent type so that the same type is not always treated first. For each type, a new chlorine solution is always made, and clean material is used.

The concentration of germs in contaminated working solution of the cleaning agents is determined on the 1st and the 10th day.

The above-described procedure is repeated daily. After 14 days, half of the spray bottles are taken out of the climatic chamber for hygienic research. This includes the determination of the total plate count and the numbers of viable enterobacteria, yeasts and fungi cells, in compliance with the description in Chapter 2.5 Microbiology.
After 24 days, the remaining spray bottles are examined hygienically in the same way.

Figure 1: Treatment of the spray bottles



Materials and cleaning agents
The three cleaning agents used are institutional cleaning agents of the type: daily cleaner, interior cleaner and sanitary cleaner. The acidity (pH) of the cleaning agents is determined in the use concentration. The cleaning agents are used with corresponding spray bottles. The cleaning agent working solutions are created according to the directions on the respective packaging.
The data for the cleaning agent working solutions, cleaning agent dosage and treatment are shown in Table 1.

Table 1: Cleaning Agents, Dosages and Treatment

Code agent	Bottle no.	Type of cleaning agent	Dosage ml/l[1]	Treatment at the end of the day	Sampling after
A	1	Daily cleaner	1	empty only	14 days
	2	Daily cleaner	1	empty only	28 days
	3	Daily cleaner	1	hygienic treatment	14 days
	4	Daily cleaner	1	hygienic treatment	28 days
B	5	Interior cleaner	5	empty only	14 days
	6	Interior cleaner	5	empty only	28 days
	7	Interior cleaner	5	hygienic treatment	14 days
	8	Interior cleaner	5	hygienic treatment	28 days
C	9	Sanitary cleaner	35	empty only	14 days
	10	Sanitary cleaner	35	empty only	28 days
	11	Sanitary cleaner	35	hygienic treatment	14 days
	12	Sanitary cleaner	35	hygienic treatment	28 days

[1] According to the instructions on the packaging

Inoculation fluid and inoculation of the spray bottles
The inoculation fluid with which the cleaning agents are contaminated is made as follows:

A half huckaback, soiled in household use, is placed in a Stomacher bag. 500ml BPW is added to this. After mixing, the mixture is incubated at 30°C for ± 15 hours. The mixture is then strained and immediately used to inoculate the cleaning agents. The plate count of the inoculation fluid is determined on the 1st and the 10th day.

At the beginning of each day, the spray bottles are filled with a weighed amount of the undiluted cleaning agent. The spray bottles are then filled with tap water to which 10 ml of inoculation fluid per litre has been added. All liquids except the inoculation fluid are pre-conditioned at 22°C. After filling, the spray head is filled with liquid by gently spraying 5 times. All the steps in the procedure described are carefully carried out in order to minimize damage to any biofilm already formed.

2.3 Hygiene in practice; field study

The field study focuses on answering the research question: Does a clean refillable spray bottle remain uncontaminated (free from bound germs) over long periods of time if during institutional use it is treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?

2.3.1 Research design of the field study

The field study is conducted at health care institutions. These institutions are given new clean spray bottles to be used in their institutional daily cleaning routines. However, even if this is not already part of the daily routine, the bottles are treated hygienically at the end of each day of use. If spray bottles have to be refilled during the working day, they must first be completely emptied. The hygienic procedure is laid down in a work instruction for the benefit of the executive employees. Before the start of the field study, the employees involved are informed about the procedure and how to handle the spray bottles on the basis of this work instruction. The spray bottles are collected after at least 4 weeks. The collected spray bottles are examined in the laboratory for quantities of bound germs.

2.3.2 Execution of field study

The method for this part of the study is schematically shown in Figure 2.

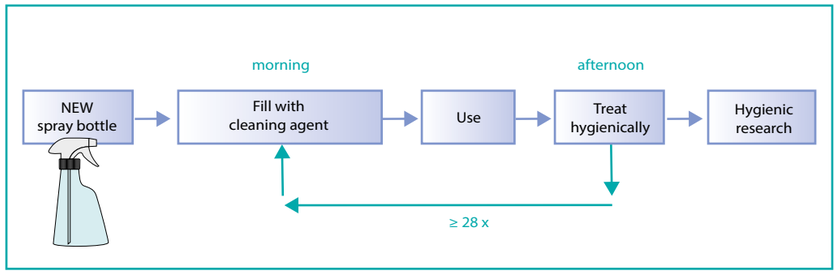


Figure 2: Treatment of spray bottles in the field study

During the preparatory phase of the study, a number of health care institutions that use refillable spray bottles in their daily cleaning routine are recruited. Institutions that are prepared to cooperate with the research are sent written information containing a description of the procedure and working method and a timetable. After their final commitment to participate is received, the institutions are visited for the delivery of the test materials and a verbal explanation of the entire procedure. After this, the field study can begin.
During execution, the spray bottles are put into use in the morning. If a spray bottle needs to be refilled during the day, it must first be emptied completely. At the end of the working day, the spray bottle is rinsed hygienically and stored in compliance with the work instructions (Appendix 7.1).

After a period of at least 4 weeks, the bottles are collected and hygienically examined.

This includes the determination of the total plate count and the quantities of viable enterobacteria, yeasts and fungi cells, in compliance with the description in Chapter 2.5 Microbiology. When collecting the used spray bottles, a sample of the diluted and undiluted cleaning agent used is also taken. The total plate count of these samples is determined.

Materials and cleaning agents

The institutions in the field study will use brands and types of spray bottles and cleaning agents they normally use in the study. The institutions will be provided with new unused bottles for the purpose of the study.
For the preparation of the chlorine solution, the institution may use its usual product, or the product made available. If necessary, dosages are adjusted to achieve the desired concentration (250 ppm) of active chlorine.

2.4 Microbiology

2.4.1 Recorvery of the germs in the biofilm

The procedure for the recovery of the bound germs is shown schematically in Figure 3.

The spray bottle is emptied and then carefully rinsed 5 times with 200 ml of sterile demineralised water, while the spray nozzle also sprays 10 ml during each rinse.

After rinsing, the empty spray bottle is filled with 90 ml of sterile liquid (PFZ) and shaken vigorously for 10 seconds, 6 times on all sides, tapped hard on a surface and finally shaken again for 10 seconds. The spray bottle is then emptied, and the remainder is poured into a combined sample. The sample is examined for quantities of enterobacteria, fungi and yeasts and the total plate count. The determinations are performed in duplicate.

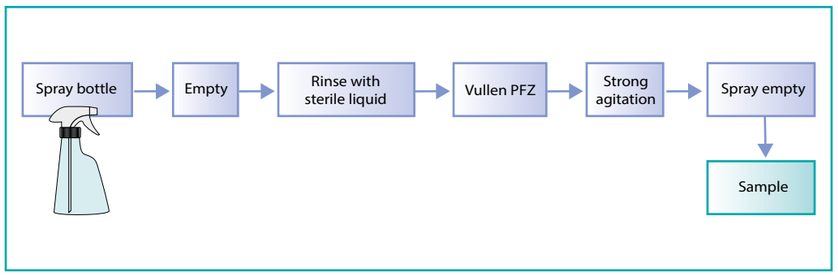


Figure 3: Procedure for determining bound germs (biofilm)

2.4.2 Germ determination

Total plate count
Dilutions 0 to –6 are spatulated onto a PCA mixing plate (Biotrading) and incubated at 30°C for 3 days. All colonies that are formed are counted.

Enteros
Dilutions 0 to –6 are spatulated onto a VRBGA mixing plate (Biotrading) and incubated at 37°C for 1 day. All colonies that are formed are counted.

Yeasts and fungi
Dilutions 0 to –6 are spatulated onto an OGGA spread plate (Biotrading) and incubated at 37°C for 1 day. Colonies are counted by species (shape and colour of the colony)

- colonies colourless transparent, fungi
- colonies large red: medium red: fermentation.

2.4.3 Data processing

The colonies on the plates are counted and the plate count is calculated using the following formula:

$$N = \frac{\sum a}{(N_1 + 0{,}1N_2)d}$$

waarbij

N = Plate count in dilution 0
Σa = Sum of the number of colonies counted
n_1 = Number of countable plates most diluted sample
n_2 = Number of countable plates most diluted sampler
d = Dilution factor n_1

CHAPTER 3 RESULTS

3.1 Laboratory simulation results

The results of the spray bottles in the laboratory simulation are summarised in Table 2; hygienic treatment, acidity and coding of the cleaning agents and average numbers of micro-organisms found in the spray bottles.

Table 2: Plate counts in spray bottles

Nr.	Code product	pH	Chlorine treatment	Sample after days	Entero LOG CFU	Total plate count LOG CFU	Yeast LOG CFU	Fungi LOG CFU
1	A	8,0	No	14	7,9	9,2	5,0	4,3
2	A	8,0	No	28	6,9	9.1	4,0	<4,0
3	A	8,0	Yes	14	<3,0	4,7	<4,0	<4,0
4	A	8,0	Yes	28	3,1	5,8	<4,0	<4,0
5	B	7,4	No	14	8,8	9,3	4,9	<4,0
6	B	7,4	No	28	9.1	9.8	5,5	<4,0
7	B	7,4	Yes	14	3,0	5,4	<4,0	<4,0
8	B	7,4	Yes	28	3.0	6,0	<4,0	<4,0
9	C	11	No	14	<3,0	5,6	<4,0	<4,0
10	C	11	No	28	4,5	5,6	<4,0	<4,0
11	C	11	Yes	14	< 3,0	<3,0	<4,0	<4,0
12	C	11	Yes	28	< 3,0	<3,0	<4,0	<4,0

3.1.1 Total plate count

For the total plate count (TPC), the number of colony-forming units (CFU) per spray bottle ranges from 4.7 LOG CFU to 7.9 LOG CFU. The value 3.0 LOG CFU is the detection limit for the trial design used.

In 10 of the 12 spray bottles, over 3.0 LOG CFU germs were found. The two spray bottles in which no germs were found were used with the sanitary cleaner and were also hygienically treated.

The results indicate a systematic difference between the degree of contamination for the hygienically treated and the untreated spray bottles with the same cleaning agent; in all cases the degree of contamination for the hygienically treated spray bottles is lower than for the comparable untreated spray bottles.

The quantity of germs in the spray bottles for which sanitary cleaner was used is lower than the comparable spray bottles with daily or interior cleaner.

After 28 days, more or an equal quantity of germs were found as in comparable spray bottles after 14 days.

3.1.2 Enterobacteria

For enterobacteria, the defined number of colony-forming units (CFU) per spray bottle is 3.0 LOG CFU to 8.8 LOG CFU. The value 3.0 LOG CFU is the detection limit for enterobacteria in the trial design used.

In 10 of the 12 spray bottles, 3.0 LOG CFU or more enterobacteria were found. The spray bottles in which no enterobacteria were found were hygienically treated or used with the sanitary cleaner.

The results indicate a systematic difference between the degree of enterobacteria for the hygienically treated and the untreated spray bottles with the same cleaning agent; in all cases the degree of contamination for the hygienically treated spray bottles is lower than for the comparable untreated spray bottles.

The quantity of enterobacteria in the spray bottles for which sanitary cleaner was dosed is lower than the comparable spray bottles with daily or interior cleaner.

3.1.3 Yeasts

For enterobacteria, the defined number of colony-forming units (CFU) per spray bottle is 4.0 LOG CFU to 5.5 LOG CFU. The value 4.0 LOG CFU is the detection limit for yeast cells in the trial design used.

In 4 of the 12 spray bottles, more than 4.0 LOG CFU germs were found. No yeast cells were detected in all the spray bottles that were hygienically treated. The same applies to the untreated spray bottles treated with sanitary cleaner that were used.

The results indicate a systematic difference between the number of germs in the hygienically treated and the untreated spray bottles for the same agent. For the interior cleaner and the daily cleaner, the degree of contamination for the hygienically treated spray bottles is lower than for the comparable untreated spray bottles.

3.1.4 Fungal germs

The value 4.0 LOG CFU is the detection limit for fungi cells in the trial design used. The number of germs is above the detection limit only for the untreated spray bottle that was used with daily cleaner A for 14 days; the excess is marginal.l.

3.1.5 Germs in the innoculation liquid

The total plate count of the inoculation fluid is determined on the 1st and the 10th day. The plate counts found are 8.7 LOG CFU /ml and 8.9 LOG CFU /ml respectively.

3.1.6 Acidity of cleaning agents

The pH of the cleaning agents is measured by the working dilution used. The measured pH (20°C) of the daily cleaner is 8.0, the interior cleaner is 7.4 and the sanitary cleaner is 11.

3.2 Field study results

The results of the field study are summarised in Table 3; hygienic treatment, institution code sample number and average numbers of micro-organisms determined in the spray bottles.

Table 4 displays the results of the measurements of the diluted and undiluted cleaning agents originating from the participating institutions.

3.2.1 Participating institutions

In total, 8 institutions declared themselves willing to participate in the study. During the preparations it emerged that one institution did not meet the preconditions of the investigation. This meant that, in the end, 7 institutions participated in the study. The group of institutions comprises 5 hospitals and 2 care homes located in the provinces of South Holland, Utrecht, Friesland, Overijssel and Gelderland.

3.2.2 Total plate count

The plate counts for the spray bottles are shown in table 3. The detection limit for the total plate count for the method used is 3 LOG CFU. The measured total plate count (TPC) in the spray bottles varies from 3.2 LOG CFU per bottle up to 7 LOG CFU. In 22 of the 38 spray bottles, the number of colony-forming units per bottle is below the detection limit (< 3 LOG CFU). No germs were found in the bottles examined for 3 of the 7 institutions.

3.2.3 Enterobacteria

The detection limit for enterobacteria in the method used is 3 LOG CFU. No quantity of enterobacteria exceeds the detection limit in any of the spray bottles.

Institution code	Sample no.	Usage indication	Sample after days	Entero LOG CFU	Total plate count LOG CFU	Yeast LOG CFU	Fungi LOG CFU
A	1 t/m 4	+	52	-	6,0/3,7/4,6/-	-	-
B	1 to 8	+/+/-/+/-/+/+/-	11	-	6,0/6,3/6,5/ 6,2/-/5,8 /5,0/-	4,1/-/5,3/ 4,2/-/-/-/-	-
C	1 to 6	+	52	-	-	-	-
D	1 to 6	+/+/-/+/+/+	52	-	7,0/6,2/-/4,5/3,8/6,6	-/-/-/-/-/5,9	-
E	1 to 5	+	52	-	-	-	-
F	1 to 5	+	52	-	-	-	-
G	1 to 5	+	30	-	-/-/5,3/3,2/4,8	-	-

Table 3: Plate count per spray bottle field study

3.2.4 Yeats

The detection limit for yeasts in the method used is 3 LOG CFU. The quantity of yeast cells found in the spray bottles varies from 4.1 LOG CFU per bottle up to 5.9 LOG CFU. In 4 of the 38 spray bottles, the number of yeast cells exceeds the detection limit (< 4 LOG CFU). No yeasts were found in the bottles examined for 5 of the 7 institutions.

3.2.5 Fungi

The detection limit for yeasts in the method used is 4 LOG CFU. The measured number of fungal germs in the spray bottles is below the detection limit for all bottles.

3.2.6 Germs and pH diluted and undiluted cleaning agent

The diluted and undiluted cleaning agents collected from the participating institutions were examined for germ concentration (total bacteria count; LOG CFU/ml) and pH. The results of the measurements are shown in Table 4.

Table 4: Germ concentrations and pH diluted and undiluted cleaning agent

Institution code	Total plate count* diluted cleaning agent LOG CFU/ML	Total plate count* diluted cleaning agent LOG CFU/ML	pH diluted agent	pH undiluted agent
A	-	-	6,3	3,6
B	-	-		7,5 / 1,5**
C	-	-	2	0,75
D	4,3	-	4,3	3,5
E	6,6	-	6,7	10,5
F	-	-	4,0	3,75
G	-	-	2,5	1,85

*detection limit of the total plate count determination is 1 LOG CFU

**an acidic and neutral undiluted product probably supplied..

CHAPTER 4
DISCUSSION AND CONCLUSIONS

4.1 Discussion

4.1.1 Effect of hygienic treatment in laboratory simulation

Hygiene spray bottles without hygienic treatment

The research shows that the daily exposure (6 hours) of the spray bottles for a longer period (14 or 28 days of use) to contaminated cleaning agent can become contaminated with micro-organisms (total plate count). The formed contamination cannot be removed by rinsing with clean tap water.

This finding is consistent with results found in VSR study Hygiene of Refillable Spray Bottles (Terpstra & Kessel, 2018). In this study, used refillable spray bottles were collected from hospitals and institutions. Micro-organisms were detected in 33 of 50 of these spray bottles. The degree of contamination appeared to vary widely.

The contamination level of the spray bottles exposed to contaminated alkaline (pH 11) sanitary cleaner is on average 3.75 LOG CFU lower than those exposed to contaminated neutral cleaners The contamination with enterobacteria and yeast cells is also lower for the sanitary cleaner. This confirms the established theory that the growth of microorganisms is inhibited by high pH.

Hygiene spray bottles with hygienic treatment

Contamination with bound germs has also been demonstrated for spray bottles that were exposed to contaminated cleaning agents in the same way as in the previous paragraph but were treated hygienically with active chlorine at the end of each day of use in compliance with the RIVM/WIP guidelines. An exception are the spray bottles that were exposed to contaminated sanitary cleaning agent; no germs were found in these spray bottles.

The contamination level of the hygienically treated spray bottles is lower than that of the comparable untreated spray bottles; the mean difference for the total plate count is > 3.45 LOG CFU. If the results for the sanitary cleaning agent are not taken into account, the average difference is 3.9 LOG CFU.

For comparison; for the authorisation of disinfectants in the medical sector, a germ reduction of the trail organisms of 5 decimal places (4 decimal places for yeasts) is required at an exposure time of 5 minutes. (Ctgb, 2003).

The results agree with the general experience that exposure to active chlorine has a hygienic effect and that this effect is reduced with germs in a biofilm (Bridler, Briandet, & Thomas, 2011).

4.1.2 Effect of hygienic treatment in the field study

In the field study, a total of 39 spray bottles were used in the normal routine over a period of 11 to 52 days at 7 health care institutions. During this period, the bottles are hygienically treated every day after use. After this period of use, germs (total bacteria count) were found in 17 of the 39 spray bottles. The infection rate was 3.8 to 7.0 LOG CFU per spray bottle. No germs were found in the bottles examined for 3 of the 7 institutions.

This implies that the use of a hygienic treatment in compliance with the RIVM/WIP guidelines in institutional practice, as applied in this study, cannot prevent some of the bottles from becoming contaminated.

In the investigation Hygiene of Refillable Spray Bottles (Terpstra & Kessel, 2018). research was conducted into the contamination of spray bottles that have been used in daily practice for at least 2 weeks. This revealed that 33 of the 50 spray bottles were contaminated; total number of germs. 19 spray bottles had an infection rate of 8.0 LOG CFU or higher. In the present study, the plate counts are in the range from 3.2 to 6.6 LOG CFU with one exception of 7 LOG CFU. This indicates that hygienic treatment prevents high levels of contamination. It should be noted that the results cannot be directly compared because the result of the present study concerns bound germs only. In the investigation Hygiene of Refillable Spray Bottles, additional research has, however, been conducted into bound germs. This showed that in the bottles examined, the numbers of bound germs and free germs were of the same order of magnitude; on average there were 0.8 LOG CFU more bound than unbound germs in the bottles. The foregoing leads to the assumption that although the hygienic treatment in the field study did not lead to uncontaminated bottles, the degree of contamination is clearly influenced; the risk of strong infections was reduced.

4.1.3 Theoretical considerations

Reinfection

Refillable spray bottles are frequently used in institutional cleaning for dosing auxiliary liquids during cleaning. When working with the spray bottles and during refilling, micro-organisms can enter the interior of the spray bottle. The conditions for outgrowth are usually favourable; the temperature of the contents will usually be room temperature or higher, while the (biodegradable) cleaning agents form an excellent food source. It is understandable and plausible that after some time a biofilm is formed in the interior of the spray bottle in which microorganisms settle and are protected against disinfectants. The germs in the biofilm will form a dynamic equilibrium with the free germs in the liquid. After refilling, the new liquid from the biofilm can be re-contaminated with germs.

If cleaning agent is applied to surfaces from contaminated bottles when cleaning, the germs present also end up on this surface. The hygienic risk of this depends on the dosage, the time the germs spent on the surface, the surface, the type of germs and the use of the surface. It is very likely that some of the germs end up in the ambient air through aerosol formation during spraying. Both of the previous hygiene risks were not further investigated in the study.

Methodology

Laboratory simulation

The spray bottles are contaminated daily in the laboratory simulation. It is unknown how and to what extent spray bottles are contaminated in the daily cleaning practice. The contamination in the laboratory simulation is expected to be a worst-case scenario.

In addition, in the laboratory simulation, the hygienic treatment was carried out strictly according to the instructions, under controlled conditions and by laboratory personnel. This is a situation that cannot be realised in daily cleaning practice. The hygienic treatment in the laboratory represents a best-case scenario.

Field study

The participating institutions were asked to carry out the hygienic treatment of the spray bottles in accordance with the instructions and the staff involved received individual explanations and illustrations. However, it is not known whether and to what extent the protocol has been deviated from. In the research *Hygiene of refillable spray bottles* has been found that a 'biofilm' once formed is only partly eradicated by a hygienic treatment.

Interpretation of plate counts

The numbers of plate counts reported in this study concern bound germs that were extracted from the spray bottles using the method described. It is not known what percentage this is of the actual number of germs contained in the spray bottles. The numbers should therefore be judged comparatively.

4.2 Conclusions

- **Research question:** Does a clean refillable spray bottle remain uncontaminated (free from bound germs) if during simulated laboratory use it is contaminated daily over long periods of time and is also treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?

The study shows that refillable spray bottles become contaminated with bound germs after a period of simulated use, where contaminated daily with contaminated cleaning agent and rinsed hygienically on a daily basis. When an alkaline sanitary cleaner is used, the contamination remains below the detection limit of the study. In comparison to spray bottles that have not been treated hygienically, the contamination (total plate count) is 3.9 decimal places lower; the hygienic treatment therefore has a significant hygienic effect. No yeasts, fungi or (with the exception of 1 exception) enterobacteria were found in the study with the hygienically treated spray bottles.

Conclusion: a hygienic treatment in compliance with the RIVM/WIP guidelines cannot prevent micro-organisms from settling in a spray bottle in a laboratory simulation, but the degree of contamination will be reduced by > 3.5 decimal places.

- **Research question**: Does a clean refillable spray bottle remain uncontaminated (free from bound germs) over long periods of time if during institutional use it is treated hygienically on a daily basis in compliance with the RIVM/WIP guidelines?

The study results show that refillable spray bottles, after a period of normal daily use and daily hygienic treatment according to the method described in this study (RIVM/WIP guidelines), do not remain free of bound germs in all situations studied. The levels of contamination found are lower than anticipated for those based on previous research Hygiene of Refillable Spray Bottles (Terpstra & Kessel, 2018) without hygienic treatment. High levels of contamination such as those found in untreated spray bottles in the aforementioned study were not found.

Conclusion: for a hygienic treatment in accordance with the RIVM/WIP guidelines in institutional cleaning practice, it is not possible for all researched institutions to prevent microorganisms from settling in a spray bottle; there is, however, strong evidence that the degree of contamination is reduced with this treatment.

4.3 Concluding obervation

During the field study, it was noticed that the cleaning was not always carried out according to the agreed procedures. In addition, the technology was not always designed in such a way that good hygiene could be expected. Although the institutions were explicitly asked to adhere to the working method of the protocol supplied and a verbal explanation was given, it is not unlikely that the difference in results between the laboratory study and the field study can be traced back (in part) to not strictly following the instructions.

Although this is not intentional, it is the reality of daily cleaning

CHAPTER 5 SUMMARY

In the past, questions have been posed regarding the hygienic condition of spray bottles used in institutional cleaning for cleaning surfaces (Bilkert M., 2016). Since no research data was available for the Dutch situation at the time regarding the hygienic condition of spray bottles, the Cleaning Research Association decided to conduct research into this area.

It led to the study Hygiene of Refillable Spray Bottles (Terpstra & Kessel, 2018). The aim of the study was to explore and determine whether spray bottles in the institutional cleaning sector are microbially contaminated, and if so, pose a hygiene risk. And additionally, if a microbial contamination does exist, to determine whether the organisms are freely found in the residual liquid in the spray bottles (free germs) or also in any biofilm (bound germs). And finally, to determine whether an existing contamination can be eliminated with a single hygienic treatment using a disinfectant (active chlorine). The study revealed that the liquid in refillable spray bottles used in institutional practice may be microbially contaminated. Germs were found in 33 of the 55 spray bottles examined. The degree of contamination ranged from 3.0 LOG CFU up to 9.0 LOG CFU per spray bottle. Furthermore, it appeared that the spray bottles contained both free germs and bound germs. The numbers of bound germs were in the same order of magnitude as the numbers of free (unbound) germs. The results further showed that a single hygienic treatment of contaminated spray bottles does not result in uncontaminated spray bottles.

The aim of this study was to investigate to what extent the hygiene of spray bottles in institutional cleaning practice improves with the application of a daily hygienic treatment in compliance with the WIP/RIVM guidelines. To achieve this, a laboratory simulation study and a field study were conducted.

In the laboratory study, the effect of the hygienic treatment was investigated in a set-up where spray bottles were exposed to an infected cleaning agent for 6 hours every day for a period of 14 and 28 days. Half of the spray bottles in the study were treated hygienically (daily) after exposure in accordance with the WIP/RIVM guidelines; the other half received no hygienic treatment. A neutral daily cleaner, a neutral interior cleaner and an alkaline sanitary cleaner were used as cleaning agents.

The spray bottles that were exposed to contaminated sanitary cleaner and treated hygienically remained uncontaminated. In all other spray bottles, contamination was found after 14 and 28 days. The degree of contamination (total plate count) of the hygienically treated spray bottles is roughly 3.5 decimal places lower than that of the untreated spray bottles.

In the field study, unused new spray bottles were issued at 7 Dutch healthcare institutions. The cleaning staff were requested to use the spray bottles in their normal daily routine. In

addition to this, the spray bottles had to be treated hygienically at the end of every working day in compliance with the hygiene guidelines of the Dutch Working Party on Infection Prevention (WIP) and the Dutch National Institute for Public Health and the Environment (RIVM). After a period varying from 11 to 52 days, the spray bottles were collected for hygienic examination. In 3 of the 7 institutions, no contamination was found in any of the spray bottles used. Contamination to a greater or lesser degree was found in the spray bottles from the other 4 institutions. The infection rate for the contaminated bottles ranged from 3.2 to 7.0 LOG CFU. Comparison of this result with previous research provides indications that the average and maximum degree of contamination is reduced by a hygienic treatment in compliance with the WIP and RIVM.

Although a daily hygienic treatment of spray bottles in compliance with the WIP/RIVM guidelines does not lead to uncontaminated bottles in all situations, the present study provides strong indications that the hygiene of spray bottles is improved by this treatment.

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CHAPTER 7 ATTACHMENTS

7.1 Appendix I hygiene treatment procedure

The bottles are cleaned daily at the end of each working day according to the following procedure:

- Prepare a fresh chlorine solution with 250 ppm of active chlorine. (Dissolve one chlorine tablet with **1.5g** active chlorine in six litres of tap water at 30±2°C,
- Empty the spray bottle and also **spray the nozzle empty**
- Rinse the spray bottle, including the spray head, carefully with clean tap water and then empty the spray bottle **and empty the nozzle**
- Immerse the rinsed bottles (nozzle loose from the bottle and filled with the chlorine solution) in the chlorine solution; make sure everything is free of air (bubbles); also spray the nozzle when immersed in the liquid
- Leave the bottles immersed for five minutes
- Then take the materials out of the container
- Rinse them with clean water
- Lay them to dry on a clean cloth or upside down on a rack until next use

7.2 Paragraph from the wip manual WIP-071030 for hospitals

5.6 Cleaning Agents

The prepared dilutions of cleaning agents should be refreshed several times daily.
Nowadays, a lot of use is made of spray bottles to spray detergent on small surfaces to be cleaned. These bottles must be emptied daily, including the spray nozzle, after the work, rinsed and then disinfected with a 250 ppm chlorine solution. Disinfection is necessary to prevent the growth of micro-organisms (pseudomonas species) in the system.

7.3 RIVM guideline

Cleaning, Disinfection and Sterilisation in Public Health Care — Standard Methods 19 September 2011

2.2.1. Instruments and objects that are contaminated with harmful micro-organisms (no blood) and can be immersed

Procedure

- Put on plastic gloves
- Clean the instrument with an all-purpose cleaner
- Rinse the instrument with clean water and dry it with a clean cloth or paper
- Alcohol dosage 70% or 250 ppm chlorine. Dissolve one chlorine tablet in six litres of luke-warm water. This is based on tablets with 1.5 grams of active chlorine per tablet. There are

also tablets on the market with 1.0 gram of active chlorine per tablet, in which case one tablet must be dissolved in four litres of water.

- Ensure that the materials to be disinfected are properly cleaned, rinsed and dried
- Immerse the cleaned materials in water with chlorine solution
- Leave the materials immersed for at least five minutes
- Take the materials out of the container with clean gloves
- Rinse them with clean water
- Place them to dry on a clean cloth
- Preferably store them in a clean drawer or cupboard
- Discard the chlorine solution after use

VSR is the independent professional cleaners platform and knowledge institute for all market parties in cleaning services. VSR aims to professionalise and objectify the cleaning sector through research, information and training.

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