

Oversætter: <http://belgium.angloinfo.com/inside/translate-online/>



Rapport

Agriton Date:03/04/2013 Ref.: PJ-13-PP- 001-1 1

T.a.v. dhr Jürgen Degraeve Bvba Agriton Nieuwkerkestraat 19 8957 Mesen

info@agriron.be Tel. 057/366 163 GSM 0477 425 686

Report on the efficiency of a biological cleaning product from Agriton when applied in three sites in Ghent.

Aim of the tests: Agriton has a biological cleaning product "Wipe and Clean " on the market of household cleaning. The City of Ghent is looking for sustainable cleaning products for public buildings and has three candidates, including Agriton, selected for testing. These tests were started on February 1, 2013 and ran for a period of 2 months. The the effects tests have to showth effects of the biological cleaning product by comparing the situation before and after the application of the cleaning agent.

The selected aereas for the Agriton test were:

- Site 1: Service Centre, Braemkasteelstraat 26 in 9050 Gentbrugge - Only use in the bathrooms on the ground floor;
- Site 2: Nursery Bees Dance, Forelstraat 147 in 9000 Gent - Application in the living areas;
- Site 3: Public toilets Baudelo, Ottogracht 2 in 9000 Gent. In order to demonstrate the efficiency of the biological cleaning product Agriton wished to be carried out by Avecom before, during and at the end of the test period. Microbiological test Avecom is a spin-off of LabMET, Ghent University and specializes in the management and optimization of microbiological processes. In the field of microbiological analysis Avecom has years of expertise.

2. Method for sampling and measurement of hygiene in close consultation between Agriton and Avecom the following samplings were proposed:

- Two samplings prior to the start and three samplings in the course of the test of 2 months (after 2.5 weeks, after 5 weeks and after 8 weeks)
- The three proposed sites in Ghent;
- Per site at each place three samplings;
- Per site whenever a sampling in three-fold;
- At each sample measurement of total aerobic plate count and various pathogens and / or indicator organisms (see below).

For checking the hygiene of the cleaned surfaces the stamp method was used, particularly the method of 3M Petrifilm (Led Techno). These small round plates are covered with a gel (selective nutrient medium for particular species or group of bacteria), which can be pressed down on the sampling surface.

To investigate the following organisms were selected for follow-up: Total plate count (Total Aerobic Count), E coli and coliforms, Enterobacteriaceae, Staphylococcus aureus.

E. coli is a member of the group of coliforms, gram-negative, non-spore-forming bacteria. E-coli is found in the intestinal tract of humans and is not in itself a pathogen. However, their presence is a good indicator of faecal contamination. In general, the coliform group of bacteria, including Enterobacteriaceae, are considered to be a good indicator of the overall hygiene. Staphylococcus aureus is a gram-positive bacterial species and a pathogen or, thus, a kind of pathogenic bacterium. The microbiological product Agriton includes anaerobic microorganisms, lactobacilli and yeasts. In order to demonstrate the presence of the cleaning agent there was, at the request of Agriton, made additional measurements of the number of Lactobacilli on the samples. A more detailed description of the 3M Petrifilm method in general, and of the measurements of the selected organisms in particular, can be found in Appendix 1. In contrast to the other measurements, the 3M™ petri films served for the measurement of the Lactobacilli to be incubated anaerobic counts were performed and the standard after 2 days of incubation. In the other CFU (Colony Forming Units) countings the counting itself was done after 1 day of incubation in the corresponding petri films, unless stated otherwise.

Avecom was responsible for both samplings (stamp method) and the microbiological measurements of the various selected microorganisms. Two samplings carried out prior to the change of the cleaning solution (control period) and three samplings, at the time of the application of the cleaning product of Agriton. The test period started on February 1, 2013. The study was launched in the last week of January 2013. The samplings were conducted on the following dates:

Control period: Tuesday, 29/01/2013 between 14 h and 15h30;

Monitoring period: Thursday, 31/01/2013 between 14 h and 15h30,

Test period: Wednesday 20/02/2013 between 14 h and 15h30,

Test period: Wednesday 06/03/2013 between 14 h and 15h30,

Test period: Wednesday 27/03/2013 between 14h and 15h30.

On each site the following spaces and surfaces were sampled:

Site 1: Dienstencentrum Gentbrugge, washroom on the ground Place A: Floor level with the middle urinal (4th floor tile from the wall), Site B: Right ladies' room: inside rim of the toilet (under the toilet seat) Location C: Floor main entrance of the toilets (perpendicular row of tiles on the left side of the entrance door).

Site 2: Nursery in Ghent, second living space Place A: Changing cushion (colored blue) Site B: Floor Fri 2 children's toilets (central) Location C: Rubber floor of the living area (about midway).

Site 3: Public toilets Baudelo in Gentplaats A: Floor level with the middle urinal (2nd floor tile from the wall), Site B: middle woman toilet: inside rim of the toilet (under the seat) Location C: Floor main entrance of the toilets (door between the main entrance and washroom). Each sampling was done at the time at about the same place and in the same order.

3. Results of the measurements

3.1. Results of the individual measurement campaigns all results of five separate measurement campaigns are detailed in the tables of the addenda.

These are:

1 Measurement campaign of checking up on 29/01: Addendum 2;

2 Measurement campaign of checking up on 31/01: Addendum 3,

3 Measurement campaign of the test period on 20/02: Addendum 4,

Measurement campaign four test period on 06/03: Addendum 5, 5 Measurement campaign of the test period on 27/03: Addendum 6.

It should be further noted that during the review period, no measurements of Lactobacilli were performed.

In the measurement of *Staphylococcus aureus* a specific 3M Petri film is used to grow.

Besides *Staphylococcus aureus* bacteria, other types (not specified by the manufacturer of 3M Petri film) stand out from the rest.

Therefore, a special drive on the overgrown petri films were made after 1 day of incubation (see also description in Addendum 1) allowing the *St. aureus* itself (after one extra day incubation) to be distinguished as a red-purple colored colony.

In none of the measurements violet colonies were observed which indicates that the pathogen was most likely not present in any single sample. For the sake of completeness measurements were made of these specific 3M where the CFU (Colony Forming Units) petri films or continue to be displayed, but they have been further described as (*Staphylococcus aureus*) which means that the specific petri films for this organism was used for the sampling, but that the grown colonies on the corresponding Petri movies were probably not *Staphylococcus aureus*.

After each measurement exercise, the results of the measurements were summarized in detail in Excel tables and sent directly to Agriton. In this manner, the intermediate results were followed up and could, if desired, be overturned. The evaluation of the results were also made in detailed reviews.

The aerobic growth of colonies on the petri 3M™ films was checked at day 1, and after 2 days of incubation. Only the measurement of Lactobacilli (anaerobic incubation) was done by default only after 2 days of incubation. In case of differences between 1 day and 2 days of incubation, the CFU numbers in both

the interim statements (Excel tables) were displayed. In the addenda and the data processing of this report, unless otherwise stated, only the results after 1 day of incubation are presented.

For some measurements, there was an exuberant growth and even discoloration of the gel (soil) from the Petri film in which individual colonies could not be counted. In those cases "TNTC" or "Too Numerous To Count" is displayed as a result.

- At the fourth sampling in public toilet (Baudelo) the floor of the entrance hall and that at the height of the urinals was moist. This sampling was done on a day with high temperatures (compared to the rest of the period). Presumably the heating was off and there was a higher humidity in the room with wet floors as a result. The results of the measurements on these floors are given to be considered. CFU showed significantly higher values, and/or indeterminate growth, and can not be considered as a "representative" of the test period.

3.2. Comparison of the measurement results for the five measurement campaigns are the mean values (and the corresponding standard deviations) of 3 CFU measurements per sampling site in tabulated.

3.2.1. Site 1: Dienstencentrum

An overview of the results of sampling of the floor at the height of the urinal in the service center is presented in Table 1. The results of the measurements carried out on a female toilet (inboard of the bathroom underneath the toilet seat), are shown in Table 2. In Table 3 are the average results of the measurements of the floor at the height of the central hall of the toilets are summarized.

Table 1. Summary of the mean values of the microbiological measurements, performed on the sample of the floor at the height of the urinals of the service center

Period	Control		Test Agriton Product		
	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	65 ± 33 2	250 ± 26 3	222 ± 35 3	132 ± 11 3	107 ± 22 3
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	205 ± 87 3	TNTC** 4	TNTC** 4
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 0 0	15 ± 4 1	37 ± 4 2	0 ± 0 0	0 ± 0 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	5 ± 8 0	6 ± 2 0	4 ± 1 0	0 ± 0 0	0 ± 1 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	74 ± 52 2	107 ± 22 3	112 ± 34 3	137 ± 29 3	70 ± 14 2

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count (more than 400 colonies per petri film)

** * Determination of the class mark: see Addendum 1

Table 2. Overview of the microbiological measurements performed on the samples from the women's toilet of the service

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	29 ± 6 1	17 ± 4 1	61 ± 26 2	57 ± 22 2	70 ± 36 2
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	50 ± 9 2	60 ± 5 2	41 ± 27 2
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 0 0	TNTC** 4	8 ± 2 0	0 ± 0 0	0 ± 0 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 0 0	12 ± 5 1	0 ± 0 0	0 ± 0 0	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	9 ± 4 0	20 ± 12 1	47 ± 29 2	44 ± 59 2	6 ± 1 1

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1

Table 3. Overview of the microbiological measurements performed on the samples from the floor of the central hall of the toilets of the service

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	130 ± 21 3	185 ± 30 3	155 ± 46 3	348 ± 97 4	95 ± 50 2
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	437 ± 32 4	192 ± 18 3	TNTC ** 4
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 0 0	158 ± 87 3	8 ± 4 0	0 ± 0 0	0 ± 1 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 0 0	11 ± 5 1	6 ± 2 0	0 ± 0 0	1 ± 1 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	13 ± 4 1	158 ± 50 3	101 ± 54 3	60 ± 18 2	80 ± 19 2

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1

3.2.2. Site 2: Nursery.

An overview of the results of the sampling of a nursing pillow in the nursery is presented in Table 4. The results of the measurements carried out on the floor at the level of the children's toilets are listed in Table 5. In Table 6 are the average results of the measurements of the floor summarized in the playroom.

Table 4. Overview of the microbiological measurements performed on the samples of a nursing pillow nursery

Period	Control		Test Agriton Product		
	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm	54 ± 12	96 ± 11	365 ± 152	68 ± 31	96 ± 37
Classifier***	2	2	4	2	0
<i>Lactobacillus</i> CFU/petrifilm	Not measured	Not measured	338 ± 80	TNTC **	TNTC **
Classifier***			4	4	4
<i>Enterobacteriaceae</i> CFU/petrifilm	0 ± 0	25 ± 9	6 ± 7	0 ± 0	0 ± 0
Classifier***	0	1	0	0	0
<i>E-coli and Colitypes</i> CFU/petrifilm	5 ± 6	0 ± 0	8 ± 7	0 ± 0	9 ± 3
Classifier***	0	0	0	0	0
<i>(St. Aureus)*</i> CFU/petrifilm	7 ± 8	75 ± 10	53 ± 19	10 ± 3	43 ± 15
Classifier***	0	2	2	1	2

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1

Table 5. Overview of the microbiological measurements performed on the samples from the floor at the height of the children's toilets nursery

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	59 ± 25 2	64 ± 20 2	321 ± 36 4	262 ± 40 3	86 ± 20 2
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	87 ± 6 2	TNTC ** 4	TNTC ** 4
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 1 0	22 ± 5 1	10 ± 8 1	0 ± 0 0	1 ± 1 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 0 0	0 ± 0 0	1 ± 2 0	0 ± 0 0	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	8 ± 6 0	28 ± 16 1	52 ± 19 2	25 ± 7 1	32 ± 19 2

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1

Table 6. Overview of the microbiological measurements performed on the samples of the rubber floor in the playroom nursery

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	9 ± 4 0	17 ± 20 1	33 ± 16 2	15 ± 7 1	56 ± 34 2
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	37 ± 38 2	50 ± 7 2	27 ± 9 1
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 0 0	4 ± 2 0	1 ± 2 0	0 ± 0 0	0 ± 0 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 0 0	0 ± 0 0	0 ± 0 0	0 ± 0 0	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	4 ± 1 0	5 ± 3 0	21 ± 11 1	16 ± 7 1	16 ± 5 1

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** Determination of the class mark: see Addendum 1

3.2.3. Site 3: Public toilets Baudelo

A summary of the results of sampling of the floor at the height of the urinals in the area of public toilets is presented in Table 7. The results of the measurements performed on a woman, toilet (below toilet seat), are shown in Table 8. In Table 9, the average results of the measurements of the floor in the central hall summarized.

Table 7. Overview of the microbiological measurements performed on the samples from the floor at the height of the urinals in public toilets (Baudelo)

Period	Control		Test Agriton Product		
	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	65 ± 18 2	47 ± 12 2	149 ± 51 3	TNTC** 4	TNTC** 4
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	143 ± 21 3	TNTC** 4	TNTC** 4
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	0 ± 0 0	12 ± 13 1	17 ± 2 1	TNTC** 4	0 ± 0 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 1 0	2 ± 0 0	6 ± 0 0	TNTC** 4	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	27 ± 2 1	81 ± 17 2	46 ± 17 2	TNTC** 4	TNTC** 4

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1 Sampling of wet floor: Not representative!

Table 8. Overview of the microbiological measurements performed on the samples of a woman lavatory public toilets (Baudelo)

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	1 ± 0 0	53 ± 41 2	203 ± 25 3	93 ± 6 2	5 ± 2 0
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	57 ± 16 2	78 ± 9 2	7 ± 2 0
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	1 ± 1 0	3 ± 5 0	11 ± 6 1	0 ± 0 0	1 ± 1 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	0 ± 0 0	0 ± 0 0	13 ± 3 1	0 ± 0 0	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	1 ± 1 0	5 ± 1 0	25 ± 7 1	19 ± 2 1	6 ± 6 0

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** Determination of the class mark: see Addendum 1

Table 9. Overview of the microbiological measurements performed on the samples from the floor at the height of the central hall of the public toilets (Baudelo)

Period	Control		Test Agriton Product		
Date	Sample 29/01	Sample 31/01	Sample 20/02	Sample 06/03	Sample 27/03
Total aerobes CFU/petrifilm Classifier***	48 ± 22 2	65 ± 30 2	49 ± 8 2	TNTC ** 4	TNTC ** 4
<i>Lactobacillus</i> CFU/petrifilm Classifier***	Not measured	Not measured	150 ± 17 3	TNTC ** 4	TNTC ** 4
<i>Enterobacteriaceae</i> CFU/petrifilm Classifier***	6 ± 3 0	18 ± 11 1	4 ± 2 0	TNTC ** 4	0 ± 0 0
<i>E-coli and Colitypes</i> CFU/petrifilm Classifier***	1 ± 2 0	0 ± 0 0	33 ± 10 2	TNTC ** 4	0 ± 0 0
<i>(St. Aureus)*</i> CFU/petrifilm Classifier***	20 ± 5 1	55 ± 5 2	61 ± 27 2	TNTC ** 4	149 ± 69 3

*CFU on the selective petri films for *Staphylococcus aureus*. However, after additional testing, these colonies were not colored purple and thus unlikely to be a colony of *St. aureus*

** TNTC: Too numerous to count

*** Determining the class mark: see Addendum 1 Sampling of wet floor: Not representative!

3.3. Evaluation of the results

- Three samples were always taken from each sampling site. For each of the different bacteria the latter are not strictly considered a repetition given the fact that the sampling surface of the three measurements were close together but never completely the same. However, layers of the results of each of the 3 measurements were always in the same order of magnitude. This can be derived both from the detailed list of all the measurement results in the addenda and the previously forwarded Excel tables summarizing the interim results and on the other deviations from the mean values with the corresponding standard.
- At the 4th measurement campaign on March 6, exceptionally high values of all measured bacteria were counted in samples from the floor (2 locations) of the public toilets of Baudelo (site 3). This was coupled with the fact that the floors were very wet at the time of sampling (believed to be due to a temporary change of the weather and the switching off of the heating). All petri films of these samplings were completely overgrown with colonies. These results are therefore regarded as not representative of the campaign and it is therefore not taken into account in evaluating the results.
- When evaluating the microbiological measurements it should be noted that an important distinction must be made between the measurement of 'harmless' bacteria on the one hand (total aerobes, *Lactobacilli* and not red purple colored colonies on the petri films for the measurement of observed *Staphylococcus aureus*) and the indicator and / or pathogenic bacteria (*E. coli* and *coliforms*, *Enterobacteriaceae* and *Staphylococcus aureus*) on the other. The biological cleaning product Agriton is in fact itself composed of bacteria and yeasts and seeks the suppression of pathogenic bacteria. The application of this cleaner even increases the total number of germs. When assigning a class rating the standard system was used for the preparation of a hyginogram with the lowest possible number of germs contemplated.
- Both between the two samplings during the audit period and between the three samplings in the test period there were some fairly large fluctuations in number of CFU/petri film per sampling location. All sampling occurred at approximately the same time of day (afternoon) but were not associated with cleaning intervals.
- The three sampling sites on the same site (service center, nursery and public toilets) played an important role regarding the number of colonies of the various measurements. In the service center, the highest numbers were measured on the floor: about the same order of magnitude of the different bacteria was present on the floor around the urinals and on the floor in the central hall. Against expectations, there were far fewer bacteria (both harmful and harmless) present in the female toilet. In the nursery, the number of germs on the floor for the children's toilets and the nursing pillow had approximately the same magnitude. Relatively low bacterial counts were measured in all samples of the rubber floor of the playing area. Apparently this flooring material contains components which suppresses bacterial growth. In these samples, exceptionally low numbers of total aerobes and *Lactobacilli* were also recovered.
- The measurements of the public toilets of the Baudelo were more or less similar to those of the toilets of the service. The highest numbers of bacteria were measured on the steel floor with relatively little difference between the floor at the height of the urinal and the floor at the height of

the central hall. Also, on the steel of the female toilet (sampling on the inside of the toilet beneath the toilet seat), there was significantly less growth of various bacteria to be measured.

- The application of the cleaning product of Agriton could be more clearly inferred from the relatively high presence of *Lactobacilli*. This was especially the case on the floors in the different sites, with the exception of the rubber floor of the nursery. The relatively high rate class for *Lactobacilli* (usually 3 to 4 for the steel of the floor) should be disregarded in view of the fact that these *Lactobacilli* have been derived partly from the cleaning product of Agriton. Used here as a negative the same observation can be made for the total number of aerobes and for not red purple colored colonies on the petri films for detection of *Staphylococcus aureus*.
- The main results of the total measurement campaign concerns the measurements of the indicator and / or pathogenic micro-organisms. As mentioned earlier it could be demonstrated (in 2 steps measurement) in all the samples by checking the Staph Express Count System that the grown colonies on this petri films *Staphylococcus aureus* were as follows: after the application of a disc no red purple discoloration of the colonies were observed which could distinguish St. aureus from the rest. Most likely this pathogenic organism was absent in all samples.
- Regarding the number of *Enterobacteriaceae*, *E. coli* and *coliforms* per site derived from the test results the following can be concluded:
 - Service Centre: At the first sampling during the monitoring period, the numbers were very low (always class 0 which is very good). However, at the second sampling during the monitoring period significant higher numbers of *Enterobacteriaceae* were measured, especially in the women's toilet and in the steel of the floor of the entrance hall. The three sets of samples during the test period with the Agriton cleaner gave still relatively low numbers of both *E. Coli*, *coliforms* and *Enterobacteriaceae*. The highest value of *Enterobacteriaceae* was measured on the floor at the height of the urinal at the first sampling of the test period and compared with 37 CFU / petri film on average. This was significantly lower than the second measurement of *Enterobacteriaceae* on the floor of the hall during the monitoring period. So the higher values measured were within the possible variations during the monitoring period.
 - Nursery: In all samples of the nursery there were few or no indicator organisms measured (mostly class 0 or 1 which is very good). There were no higher numbers to be measured in the samples of the test period in comparison with the corresponding samples of the control period. Thus, the results were consistently very good.
 - Public toilets (Baudelo): The samples of wet floors (fourth sample) were not taken into consideration. During the monitoring period, the numbers of *Enterobacteriaceae*, *E-coli* and *coliforms* were still very low (class 0 or 1 which is very good). This was also the case in most of the samples of the test period.

Only at the third sampling at 20/02 a slight increase of *E-coli* and *coliforms* was measured in the samples of the female toilet (13 CFU / petri film versus 0 CFU / petri film in all other samples) and the floor of the entrance hall (33 CFU / petri film versus maximum 1 CFU / petri in all other film samples). Similarly, the measurements of the *Enterobacteriaceae* were highest in the third of the sampling at the level of the floor of the female urinals and toilet. The differences in numbers of these indicator organisms between the control samples and test samples were very limited.

In general, the results of the total measurement show that the application of the biological cleaning product of Agriton in the hygienic area gave good results in comparison with the prior cleaning product used. There was no significant increase in indicator and/or pathogenic microorganisms detected in the samples from the different sites. The hygienogram completed on the basis of the indicator and/or pathogenic microorganisms always gave good to very good results during the test period with the Agriton cleaning product.

By the use of a biological product, which in itself is composed of, among other components, bacteria and yeasts, there is an increase in the total number of germs to be measured. However, they are harmless bacteria that also help to suppress the growth of pathogens. Thus, the standard class rating system and the associated layout of a hygienogram can not be fully applied here and was therefore only used for the indication of pathogenic microorganisms.

Addendum 1:

General principles of 3M Petrifilm™

The surface monitoring was based on the 3M Petrifilm method. It is a fast, simple, reliable and reproducible method which can be used for filling in hygienograms. More information about this method can be found on www.ledtechno.be.

The Petrifilm method consists of a film with a surface area of 20 cm which, upon hydration, is contacted with the surface to be analyzed in contact. After sampling the petri films they are incubated at 37C for at least 24 h. Afterwards the number of colonies are counted on the petri plates and expressed as CFU (colony-forming units or colony forming units) / petri film.

There are 3M Petrifilm plates for different bacteria or groups of bacteria as well as fungi and yeasts. For each type of 3M Petrifilm the gel consists of a specific soil on which the desired bacteria can grow selectively and thus be distinguished.

3M Petrifilm™ plates for the Agriton Testing

The following sheets were used for the tests:

- *E. coli* and *coliform* Count Plates;
- *Enterobacteriaceae* Count Plates;
- *Staph* Express Count Systems (plate and disc)
- *Lactobacillus* Count Plates (anaerobic incubation)
- Total Aerobic Count Plates (same plates as those of *Lactobacillus* but using aerobic incubation)

The Petrifilm plates for *E. coli* and *coliform* containing Violet Red Bile (VRB) nutrients, a cold-water-soluble gel, a sensitive indicator BCIG-glucuronidase (for the detection of *E. coli*) and a tetrazolium indicator that facilitates colony count. The top film traps the gas produced by the lactose fermenting *coliforms* and *E. coli*. During the test all colonies were counted, and there was no distinction between *E.coli* and *coliforms*.

For *Enterobacteriaceae* the 3M Petrifilm plates were used. The plates contain a pH-indicator showing red in the presence of acid (glucose fermentation) and an indicator turning yellow in the presence of gas. As a result, the colonies of acid-or gas-producing *Enterobacteriaceae* is relatively easy to distinguish.

For the measurement of *Staphylococcus aureus* the 3M Petrifilm Staph Express Count System was used which consists of a Petrifilm Plate Count and Staph express a Petrifilm Staph express disc. The plate consists of a chromogenic, modified Baird-Parker medium that is selective for *Staphylococcus aureus*. The latter appear on this medium as a red-violet colonies. However, other than red-violet colonies are able to grow on these plates. An additional test is therefore sometimes necessary to make *Staphylococcus aureus* distinguishable. The disc has been developed for the determination of deoxyribonuclease (DNase) reactions, which are specific for the *Staphylococcus aureus* of the Staph Express count Petrifilm plate. It contains toluidine blue O DNase that makes comments more visible. The disk must be used when other than red-violet colonies on the plate are provided.

For the measurement of *Lactobacilli*, Petrifilm Aerobic Count Plate with a MRS broth dilutant were used. After anaerobic incubation, the *Lactobacilli* were perceptible as red to red-brown colonies, whether or not in combination with gas bubbles. The same type of petri films was also used for the measurement of the total aerobic plate count (aerobic incubation).

Evaluating the counts at the layout of the hygienogram based on the 3M Petrifilm measurements, the following classification is used (stamp surface 20 cm²):

Number of colonies	Class
<10	0
10-30	1
31-100	2
101-300	3
>300	4

The quality of the cleaning was calculated on the basis of the average of the classes of the 3 measurements per indicator organisms, particularly

Average classifier	Quality
<0,5	Very good
0,5-1	Good
1-1,5	Satisfactory
1,5-2	Dissatisfactory
2-2,5	Poor
>2,5	Very poor

Addendum 2:

Results of the measurements on 29/01/2013 - CONTROL PERIOD

Site 1: Dienstencentrum

1 DAY AFTER INCUBATION CFU / petri film

Site 1 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	41	102	52	65	33
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	122	80	19	74	52
<i>E-coli and coliforms</i>	0	0	14	5	8

Site 1 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	35	30	23	29	6
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	13	9	5	9	4
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 1 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	113	125	153	130	21
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	16	13	9	13	4
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 2: Nursery

Site 2 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	49	68	46	54	12
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	16	2	3	7	8
<i>E-coli and coliforms</i>	0	4	12	5	6

Site 2 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	38	51	87	59	25
<i>Enterobacteriaceae</i>	0	1	0	0	1
<i>(St. Aureus)</i>	5	3	15	8	6
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 2 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	9	13	5	9	4
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	5	4	4	4	1
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3: Public toilets

1 DAY AFTER INCUBATION CFU / petri film

Site 3 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	50	85	59	65	18
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	25	29	26	27	2
<i>E-coli and coliforms</i>	0	1	0	0	1

Site 3 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	1	1	1	1	0
<i>Enterobacteriaceae</i>	0	0	2	1	1
<i>(St. Aureus)</i>	1	1	0	1	1
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	33	73	37	48	22
<i>Enterobacteriaceae</i>	9	3	5	6	3
<i>(St. Aureus)</i>	15	25	19	20	5
<i>E-coli and coliforms</i>	0	3	0	1	2

Addendum 3:

Results of the measurements on 31/01/2013 - CONTROL PERIOD

Site 1: Dienstencentrum

1 DAY AFTER INCUBATION CFU / petri film

Site 1 A	Count 1	Count 2	Count 3	Average	Standard deviation
Total aerobes	270	220	260	250	26
Enterobacteriaceae	15	12	19	15	4
(St. Aureus)	102	132	88	107	22
E-coli and coliforms	5	8	6	6	2

Site 1 B	Count 1	Count 2	Count 3	Average	Standard deviation
Total aerobes	15	21	14	17	4
Enterobacteriaceae	TNTC*	TNTC*	TNTC*	(Discoloration of the gel)	
(St. Aureus)	18	9	33	20	12
E-coli and coliforms	18	8	11	12	5

- TNTC: Too numerous to count (too many colonies)

Site 1 C	Count 1	Count 2	Count 3	Average	Standard deviation
Total aerobes	185	155	215	185	30
Enterobacteriaceae	60	190	225	158	87
(St. Aureus)	110	155	210	158	50
E-coli and coliforms	15	12	5	11	5

Site 2: Nursery

Site 2 A	Count 1	Count 2	Count 3	Average	Standard deviation
Total aerobes	96	85	106	96	11
Enterobacteriaceae	35	19	21	25	9
(St. Aureus)	85	66	73	75	10
E-coli and coliforms	0	0	0	0	0

Site 2 B	Count 1	Count 2	Count 3	Average	Standard deviation
Total aerobes	41	71	79	64	20
Enterobacteriaceae	19	20	28	22	5
(St. Aureus)	11	41	33	28	16
E-coli and coliforms	0	0	0	0	0

Site 2 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	16	17	18	17	1
<i>Enterobacteriaceae</i>	2	5	4	4	2
<i>(St. Aureus)</i>	3	9	4	5	3
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3: Public toilets

1 DAY AFTER INCUBATION CFU / petri film

Site 3 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	60	44	36	47	12
<i>Enterobacteriaceae</i>	27	3	5	12	13
<i>(St. Aureus)</i>	88	62	93	81	17
<i>E-coli and coliforms</i>	2	2	2	2	0

Site 3 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	73	81	6	53	41
<i>Enterobacteriaceae</i>	0	0	8	3	5
<i>(St. Aureus)</i>	4	5	6	5	1
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	92	71	32	65	30
<i>Enterobacteriaceae</i>	8	29	18	18	11
<i>(St. Aureus)</i>	55	50	60	55	5
<i>E-coli and coliforms</i>	0	0	0	0	0

Addendum 4:

Results of the measurements on 20/02/2013 - TEST PERIOD

Site 1: Dienstencentrum

1 DAY AFTER INCUBATION CFU / petri film

Site 1 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	225	255	185	222	35
<i>Lactobacilli</i>	145	165	305	205	87
<i>Enterobacteriaceae</i>	42	34	36	37	4
<i>(St. Aureus)</i>	138	74	124	112	34
<i>E-coli and coliforms</i>	3	4	5	4	1

Site 1 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	68	83	33	61	26
<i>Lactobacilli</i>	50	41	58	50	9
<i>Enterobacteriaceae</i>	6	8	10	8	2
<i>(St. Aureus)</i>	16	51	73	47	29
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 1 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	115	145	205	155	46
<i>Lactobacilli</i>	400	450	460	437	32
<i>Enterobacteriaceae</i>	5	12	8	8	4
<i>(St. Aureus)</i>	162	61	79	101	54
<i>E-coli and coliforms</i>	8	6	4	6	2

Site 2: Nursery

Site 2 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	190	440	465	365	152
<i>Lactobacilli</i>	250	360	405	338	80
<i>Enterobacteriaceae</i>	5	12	8	8	4
<i>(St. Aureus)</i>	162	61	79	101	54
<i>E-coli and coliforms</i>	8	6	4	6	2

Site 2 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	332	280	350	321	36
<i>Lactobacilli</i>	89	81	92	87	6
<i>Enterobacteriaceae</i>	19	3	8	10	8
<i>(St. Aureus)</i>	68	58	31	52	19
<i>E-coli and coliforms</i>	0	4	0	1	0

Site 2 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	18	32	50	33	16
<i>Lactobacilli</i>	11	19	81	37	38
<i>Enterobacteriaceae</i>	0	0	3	1	2
<i>(St. Aureus)</i>	12	18	33	21	11
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3: Public toilets

1 DAY AFTER INCUBATION CFU / petri film

Site 3 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	97	152	198	149	51
<i>Lactobacilli</i>	121	163	144	143	21
<i>Enterobacteriaceae</i>	16	15	19	17	2
<i>(St. Aureus)</i>	66	38	34	46	17
<i>E-coli and coliforms</i>	8	6	4	6	2

Site 3 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	208	176	225	203	25
<i>Lactobacilli</i>	72	59	41	57	16
<i>Enterobacteriaceae</i>	18	10	6	11	6
<i>(St. Aureus)</i>	17	30	28	25	7
<i>E-coli and coliforms</i>	12	16	10	13	3

Site 3 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	39	54	53	49	8
<i>Lactobacilli</i>	158	131	162	150	17
<i>Enterobacteriaceae</i>	4	5	2	4	2
<i>(St. Aureus)</i>	55	38	91	61	27
<i>E-coli and coliforms</i>	25	31	44	33	10

Addendum 5:

Results of the measurements on 06/03/2013 - TEST PERIOD

Site 1: Dienstencentrum

1 DAY AFTER INCUBATION CFU / petri film

Site 1 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	139	138	119	132	11
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	103	151	156	137	29
<i>E-coli and coliforms</i>	1	0	3	1	2

** TNTC: Too numerous to count

Site 1 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	32	65	73	57	22
<i>Lactobacilli</i>	56	59	66	60	5
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	7	13	112	44	59
<i>E-coli and coliforms</i>	0	2	0	1	1

Site 1 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	325	265	455	348	97
<i>Lactobacilli</i>	208	196	172	192	18
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	47	80	53	60	18
<i>E-coli and coliforms</i>	11	12	13	12	1

Note: Lactobacilli were counted by standard after 2 d incubation

Because of unclear growth measured after 1 day of incubation, the measurement of E-coli and coliforms was performed after 2 days of incubation

Site 2: Nursery

Site 2 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	91	33	79	68	31
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	11	12	7	10	3
<i>E-coli and coliforms</i>	48	8	51	36	24

** TNTC: Too numerous to count

Site 2 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	265	300	220	262	40
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	22	33	19	25	7
<i>E-coli and coliforms</i>	21	44	38	34	12

** TNTC: Too numerous to count

Site 2 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	11	23	12	15	7
<i>Lactobacilli</i>	48	45	58	50	7
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	9	23	16	16	7
<i>E-coli and coliforms</i>	1	3	5	3	2

Because of unclear growth measured after 1 day of incubation, the measurement of E-coli and coliforms was performed after 2 days of incubation

Site 3: Public toilets

WET FLOOR! CFU / petri film

Site 3 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	TNTC**	TNTC**	TNTC**		
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	Growth matter not countable				
<i>(St. Aureus)</i>	TNTC**	TNTC**	TNTC**		
<i>E-coli and coliforms</i>	TNTC**	TNTC**	TNTC**		

** TNTC: Too numerous to count

Site 3 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	94	98	86	93	6
<i>Lactobacilli</i>	72	88	74	78	9
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	21	17	18	19	2
<i>E-coli and coliforms</i>	1	3	9	4	4

WET FLOOR!

Site 3 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	TNTC**	TNTC**	TNTC**		
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	Growth matter not countable				
<i>(St. Aureus)</i>	TNTC**	TNTC**	TNTC**		
<i>E-coli and coliforms</i>	TNTC**	TNTC**	TNTC**		

Because of unclear growth after 1 day of incubation, the measurement of E-coli and coliforms was performed after 2 days of incubation

Addendum 6:

Results of the measurements on 27/03/2013 - TEST PERIOD

Site 1: Dienstencentrum

1 DAY AFTER INCUBATION CFU / petri film

Site 1 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	85	107	128	107	22
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	73	55	82	70	14
<i>E-coli and coliforms</i>	0	0	0	0	0

**TNTC: Too numerous to count

Site 1 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	86	96	29	70	36
<i>Lactobacilli</i>	21	31	72	41	27
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	5	7	6	6	1
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 1 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	149	83	52	95	50
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	1	0	0	0	1
<i>(St. Aureus)</i>	59	86	95	80	19
<i>E-coli and coliforms</i>	1	1	2	1	1

**TNTC: Too numerous to count

Site 2: Nursery

Site 2 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	156	55	108	96	37
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	41	30	59	43	15
<i>E-coli and coliforms</i>	11	10	6	9	3

**TNTC: Too numerous to count

Site 2 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	108	80	70	86	20
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	2	0	0	1	1
<i>(St. Aureus)</i>	45	41	11	32	19
<i>E-coli and coliforms</i>	0	0	0	0	0

**TNTC: Too numerous to count

Site 2 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	95	43	31	56	34
<i>Lactobacilli</i>	18	27	35	27	9
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	17	21	11	16	5
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3: Public toilets

1 DAY AFTER INCUBATION CFU / petri film

Site 3 A	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	TNTC**	TNTC**	TNTC**		
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	TNTC**	TNTC**	TNTC**		
<i>E-coli and coliforms</i>	0	0	0	0	0

**TNTC: Too numerous to count

Site 3 B	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	5	7	3	5	2
<i>Lactobacilli</i>	7	9	6	7	2
<i>Enterobacteriaceae</i>	0	1	2	1	1
<i>(St. Aureus)</i>	1	3	13	6	6
<i>E-coli and coliforms</i>	0	0	0	0	0

Site 3 C	Count 1	Count 2	Count 3	Average	Standard deviation
<i>Total aerobes</i>	277	184	251		
<i>Lactobacilli</i>	TNTC**	TNTC**	TNTC**		
<i>Enterobacteriaceae</i>	0	0	0	0	0
<i>(St. Aureus)</i>	223	135	88	149	69
<i>E-coli and coliforms</i>	0	0	0	0	0

**TNTC: Too numerous to count