FACHHOCHSCHULE DEGREE COURSE
BIO- AND ENVIRONMENTAL TECHNOLOGY

Study of the cleaning effects of EM·Effective® Microorganisms

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by

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Dissertation tutor

DI ROBERT BURGHOLZER

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My sincerest thanks also go to my family, who made my studies at the Fachhochschule Wels possible, as well as to my study colleagues and friends, who accompanied me during my course.

Wels, September 2006

Daniel Haslinger

Oath

I promise to serve the sciences in truth, to further their objectives, to use the knowledge gained from my studies with responsibility and thus to contribute to the solution of the problems of human society.

I promise to consider the general good with regard to my actions, to protect the natural bases of life, to maintain my associations with the Fachhochschule and to prove myself worthy of my academic title.

_____

Name: Daniel Haslinger
Matriculation number: 02/1/0112/014

Abbreviations

ATP	Adenosintriphosphate
AMP	Adenosinmonophosphate
CCP	Critical Control Point
CV	Coefficient of variation
DGHM	Deutschen Gesellschaft für Hygiene und Mikrobiologie
DIN	Deutsches Institut für Normung
EM	Effective® Microorganisms
fg	Femtogram ($1x10^{-15}$ g)
GKZ	Total bacteria total
HACCP	Hazard Analysis and Critical Control Point
h	Hours
KBE	Colony Forming Units
MO	Microorganisms
RLU	Relative Light Units
RODAC	Replicate Organism Direct Agar Contact
TVC	Total Colony Counts
ÖGHMP	Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin

Abstract

EM·Effective® Microorganisms include a large number of differing microorganisms, which by means of up- and degrading processes, are capable of influencing organic substances in such a manner that a life supporting process is created. Such mixtures of active substances are designated as EM·Effective® Microorganisms and have already achieved positive results in diverse areas of application. In eMC® Cleaner the effectiveness of these microorganisms is enhanced by the addition of various biological additives, e.g. enzymes, sugar cane molasses and fruit seed extracts. This dissertation is intended to clarify if, when used as a cleaning agent, EM·Effective® Microorganisms are in any way less efficient and effective than chemical equivalents.

In order to examine the cleaning effects, surfaces were cleaned and examined for dirt using ATP measurement and conventional, microbiological methods. These controlled tests were completed in various facilities and buildings. As comparative cleaning agents, products were employed that are used in the various test objects for cleaning subject to the instructions for use supplied by the manufacturers. In addition, a disinfectant meeting the requirements of the ÖGHMP and DGHM was utilised as a reference point in all the test objects. The methods and materials employed, as well as the evaluation of the results corresponded with generally applied standards.

The results show that directly after the cleaning process, the eMC® and comparative cleaning agents provided virtually identical results and were only slightly surpassed by the disinfectant. The results also indicated that the test surfaces subjected to eMC® Cleaner remained cleaner for longer, than those treated with the comparative agents. Above all, this was the case 24 and 48 hours after cleaning, where the absolute frequency of the results of the eMC® Cleaner were in the 4 bacteria count class. In the comparable products, this value amounted to bacteria count class 5.

CONTENTS

1. Introduction

1.1. EM·Effective® Microorganisms

Professor Teruo Higa from Ryukyu University, Okinawa, developed the concept of EM·Effective® Microorganisms in the 1970s. This concept states that a combination of around 80 differing microorganisms is capable of influencing putrefying ("life hostile") organic substances in such a way that a life-promoting process results. In the case of eMC® Cleaner, these processes are supported by biological additives, e.g. enzymes, sugar cane molasses and ethereal oils. In this connection, the microorganisms can be divided into three groups:

- Negative microorganisms (decomposing, degenerative, putrefactive)
- Positive microorganisms (constructive, regenerative, fermentative)
- Neutral microorganisms (opportunistic "fellow travellers")

Higa focused largely on cultures, which are employed during the production of soured milk foods. The microorganisms contained in the EM·Effective® Microorganisms consist mainly of lactic acid bacteria, yeast and photosynthesis bacteria, i.e. positive microorganisms.

The effects of EM can be explained by the dominance principle. Both positive and negative microorganisms can predominate and the opportunistic microorganisms support the group dominating within a milieu. Consequently, even a small quantity of microorganisms is capable of steering processes in a certain environment (water, soil, air, intestines, nutritional solutions, etc.) in the desired direction.

In his book, Professor Higa describes numerous applications for EM, e.g. in the nutritional sector, agriculture, horticulture, etc., which have led to excellent results. EM technology is already in extensive use in Asia, as opposed to the USA and Europe, where the upturn in the EM technology sector first commenced in the 1990s. However, it should be added that this upswing is still in progress and new applications are constantly being added.[i]

1.2. E. coli

E.coli is a gram-negative, rod-shaped, acid-forming and peritrichal, flagellated bacterium, which inhabits human and animal intestines and amounts to around 1% of the bowel flora. Outside the intestines, *E. coli* is seen as a bacterial indicator for the faecal pollution of water, food and surfaces. *E .coli* was named after Theodor Escherich, who discovered it in 1919 and today it numbers among the best-documented organisms in the world. [ii]

Indeed, should hygiene and cleanliness be the object of discussion, then these terms are currently closely linked with *E. coli*.

1.3. Coliform bacteria

Coliform bacteria constitute a heterogeneous, enterobacterial group, which includes *Escherichia, Citrobacteria, Salmonella, Enterobacteria* and *Klebsiella*, to name but a few.

Family	Genus	Type
Enterobacteriaceae	*Escherichia*	*Escherichia coli*
	Klebsiella	*Klebsiella pneumoniae*
	Salmonella	*Salmonella enteritidis*
	Enterobacteria	*Enterobacter amnigenus*
	Citrobacteria	*Citrobacter freundii*

Table 1 Family, genus and type of standard coliforms

The majority of definitions of coliform bacteria are based largely on general biochemical characteristics. Coliform bacteria are described as constituting all aerobic and facultative anaerobic, gramnegative, non-spore forming bacilli, which ferment lactose within 48h at 35°C, creating acids and gas, or all aerobic and many facultative anaerobic, gramnegative, non-spore forming bacilli, which form a red colony with a metallic shine within 24h at 35°C on an endoagar containing lactose.[iii]

However, as a result of improved microbiological diagnostics, the term coliform bacteria has altered:

Prior to 1994	Report 71 (1994)	Enzyme-based (ß-Galactosidase)
Degradation of lactose into gas and acids within 24-48h at 36±2°C, thermotolerant or faecal coliforms (44.5±0.2°C).	Definition of coliforms was altered to the formation of acid from lactose.	Now bacteria containing the ß-Galactosidase gene (lac Z gene) are also included in the coliform bacteria group.
Escherichia	*Escherichia*	*Escherichia*
Klebsiella	***Klebsiella***	***Klebsiella***
Enterobacteria	***Enterobacteria***	***Enterobacteria***
Citrobacteria	***Citrobacteria***	***Citrobacteria***
	Yersinia	***Yersinia***
	Serratia	***Serratia***
	Hafnia	***Hafnia***
	Pantoea	***Pantoea***
	Kluyvera	***Kluyvera***
		Cedecea

		Ewingella
		Moellerella
		Leclercia
		Rahnella
		Yokenella

Genus in bold type = coliforms that are found both in the environment and in human faeces.

Genus in bold type and underlined = coliforms, which are mainly found in the environment.

Table 2 Development of the classification of coliform bacteria[iv]

The coliform concept includes all coliform bacteria and therefore also encompasses bacteria, which are not of faecal origin and do not pose a health problem. Therefore, the presence of coliform bacteria only permits the suspicion of the faecal pollution of water, foods or surfaces, but this may not necessarily be the case.

1.4. Moulds

These belong to the mushroom or fungi family (Eukaryotes) and possess a cytoskeleton, mitochondrial, endoplasmatic reticulum (ER) and, as opposed to bacteria (Prokaryotes), in general also have a genuine cell nucleus.

Their multiplication mostly occurs pathogenically via so-called spores, which are to be found virtually everywhere in the air. These are also referred to as conidia in the case of known mould forming genera of tubular fungi (Ascmycetes) such as *Penecillium* and *Aspergillus.*

In scientific terms, there is no separate group of moulds, but nonetheless many fungi genera such as *Mucor* (capitulum mould), *Rhizopus* (common

bread mould), *Aspergillus* (watering can mould), *Cladosporium* and *Penicillium* (brush mould) are classified as belonging to the moulds.

A differentiation is frequently made between moulds (e.g. starter cultures such as Penicillium nalgiovense on cheese) and harmful, mycotoxic moulds and spores, which can cause allergies in sensitive persons.[v]

1.5. Yeasts

Like moulds, yeasts number among the fungi, but are constantly monocellular and multiply by budding or division. As opposed to moulds, yeasts have either partially or totally lost the ability to form hyphai. However, as yeasts belong to the eukaryotes, in general they are significantly larger than bacteria and due to their cell organelle, also have greater similarities to higher organisms (animal and plant cells) than bacteria.

Yeasts are used for a diversity of important commercial purposes. In addition to the production of bread, beer and wine, they are employed for numerous biotechnological applications for the development of a variety of raw and inactive ingredients in the pharmaceutical field.[vi]

2. Objective

2.1. Assignment

As no scientific tests exist with regard to the cleaning effects of the eMC® Cleaner (Messrs Multikraft), these were to be completed in the course of this dissertation. The tests incorporated the examination of the overall bacteria total, coliform and (mould) fungi by means of classic, microbiological methodology (impression test) and bioluminescence (ATP measurement). The completion of these experiments was divided into three phases. As no authorisation is required for domestic cleaning agents in Austria, the provision of meaningful results using the aforementioned methods was attempted.

2.1.1 Description of Phase 1

This phase involved the testing of the cleaner concentration in order to subsequently clean five test objects using the diluted cleaner. At the same time, a comparison was made between eMC® Cleaner and a standard, comparable cleaning agent. This initial, or laboratory phase, involved two private kitchens and three freely selected laboratory areas at the Fachhochschule Wels.

2.1.2 Description of Phase 2

The second phase saw the completion of testing on five test objects selected by *Messrs Multikraft*. In this phase, examinations were completed relating to the test surfaces cleaned with eMC® Cleaner, which were in normal daily use, as opposed to the laboratory test surfaces from Phase 1, which remained untouched during the 3-day sampling test period.

2.1.3 Description of Phase 3

The third and thus concluding phase, served the verification of the results from the preceding test phases. In addition, during the final phase, the cleaning effect of the eMC® Cleaner was compared with standard cleaning agents and a disinfectant. Phase 3 included a total of three test runs using the five test objects. During these three runs, the layout of the test surfaces was altered in such a way that each test surface was tested using each of the three cleaners. This was intended to provide information, as to whether certain surfaces become more soiled than others during normal use.

3. Materials and Methods

3.1 Materials employed

Designation	Type	Brand
Nutrient medium	Envirocheck® Rodac Blister TVC	Merck
Nutrient medium	Envirocheck® Contact C	Merck
SystemSure II	Ultrasnap ATP swab	Hygiena
Descocid	Disinfectant	Antiseptica
Universal cleaner❶*	Household cleaner	Henkel
Universal cleaner❷	Alcohol-based cleaning agent	Stangl
Rinsing and cleaning agent❸	Prilon	Ecolab
Alcohol-based cleaning agent ❹	Alcosan	Gruber
Fat solvent❺	Craft	Gruber

Table 3 Materials employed

*…subsequently designated as comparative cleaners ❶-❺.

3.2. Methods employed

3.2.1. Determination of the total bacteria, mould and yeasts on surfaces

The *Envirocheck® Rodac Blister TVC* nutrient medium served to determine the total number of aerobic, mesophilic bacteria. Apart from bacteria, this quantitative process also proved the presence of yeasts and moulds. Subsequent to the taking of samples, the *Rodac plates* 68 ± 4 h were incubated at 30°C. For the better identification of moulds and yeasts, the plates were further incubated for 24-68 h at 30 °C.

Following sufficient incubation of the *Rodac plates*, the colonies on the 25 cm^2 agar surface were counted and then extrapolated for 100 cm^2. The identification of the individual MOs took place according to standard microbiological methods. The differentiation between yeasts and moulds is of special importance to the assignment involved in this dissertation and therefore this was completed using an underlight microscope.

The nutrient medium employed on the Rodac plates corresponded with the Draft European Standard CEN/TC 243/WG 2 (German version prEN 1632-3:1994);(Table 4).

The basis medium contains disinhibition agents in order to inactivate antibiotics (*CASO Agar, Merck Art.Nr.1.05458*). [vii]

Substance	Mass concentration [g/l]
Casein peptone	15.0
Soya peptone	5.0
NaCl	5.0
Tween 80	5.0
Lecithin	0.7
Sodium thiosulphate	0.5
L-histidine	1.0
Agar-agar	20.5

Table 4 Rodac TVC plate nutrient medium composition

According to DIN 10113-3 (*Deutsches Institut für Normung 1997*), for better comparison, the count results can be subjected to various methods as shown in Table 5.

KBE/plate (25cm^2)	Bacteria count groups
0	0
1-3	1
4-10	2
11-30	3
31-60	4
> 60 colonies, but non-confluent	5
Lawn growth, confluent, and >300	6

Table 5 Evaluation classes according to DIN 10113-3

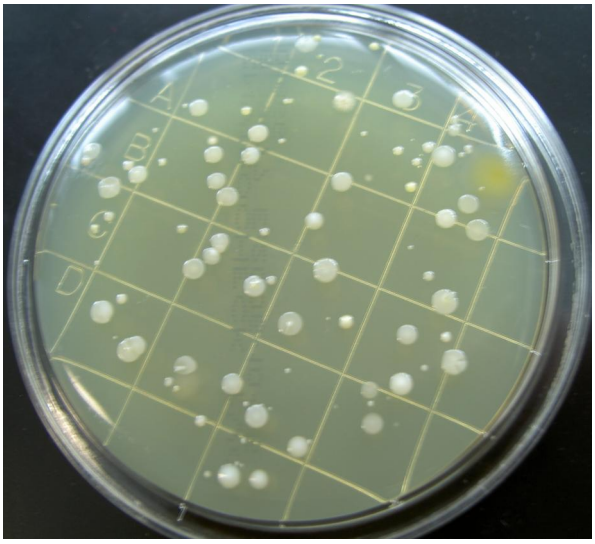

Fig. 1 Bacteria count group 5 (non-confluent)

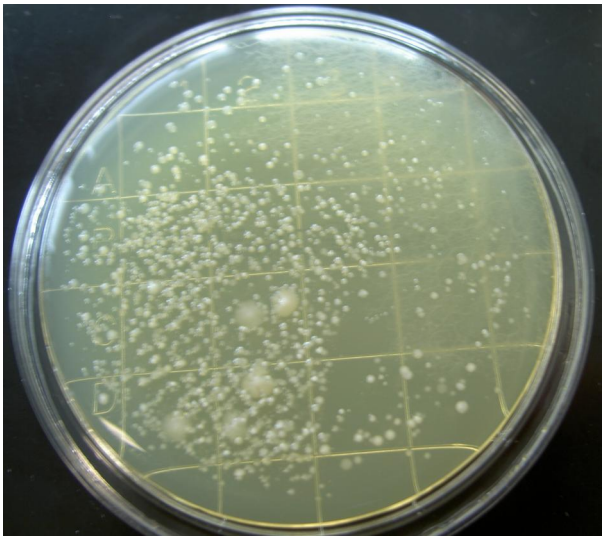

Fig. 2 Bacteria count group 6 (confluent)

As previously mentioned, authorisation is not obligatory for domestic cleaners and therefore there is no standard procedure with regard to the examination of the effectiveness of these cleaning agents.

Nonetheless, I believe an evaluation of the results using DIN 10113-3 to be both permissible and useful, although as a rule, this regulation is employed for the determination of surface bacteria content on fixtures and utensils in the foods sector.

3.2.2. Determination of the total coliforms/E.coli on surfaces

Envirocheck® Contact C slides consist of two differing test surfaces. Plate count agar has been applied to test surface 1 for the determination of the total aerobic bacteria count.

Following 48-hour incubation at 37°C in the laboratory, evaluation took place using a semi-quantitative process, whereby in accordance with five evaluation classes, the growth thickness of the nutrient medium carrier

was classified as ranging from "very small" to "very large" in line with the comparative sample from the nutrient manufacturer.

Bacterial growth	Very small	Small	Moderate	Large	Very large
Aerobic bacteria count in KbE/cm^2	3.5	17	58	140	350

Table 6 Envirocheck Contact C evaluation classes for plate count agar

Chromocult® coliform agar is applied to surface 2 to prove the presence of coliforms and E.coli as evidenced by Table 7. The evaluation of testing surface 2 took place in a manner analogous to that employed for test surface 1.

Organism	Plate count agar	Chromocult® coliform agar
E.coli ATCC 11775	Good growth	Good growth; dark blue-violet colonies
C.freundii ATCC 8090	Good growth	Good growth; rose pink colonies
E.coli 0157:H7 ATCC 35150	Good growth	Moderate/ good growth; pink-red colonies
S.enteritidis ATCC 13076	Good growth	Good growth; colourless colonies

Table 7 Bacteria growing on Envirocheck® Contact C

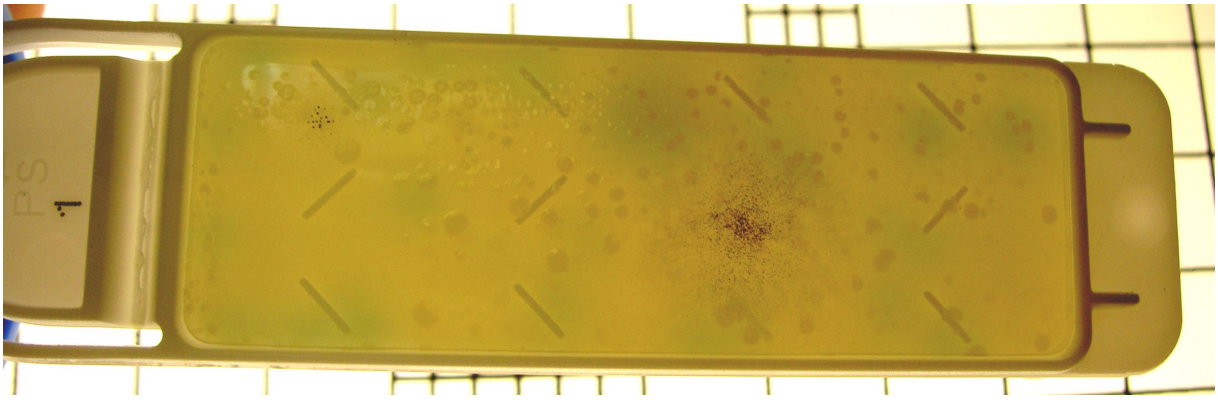

Fig. 3 Contact C slide test surface 1 (plate count agar) with strong bacterial growth

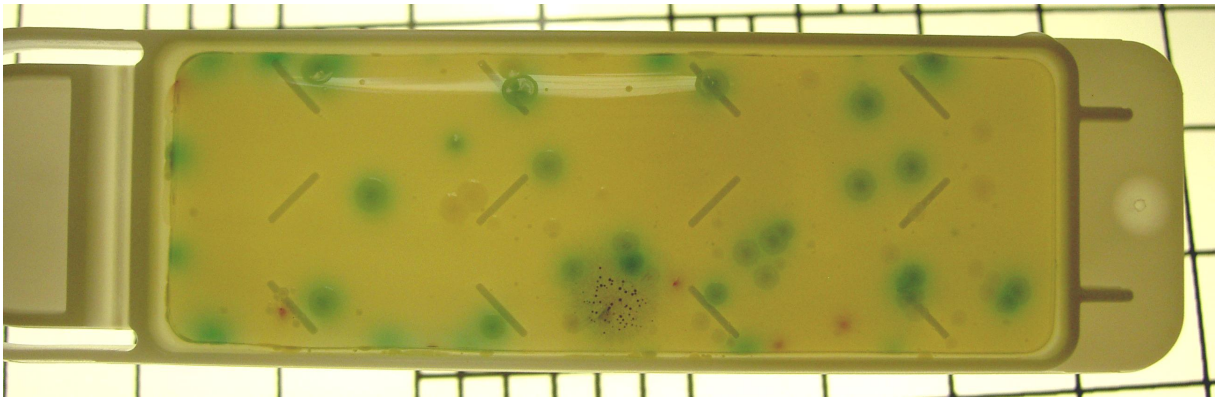

Fig. 4 Contact C slide test surface 2 (Chromocult® coliform agar) with strong bacterial growth

3.2.3. ATP measurement

The *System SURE II* surface sampling device is a sealed piece of apparatus for use with the *System SURE II* luminometer. This measurement device is particularly suitable for product quality and HACCP checks on production equipment, surfaces, water samples and other areas in which hygiene and cleanliness are especially important.

ATP is a universal energy molecule, which is found in all animal, plant, bacteria, mould and yeast cells. ATP can also occur in product and food residues, which contain more ATP than microbiological impurities.

The measurement principle is based on bioluminescence, which allows certain living things to generate light either directly, or with the help of symbiotes. The luciferines contained in the *Ultrasnap ATP swab* are induced to emit energy in the form of light by means of exerogenous oxidation with ATP (bioluminescence; see Fig. 5). This reaction is then catalysed by luciferases, which are also contained in the *Ultrasnap ATP swab.* When the test swab comes into contact with the luciferine/luziferase reagence in the *Ultrasnap* testing device, light is emitted in direct ratio to the quantity of ATP present. The *System Sure II* device measures the volume of light generated and provides the measurement result in RLU within seconds. [viii]

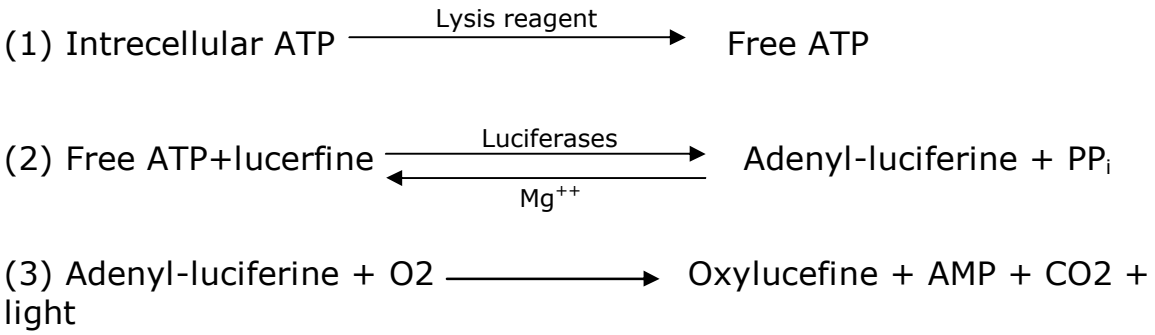

Fig. 5 Bioluminescence reaction principle

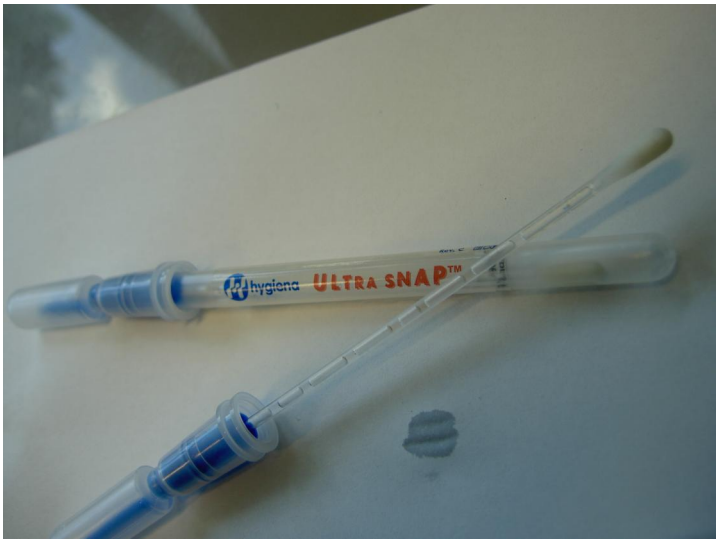

Fig. 6 Ultrasnap ATP swab

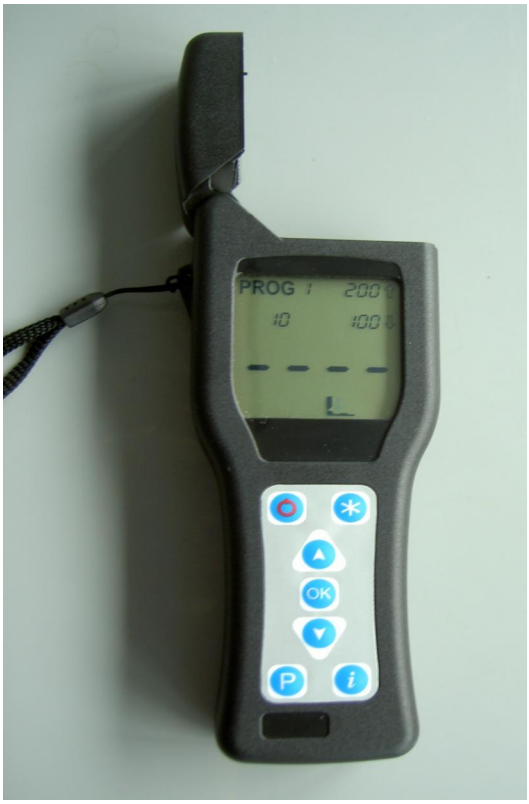

Fig. 7System Sure II - luminometer

On average, the ATP content of bacteria amounts to 1fg/cell. Yeast cells contain approximately 30 times this amount and somatic cells around 100 time more ATP than bacteria cells. By contrast, *Bacillus cereus* only contains about 0.1 fg/cell and spores no ATP.[ix]

3.3. Completion of surface testing

The sizes of the selected testing surfaces amounted to 1 m^2 ±0,2 m^2. Prior to the taking of the first sample, any possible dust and dirt was removed from these surfaces using disposable cloths. Initial sampling then followed (point in time t_0) in line with the procedure shown in Fig. 1.

The test surfaces were then cleaned with the respective cleaning solutions using disposable cloths and samples again taken (point in time

t_1). The other samples (t_2-t_x) were taken at established points in time following each test phase subject to adherence to the schedule contained in Fig.1, in order to ensure the comparability of the results.

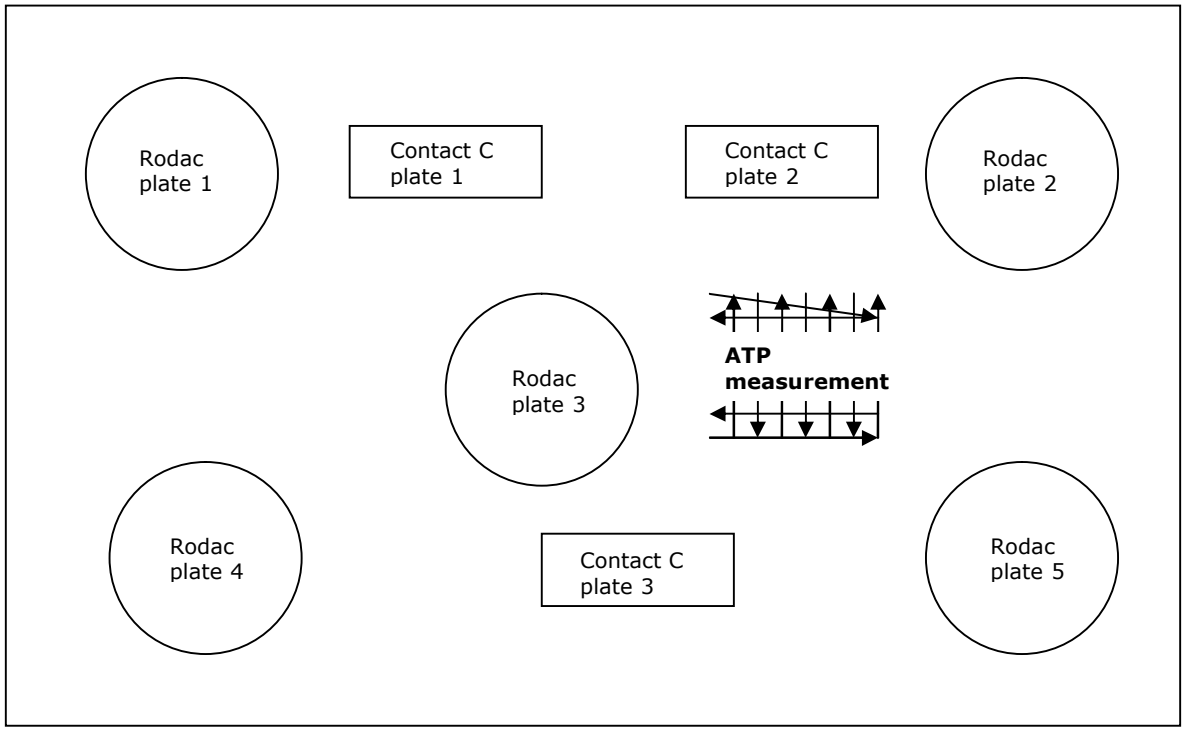

Fig. 8 Schematic diagram of the test layout

3.4. Reproduceability and sensitivity of the methods employed

Characteristic	Microbiology	ATP bioluminescence
Acceptance	High acceptance worldwide in the foods industry	High acceptance in the UK and parts of Europe, levels increasing
Methods/basic principle	Microorganisms are contacted from a surface and bred	ATP from microorganisms and foods are analysed according to enzyle reaction using a luminometer
Test duration	18-48 hours	2 minutes

Sensitivity with standard E. coli test	10^0 cells	10^4 cells
Reproducibility with clean/marginally clean surfaces	CV 60-191%	CV 19-31%
Workplace requirements	Laboratory necessary	Laboratory unnecessary
Employee requirements	Microbiological training necessary	Little or no training necessary

Table 8 Reproduceability/sensitivity of the methods employed

Measurement reproducibility can be defined as the ability to achieve the same result under identical conditions and with the same level of biological impact, irrespective of the person carrying out the testing.

Reproducibility is defined by the "coefficient of variation" (CV) or through the relative standard deviation (SD). The CV is calculated as follows[x]

$$CV = \frac{SDx100}{Average}$$

The standard deviation (SD) is expressed as a percentage of the average and the lower the CV, the greater the precision and hence the reproducibility of the results.

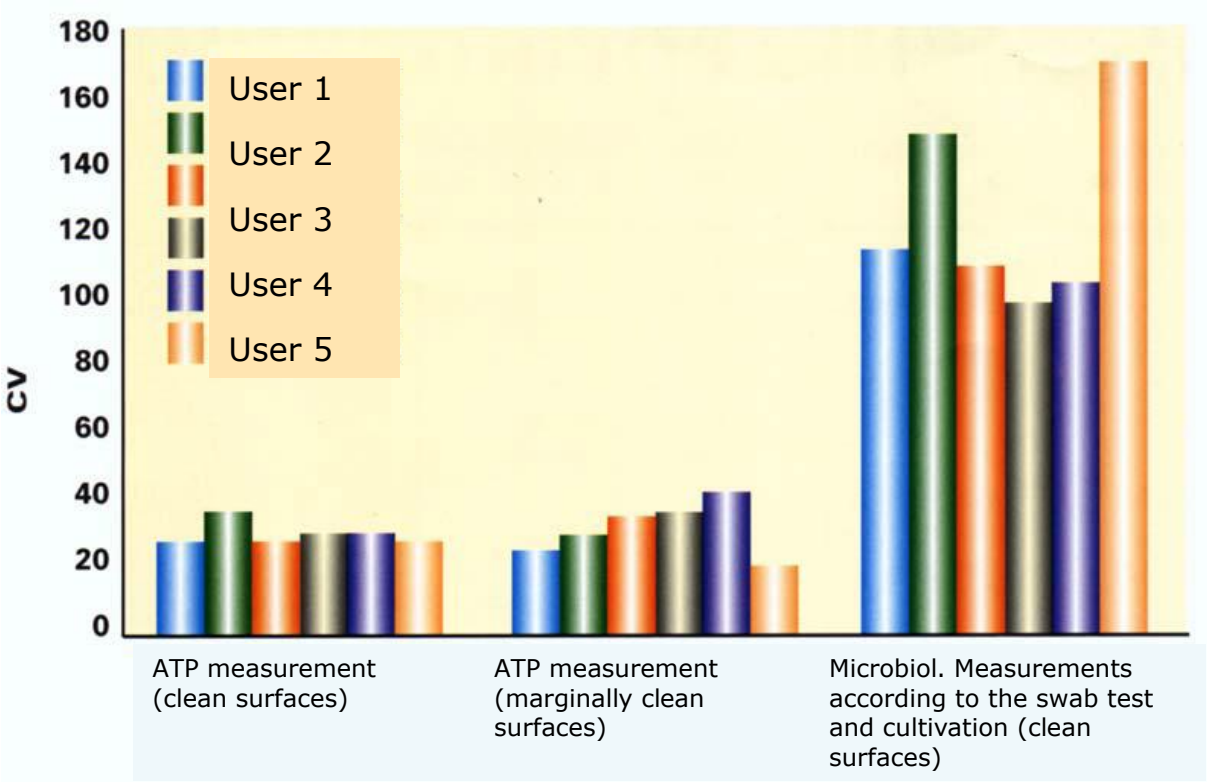

Fig. 9 Comparison of the reproducibility of microbiological and ATP bioluminescence measurements

4. Results

4.1 Phase 1

Table 9 shows that eMC® Cleaner in a dilution of 1:100 achieved a superior cleaning result at the t_1 time point. The optical impression also communicated a better cleaning result as compared to the 1:1000 solution. The subsequent tests were therefore all carried out with the 1:100 dilution.

Figs 10-21 show the temporal sequence of KBE and ATP. The coliform load amounted to "very small" on all test surfaces and at all times (*coliform key: see page 20, Table 6*). Moulds only grew singly.

Test surface 0	EM dilution			
	1:100		1:1000	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	62	172	46	373
1	14	20	18	22
2	23	12	9	24
4	25	36	15	10
22	17	16	22	31

Table 9 Dilution comparison

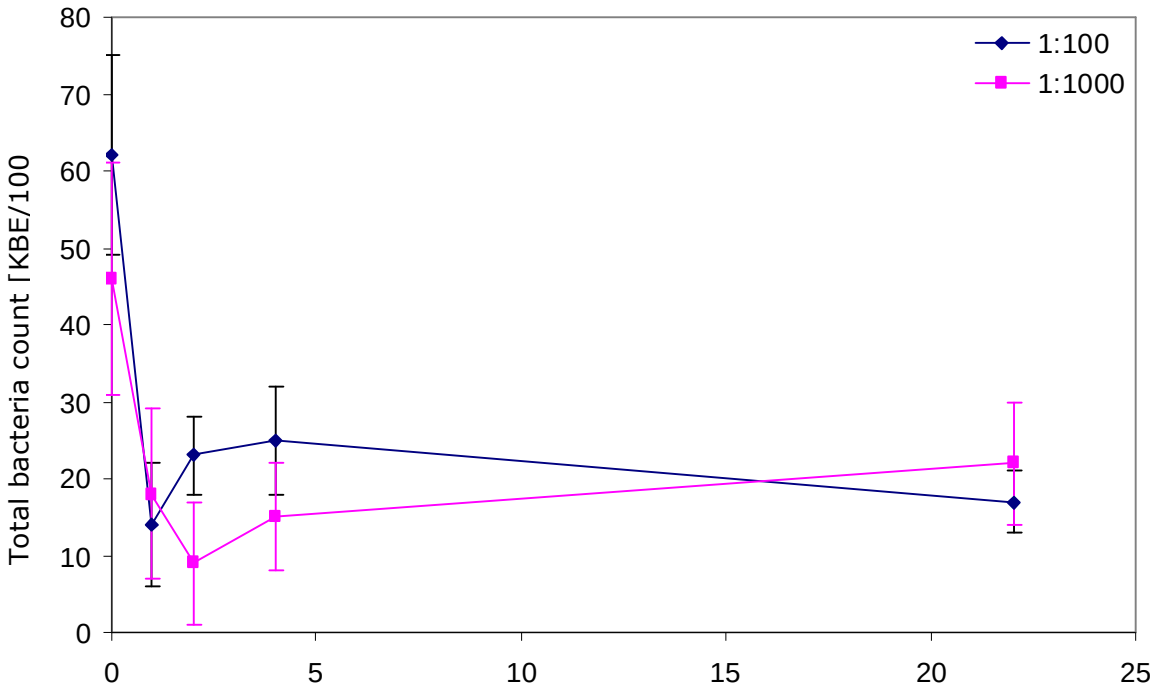

Fig. 10 Dilution comparison 1:100/1:1000 - temporal KBE development

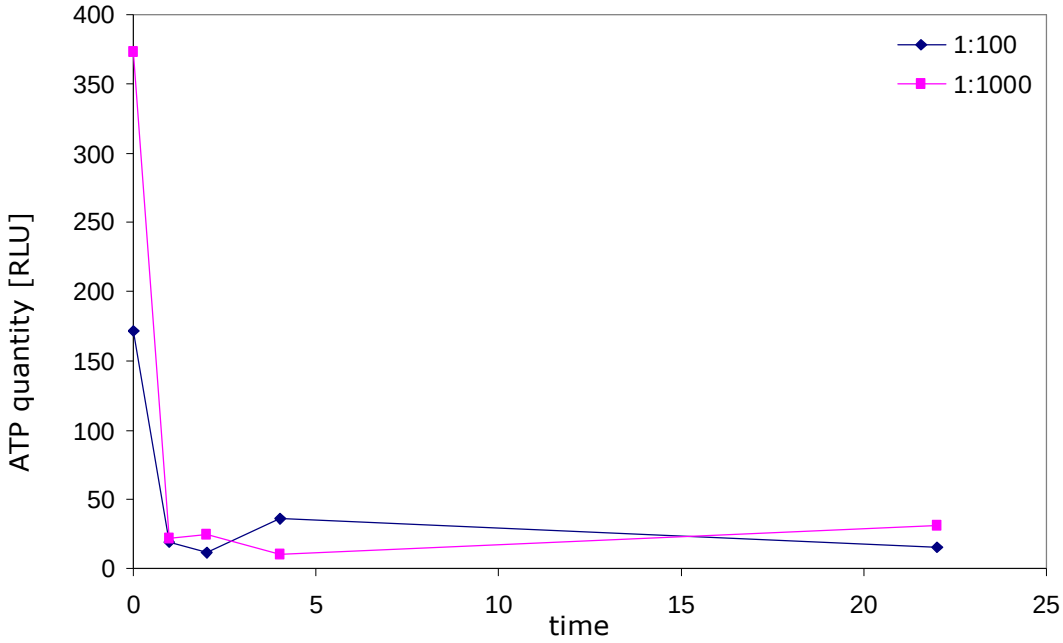

Fig. 11 Dilution comparison 1:100/1:1000 - temporal ATB development

Test surface 1	EM		Comp. cleaner	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	315	315	530	196
1	82	6	1200	37
12	234	43	1200	113
24	341	65	1200	227
48	386	137	1200	250

Table 10 Phase 1 – private kitchen 1

Fig. 12 shows that in the case of the comparative cleaner ❶, the selected dilution (1:100) was too low to reduce the number of bacteria and to sufficiently clean the surface.

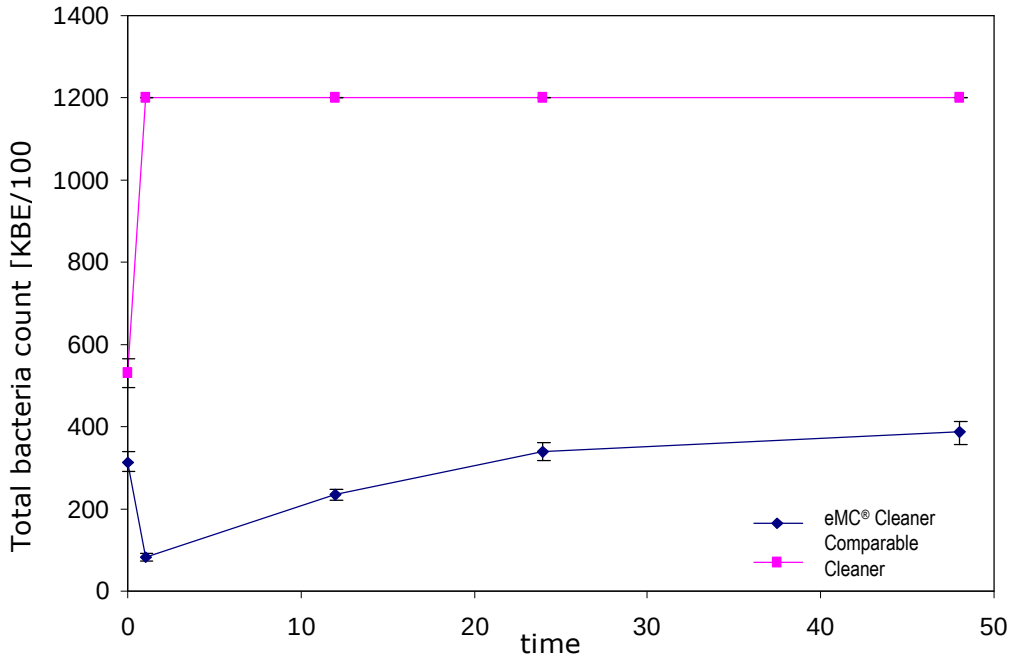

Fig. 12 Test surface 1 - temporal KBE development

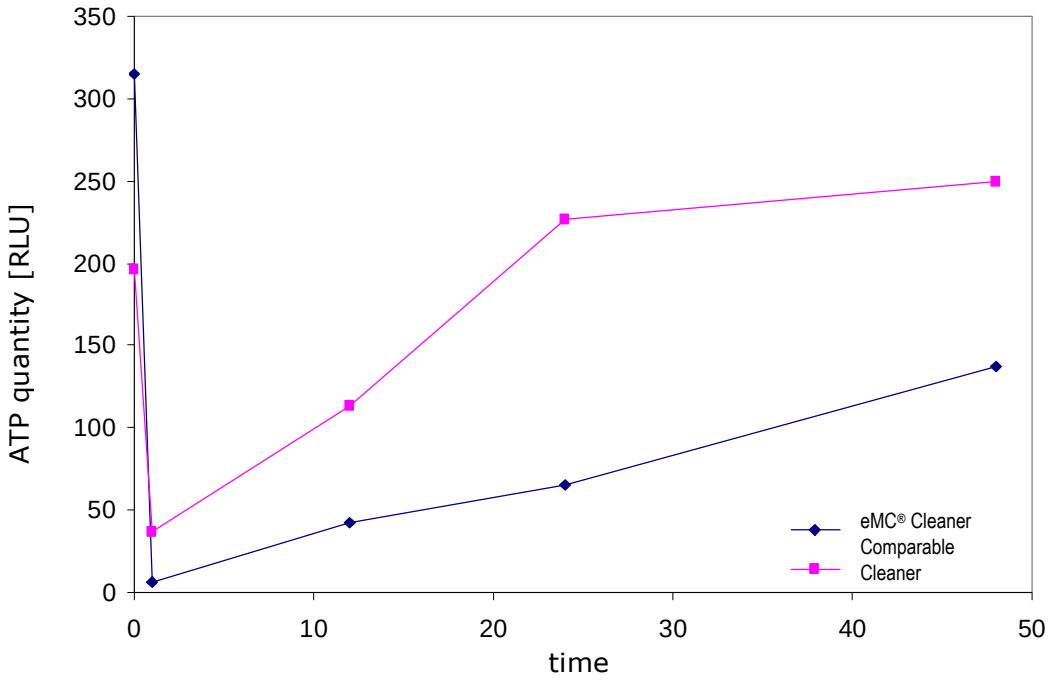

Fig. 13 Test surface 1 - temporal ATB development

Test surface 2	EM		Comp. cleaner	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	167	176	174	203
1	8	3	13	3
12	83	4	47	5
24	31	12	13	9
48	40	15	31	32

Table 11 Phase 1 – microbiological laboratory

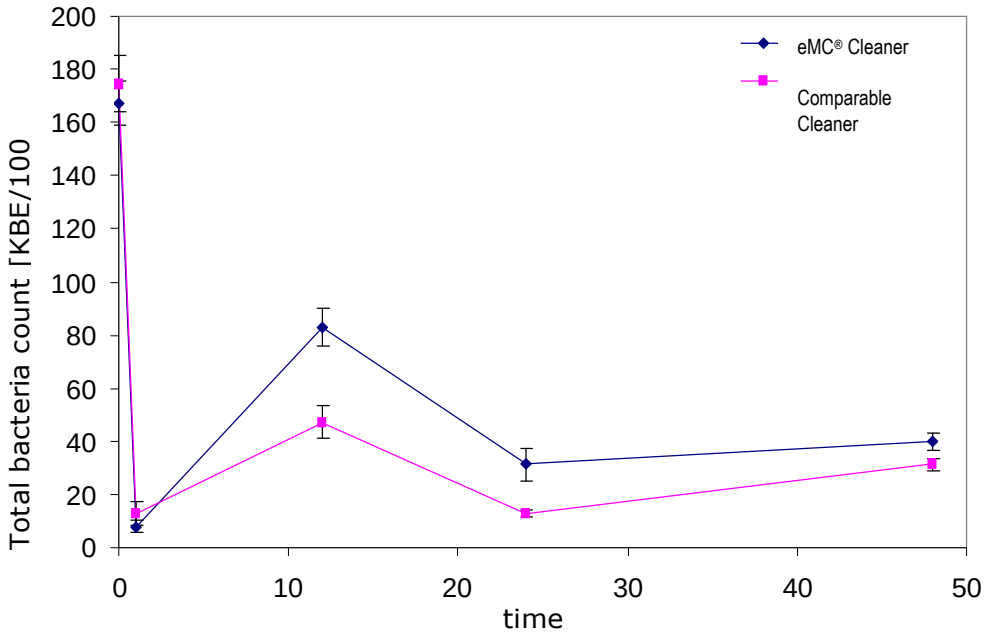

Fig. 14 Test surface 2 - temporal KBE development

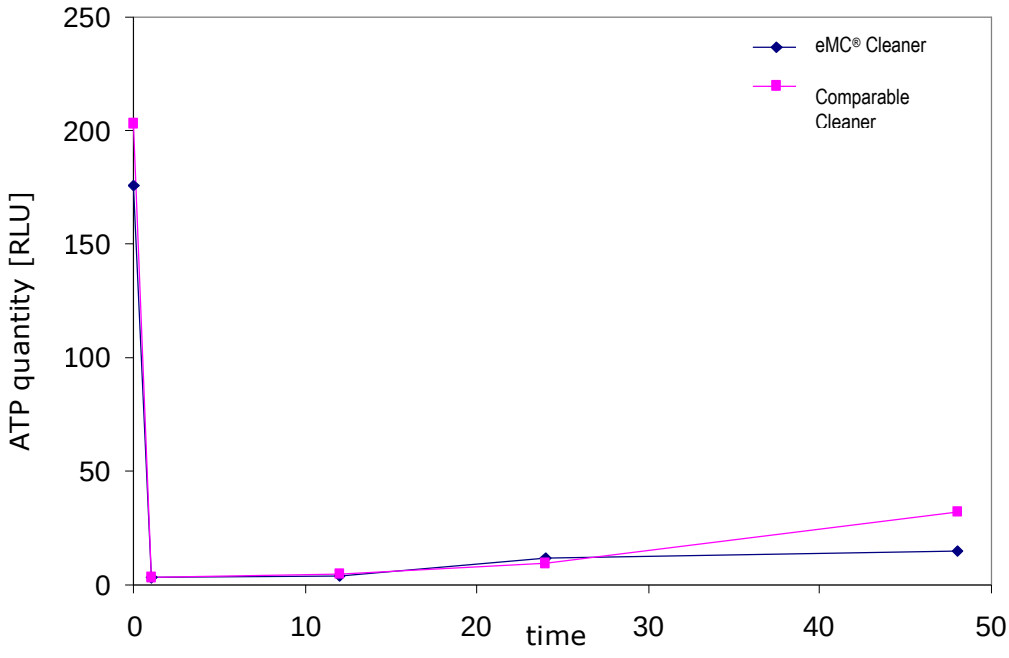

Fig. 15 Test surface 2 - temporal ATP development

Test surface 3	EM		Comp. cleaner	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	238	228	229	241
1	5	3	18	5
12	65	8	99	18
24	60	25	130	32
48	77	33	135	52

Table 12 Phase 1 – chemical laboratory

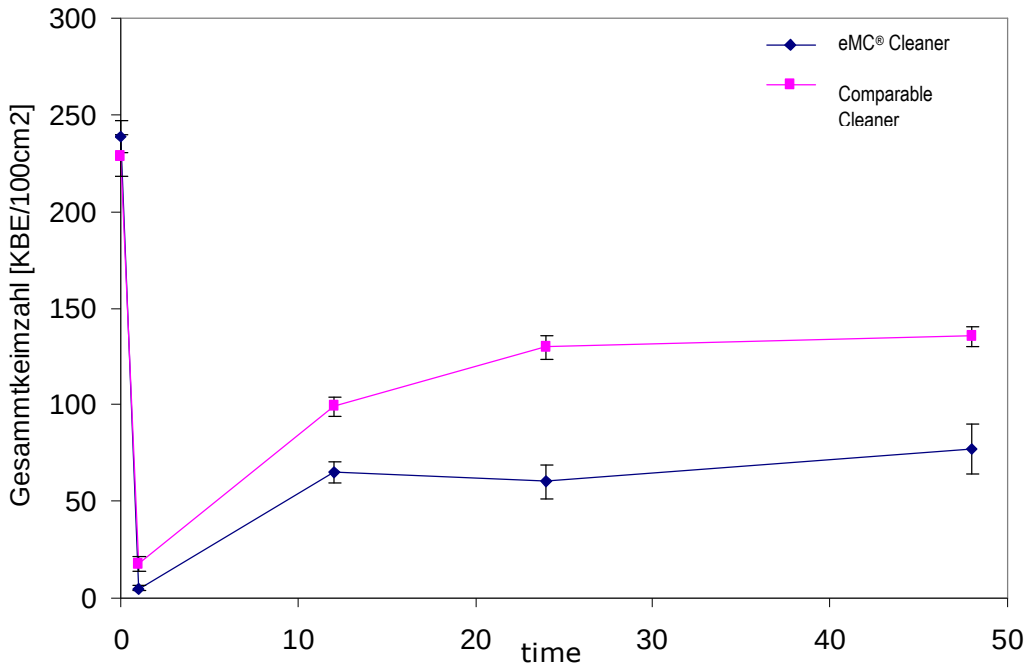

Fig. 16 Test surface 3 – temporal KBE development

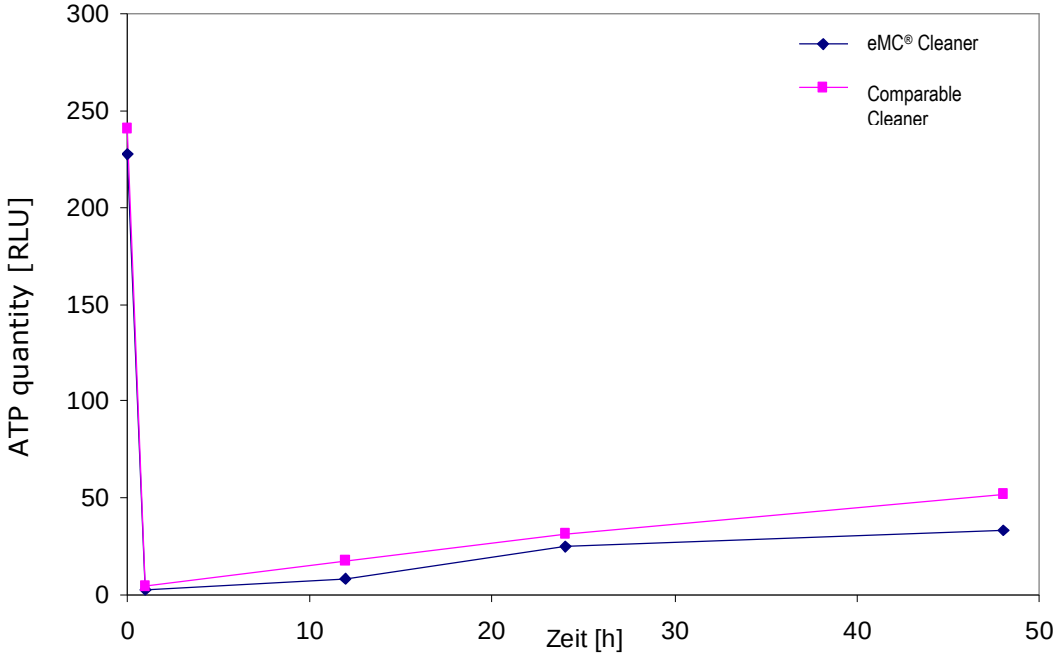

Fig. 17 Test surface 3 - temporal ATP development

Test surface 4	EM		Comp. cleaner	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	273	330	246	292
1	14	3	10	5
12	84	27	99	46
24	134	30	154	67
48	125	45	221	102

Table 13 Phase 1 – biotechnical centre

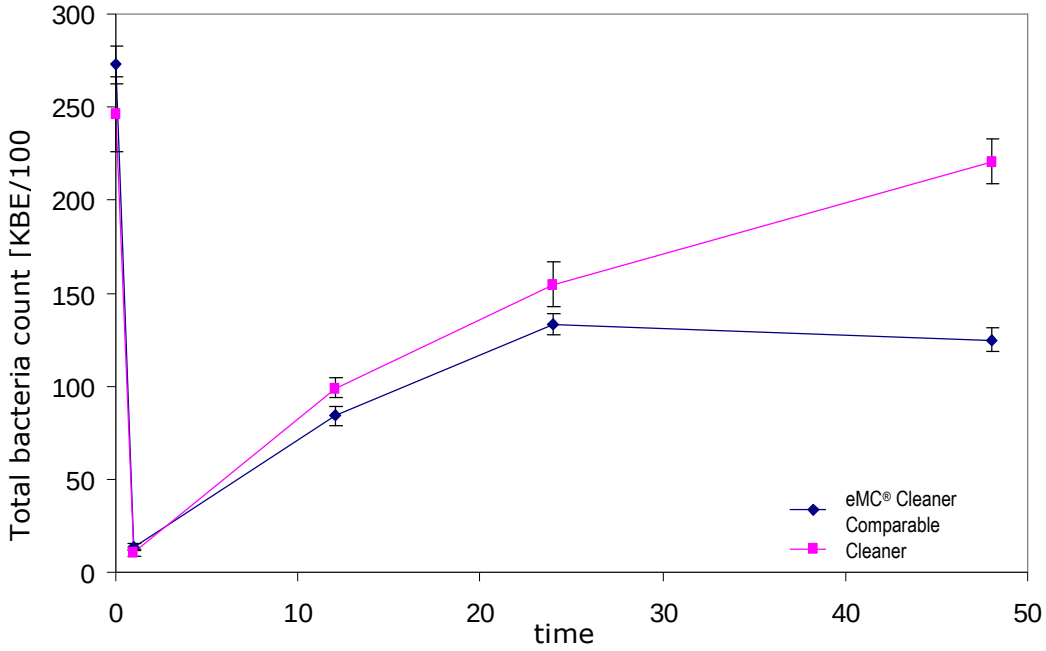

Fig. 18 Test surface 4 – temporal KBE development

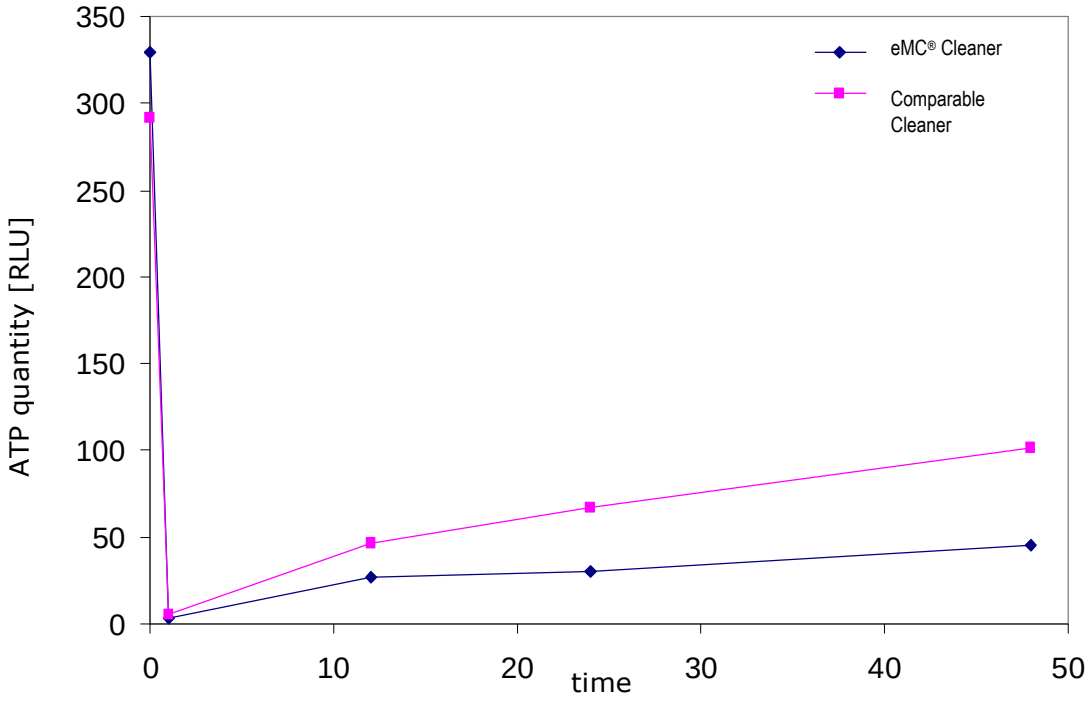

Fig. 19 Test surface 4 – temporal ATP development

Test surface 5	EM		Comp. cleaner	
Time [h]	KBE/100cm²	RLU	KBE/100cm²	RLU
0	372	330	328	292
1	22	3	34	5
12	59	27	90	46
24	115	30	122	67
48	108	45	133	102

Table 14 Phase 1 – private kitchen 2

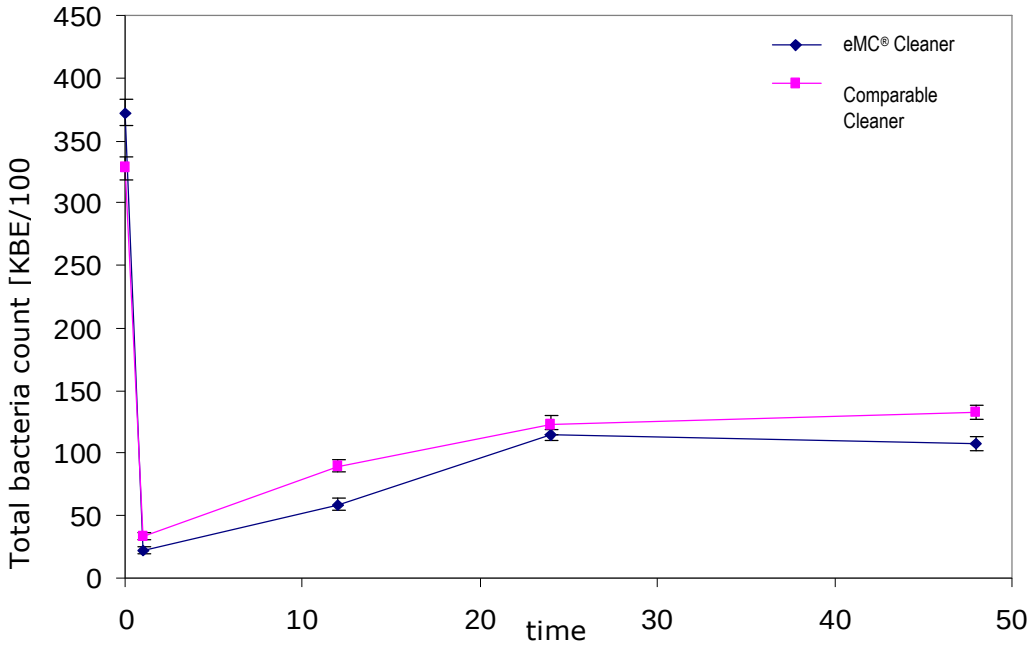

Fig. 20 Test surface 5 - temporal KBE development

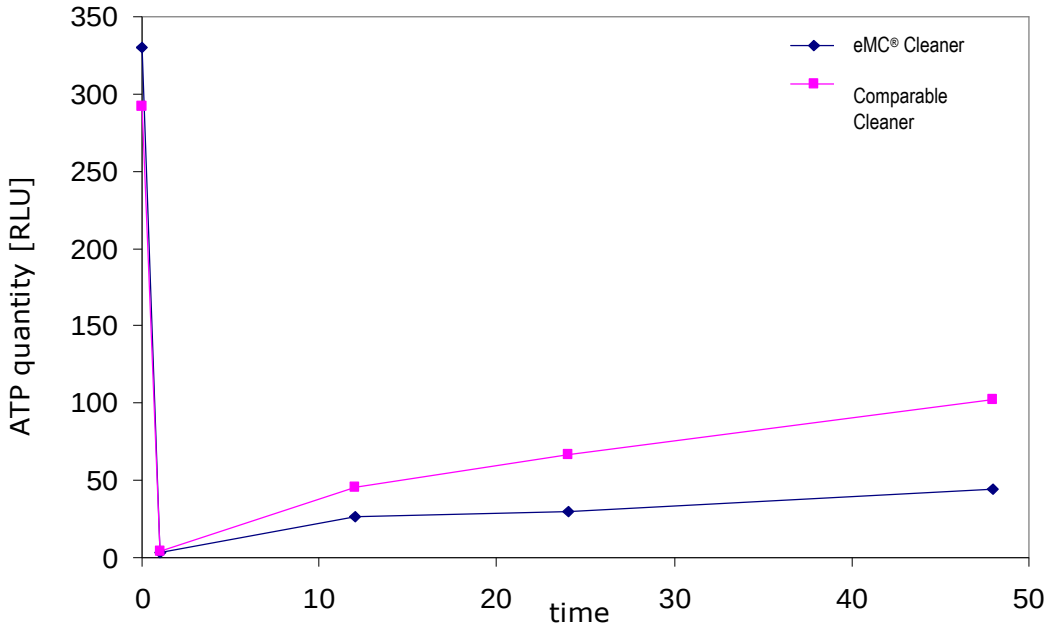

Fig. 21 Test surface 5 - temporal ATP development

4.2 Phase 2

Figs 22-26 show how temporal KBE and ATP development progressed as in Phase 1. After cleaning, no coliform presence on the test surfaces was proven. Prior to cleaning the impact range extended from "very small" to "moderate" (coliform key: see page 20, Table 6). Moulds were only singly proven.

Test surface 6	EM	
Time [h]	KBE/100cm²	RLU
0	207	47
1	15	3
24	23	44
48	64	20

Table 15 Phase 2 – PVA 1

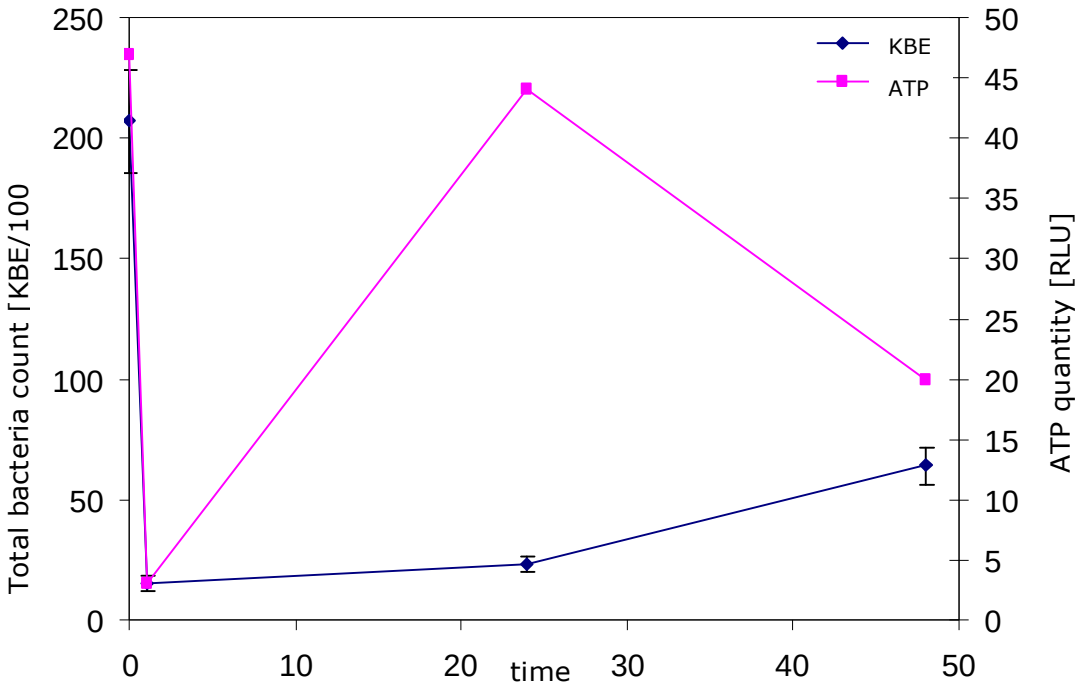

Fig.22 Test surface 6 – KBE and ATP volume progression

Test surface 7	EM	
Time [h]	KBE/100cm^2	RLU
0	228	46
1	58	2
24	74	35
48	100	44

Table 16 Phase 2 – atrium

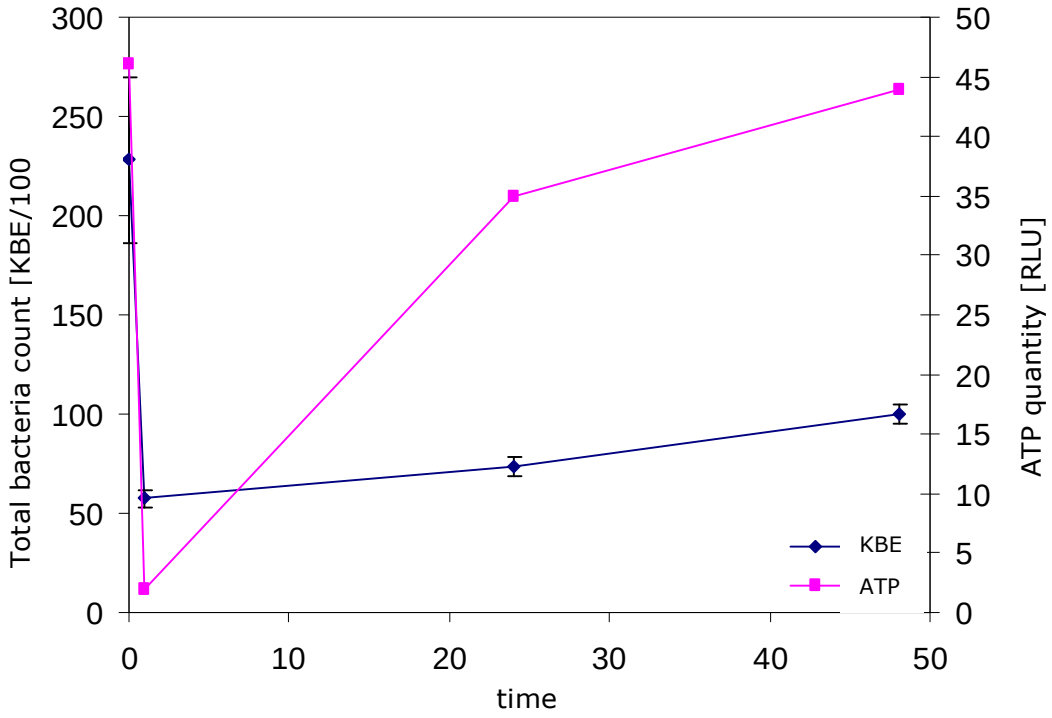

Fig.23 Test surface 7 – KBE and ATP volume progression

Test surface 8	EM	
Time [h]	KBE/100cm²	RLU
0	166	90
1	12	4
24	57	12
48	96	45

Table 17 Phase 2 – PVA 2

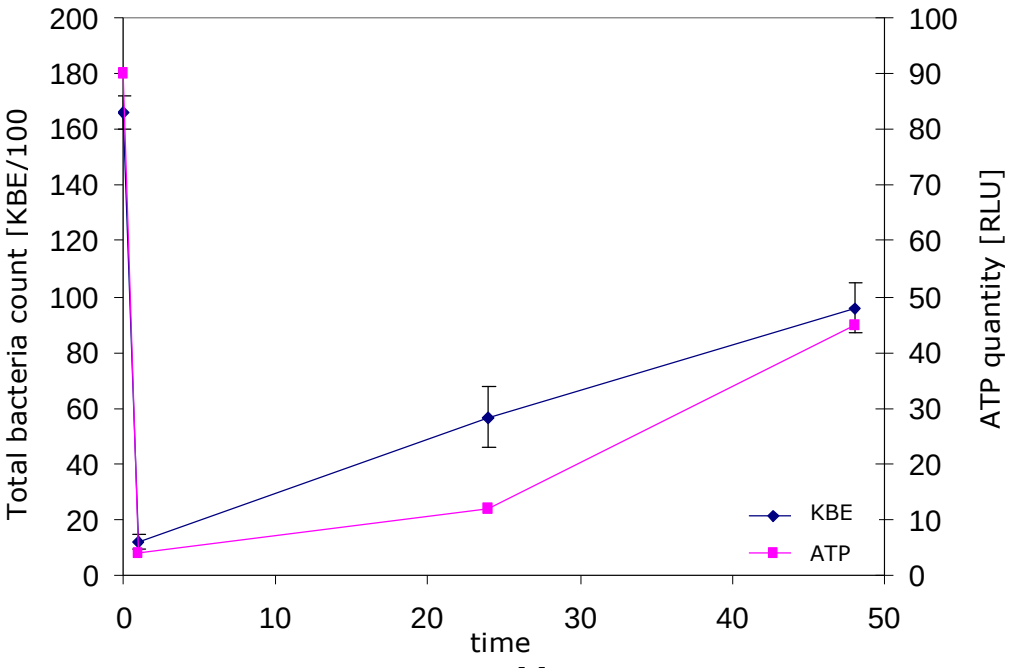

Fig.24: Test surface 8 – KBE and ATP volume progression

Test surface 9	EM	
Time [h]	KBE/100cm²	RLU
0	102	41
1	6	2
24	38	21
48	74	25

Table 18 Phase 2 – Parkhotel

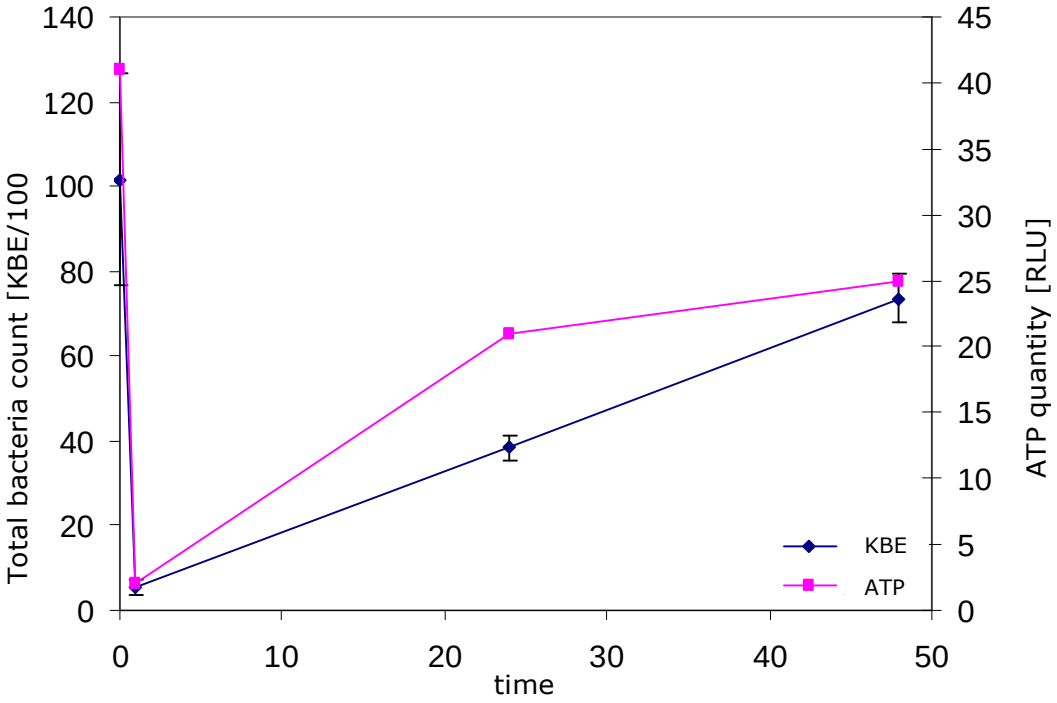

Fig.25 Test surface 9 – KBE and ATP volume progression

Test surface 10	EM	
Time [h]	KBE/100cm²	RLU
0	44	9
1	22	2
24	22	8
48	47	21

Table 19 Phase 2 – Wels Hospital/Pathology II

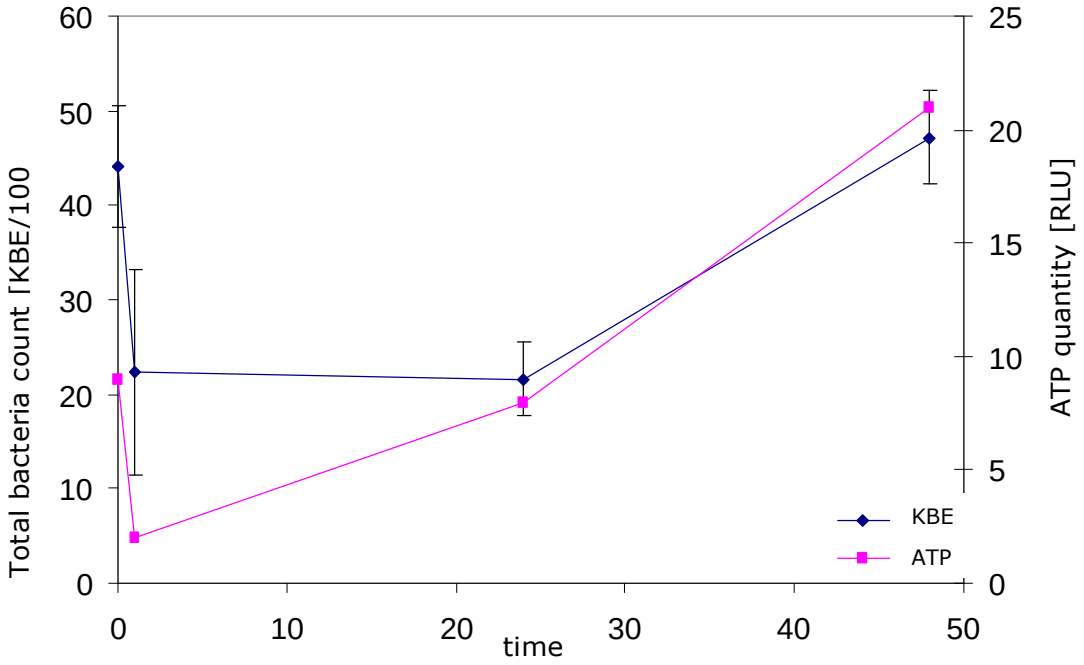

Fig.26 Test surface 10 – KBE and ATP volume progression

4.3 Phase 3

Test object	EM cleaner	Comp. cleaner	Disinfectant
PVA 1	eMC 1:100	Comp. cleaner❷	Descocid
Atrium	eMC 1:100	Comp. cleaner ❸	Descocid
PVA 2	eMC 1:100	Comp. cleaner ❹	Descocid
Parkhotel	eMC 1:100	Comp. cleaner ❺	Descocid
Wels hosp.	eMC 1:100	-	Descocid

Table 20 List of the cleaners employed

Figs 27, 28 and 29 show the distribution of the various bacteria count groups immediately following the cleaning of the test surfaces (t_1), 24 hours later (t_2) and 48 hours after cleaning (t_3).

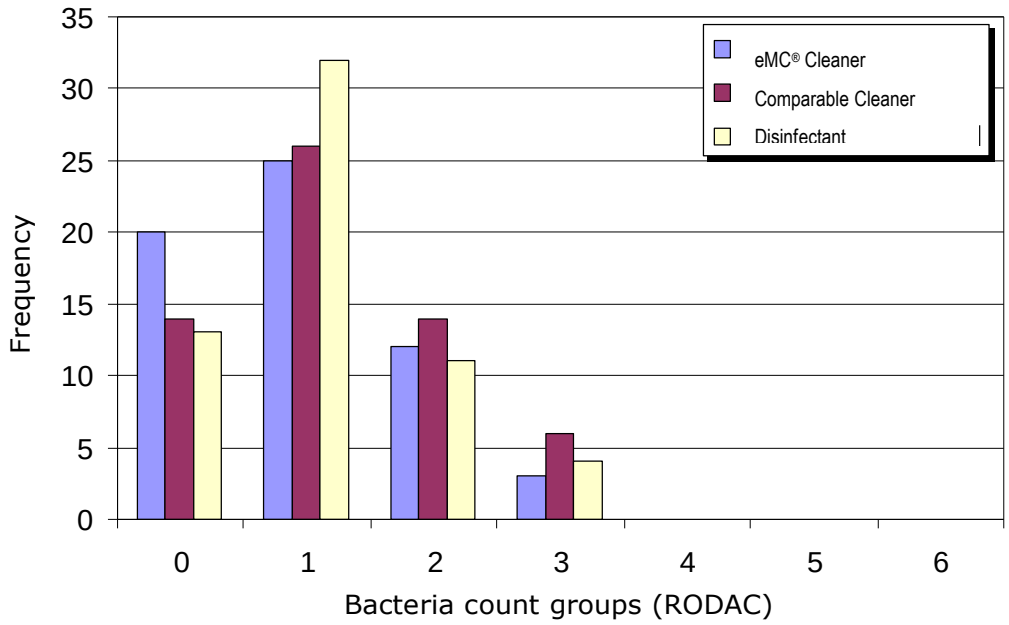

Fig.27 Frequency of occurrence of the individual bacteria count groups pursuant to DIN 10113-3 (after t_1)

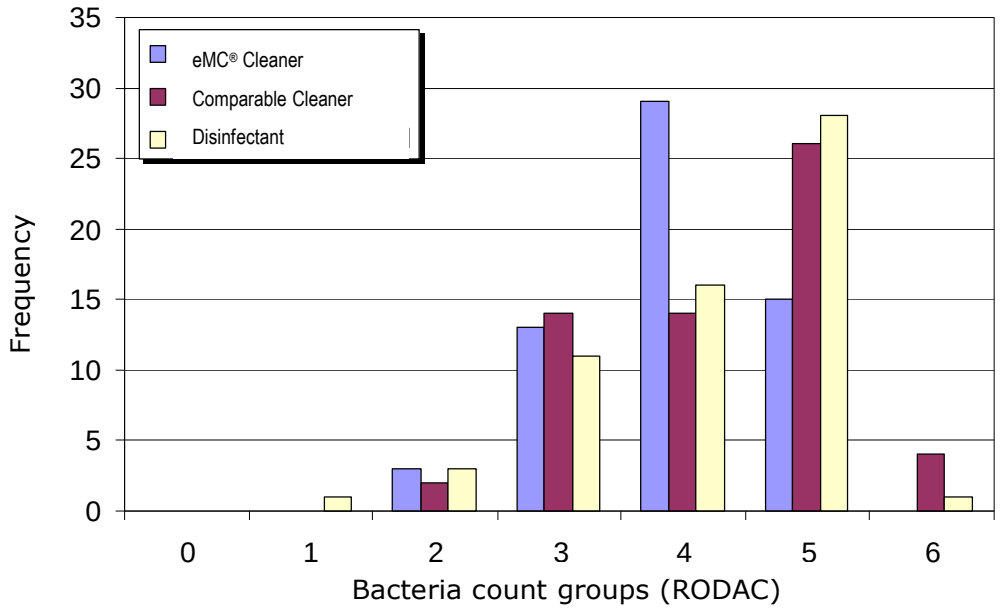

Fig.28 Frequency of occurrence of the individual bacteria count groups pursuant to DIN 10113-3 (24 hours after cleaning t_2)

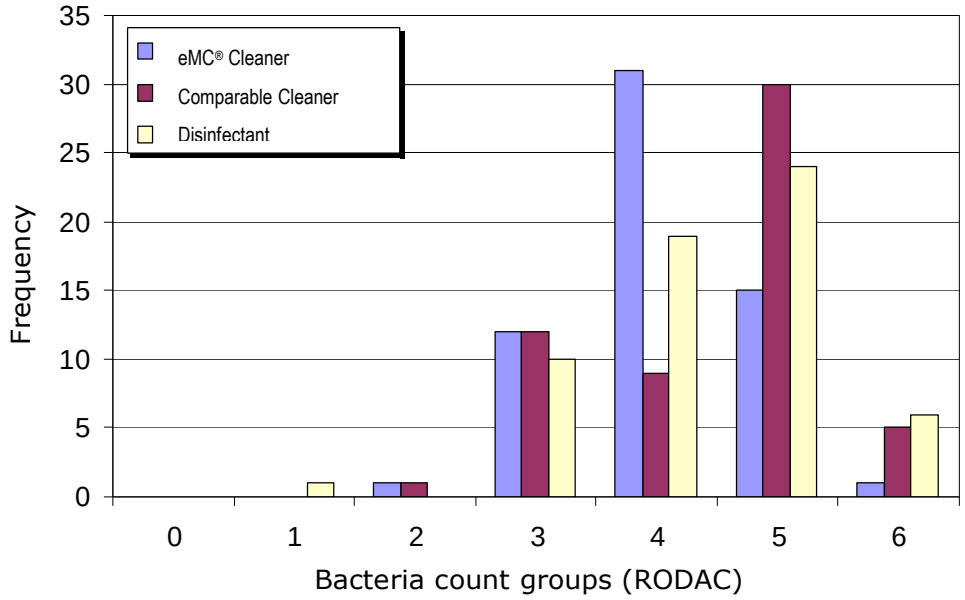

Fig.29 Frequency of occurrence of the individual bacteria count groups pursuant to DIN 10113-3 (48 hours after cleaning t_3)

Key: see page 19 Table 5

4.3.1. Phase3_1

Figs 30-39 show how temporal KBE and ATP development progressed. Prior to cleaning, the impact range extended from "very small" to "moderate". After cleaning, the coliform levels on all surfaces were "very small" or undetectable (coliform key: see page 20, Table 6). Moulds accounted for 30-90% of the total bacteria count at all the testing times.

Test surface 6	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/10 0cm²	RLU
0	315	315	513	38	513	38
1	82	6	25	5	25	5
24	234	43	126	25	126	25
48	341	65	206	24	206	24

Table 21 Phase 3_1 – PVA 1

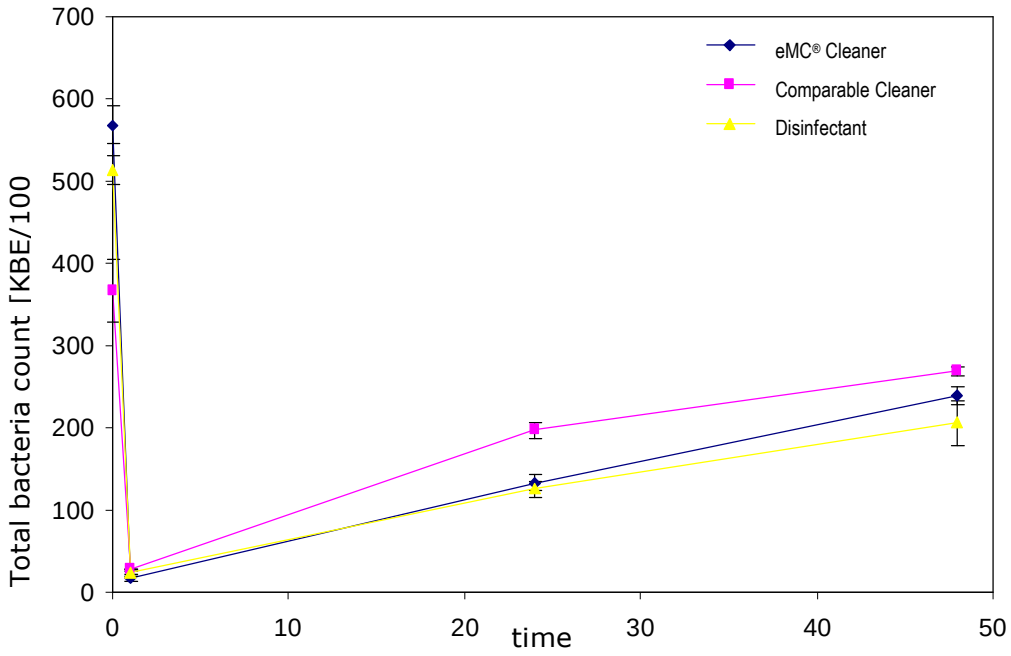

Fig.30 Test surface 6 – temporal KBE development

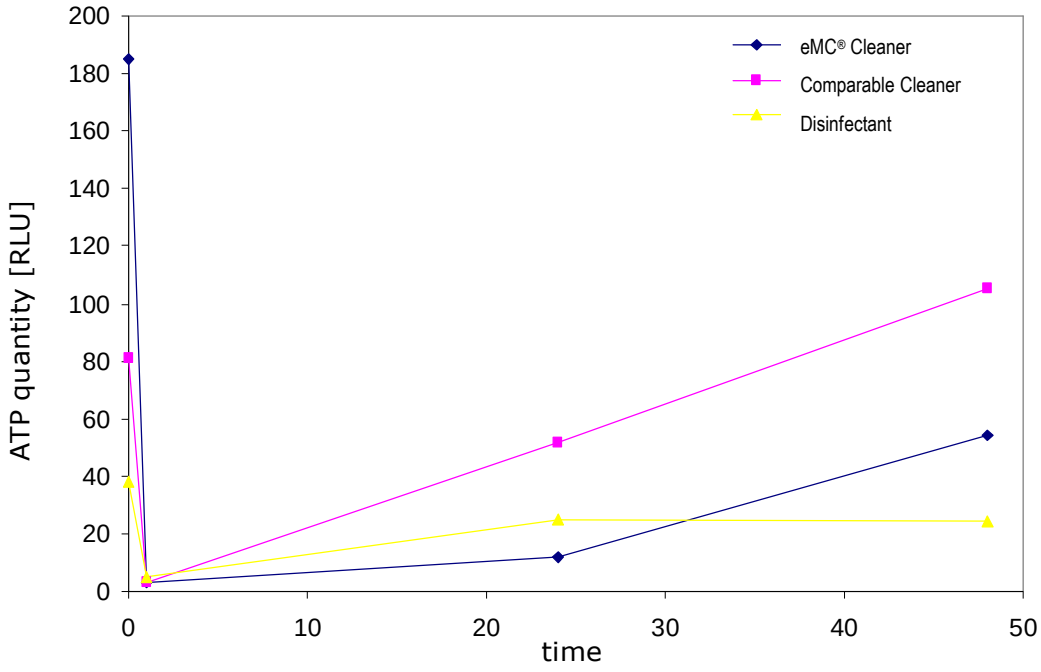

Fig.31 Test surface 6 - temporal ATP development

Test surface 7	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	405	6	271	72	332	292
1	4	1	22	1	7	0
48	198	11	345	18	313	84

Table 22 Phase 3_1 - atrium

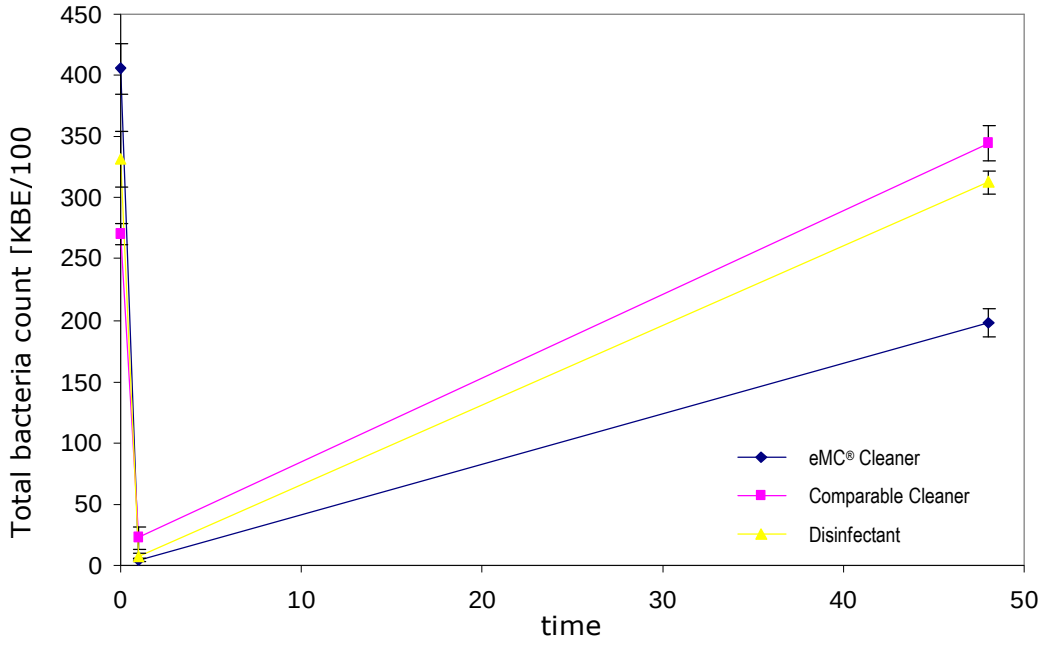

Fig.32 Test surface 7 – temporal KBE development

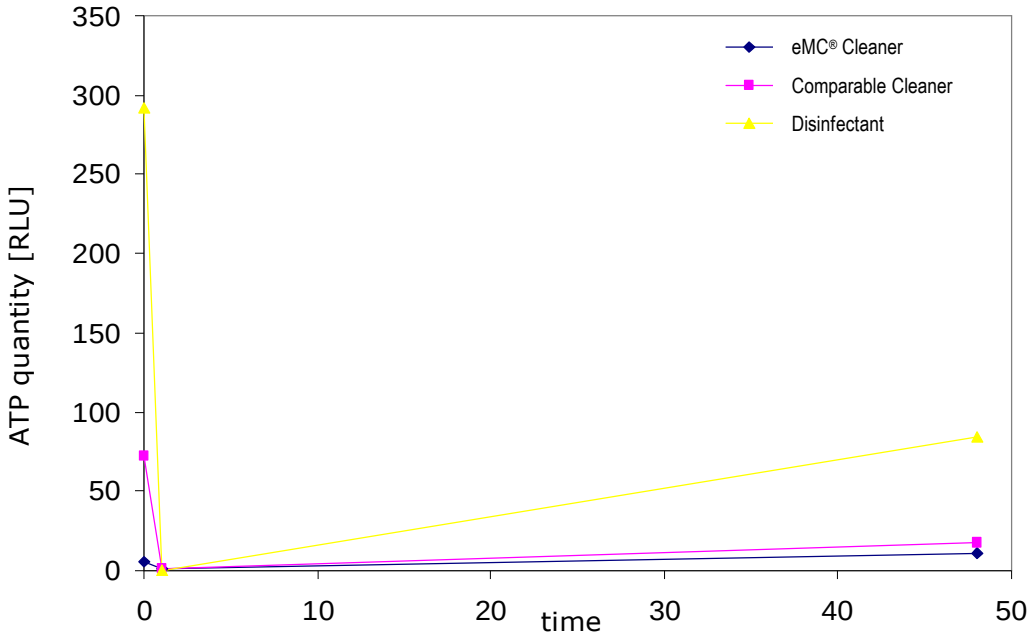

Fig.33 Test surface 7 - temporal ATP development

Test surface 8	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	180	39	188	14	154	22
1	20	3	10	3	18	4
24	51	2	138	4	41	1
48	104	4	114	23	75	1

Table 23 Phase 3_1 – PVA 2

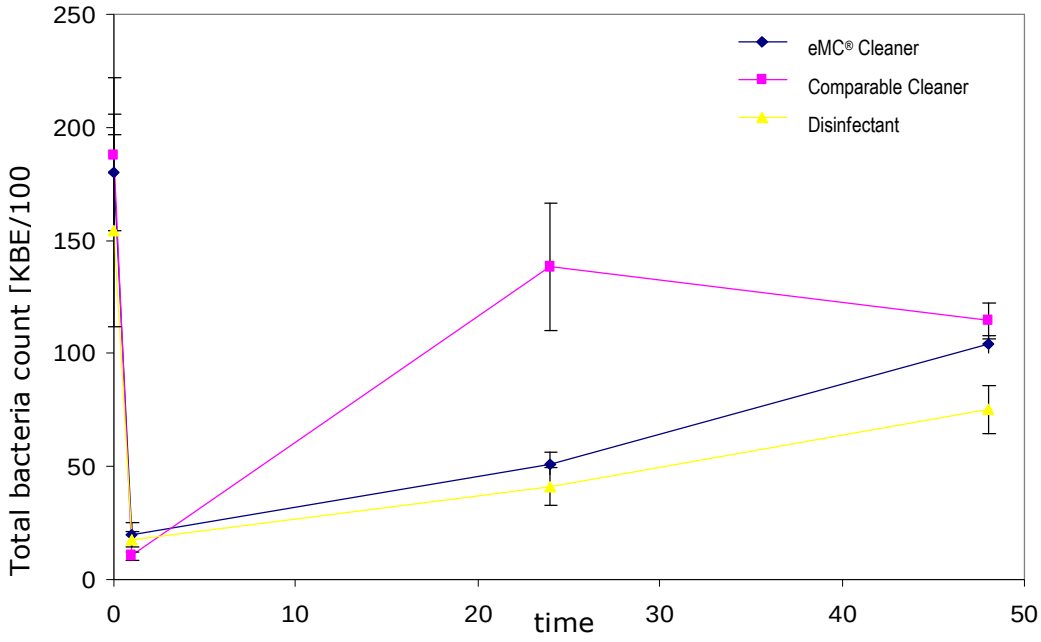

Fig.34 Test surface 8 – temporal KBE development

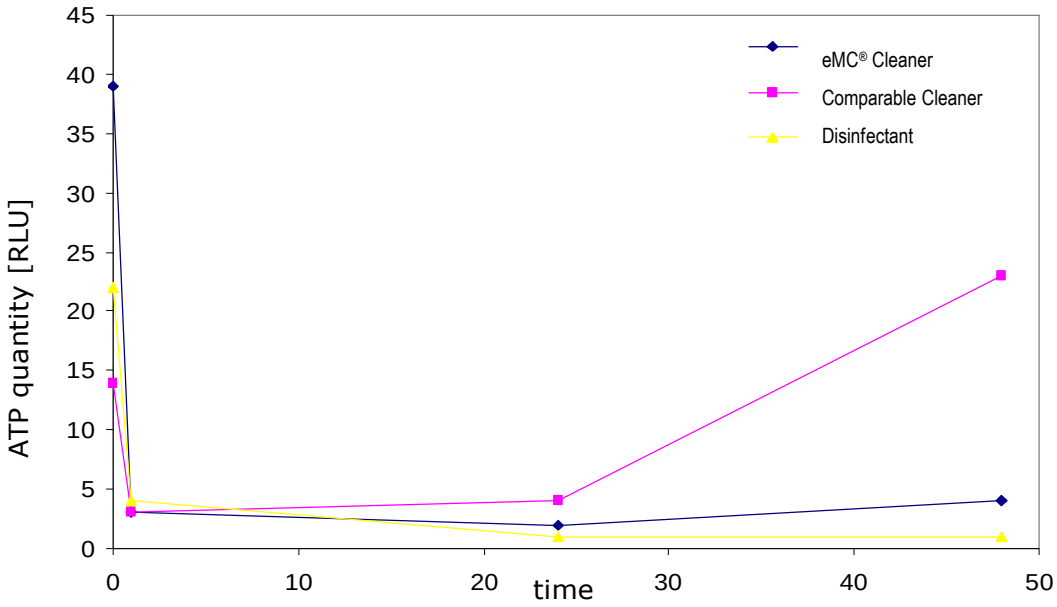

Fig.35 Test surface 8 - temporal ATP development

Test surface 9	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	456	90	257	18	616	717
1	6	4	50	30	12	6
24	174	4	136	28	235	17
48	244	75	143	41	279	28

Table 24 Phase 3_1 – Parkhotel

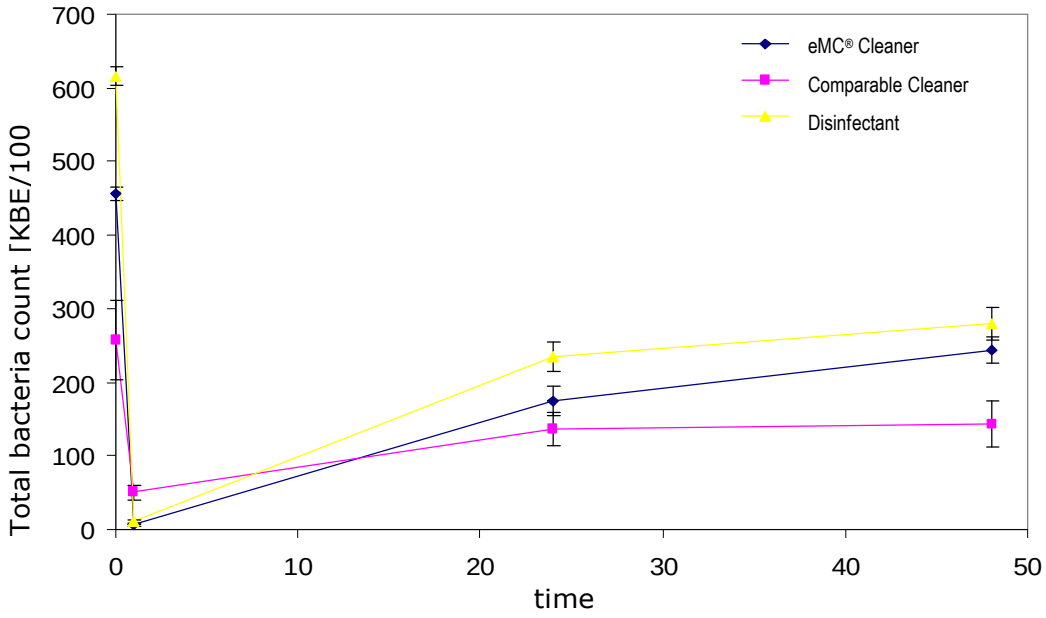

Fig.36 Test surface 9 – temporal KBE development

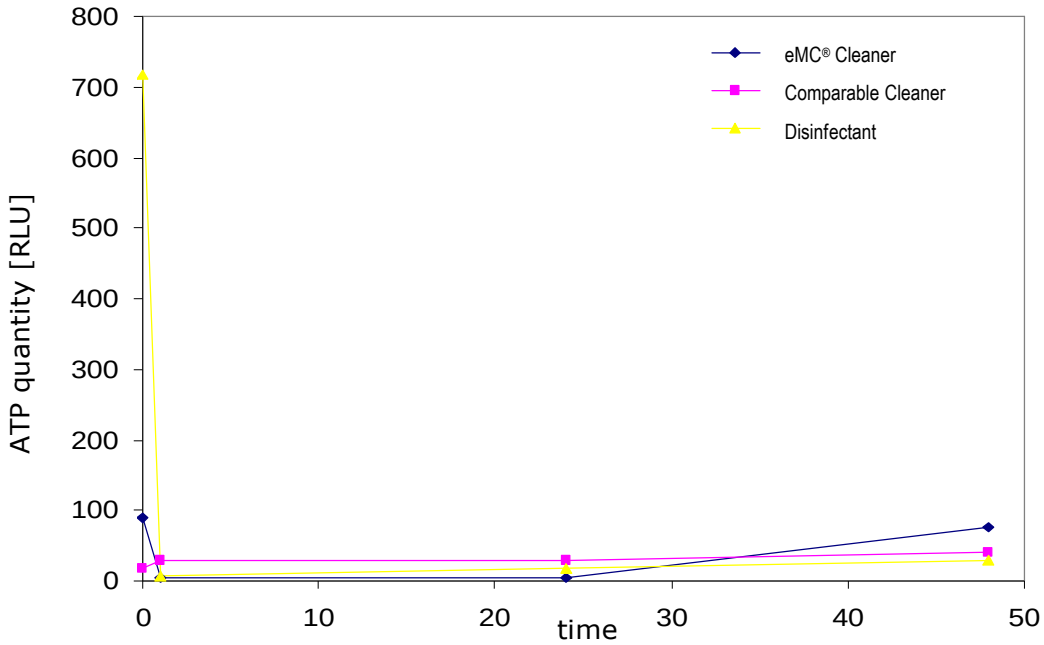

Fig.37 Test surface 9 - temporal ATP development

Test surface 10	EM		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	405	6	332	292
1	4	1	7	0
48	198	11	313	84

Table 25 Phase 3_1 – Wels Hospital/Pathology II

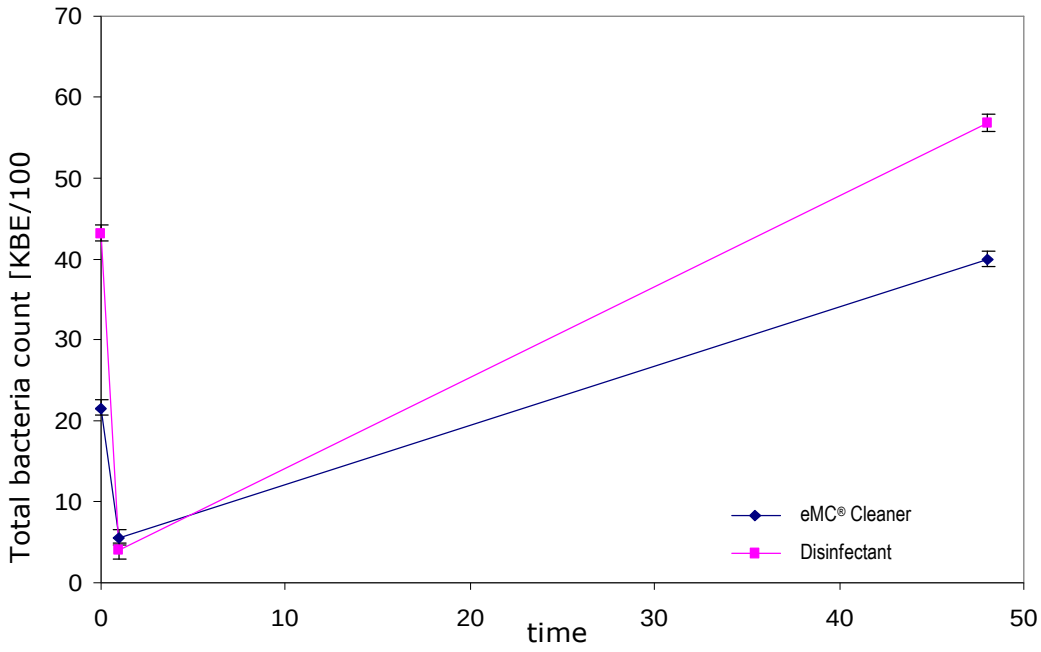

Fig.38 Test surface 10 – temporal KBE development

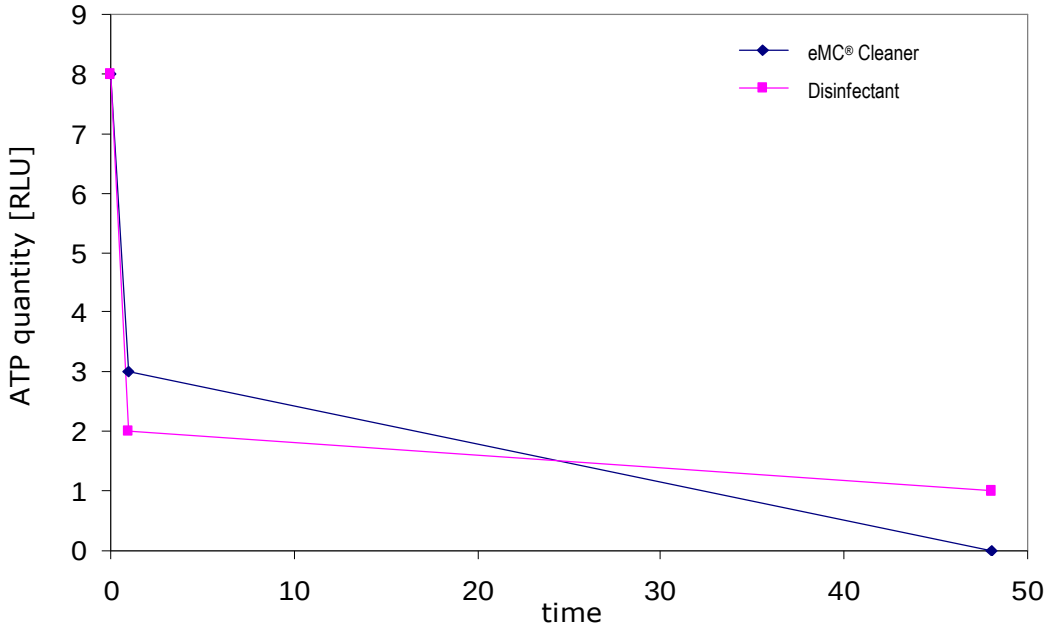

Fig.39 Test surface 10 - temporal ATP development

4.3.2. Phase 3_2

Figs 40-49 show how temporal KBE and ATP development progressed. Prior to cleaning, the impact range extended from "very small" to "moderate". After cleaning, the coliform levels on all surfaces were "very small" or undetectable (coliform key: see page 20, Table 6). Moulds accounted for 30-90% of the total bacteria count at all the testing times.

Test surface 6	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	320	367	423	46	673	291
1	34	0	40	3	33	4
24	420	12	354	8	582	10
48	231	32	1062	54	443	11

Table 26 Phase 3_2 – PVA1

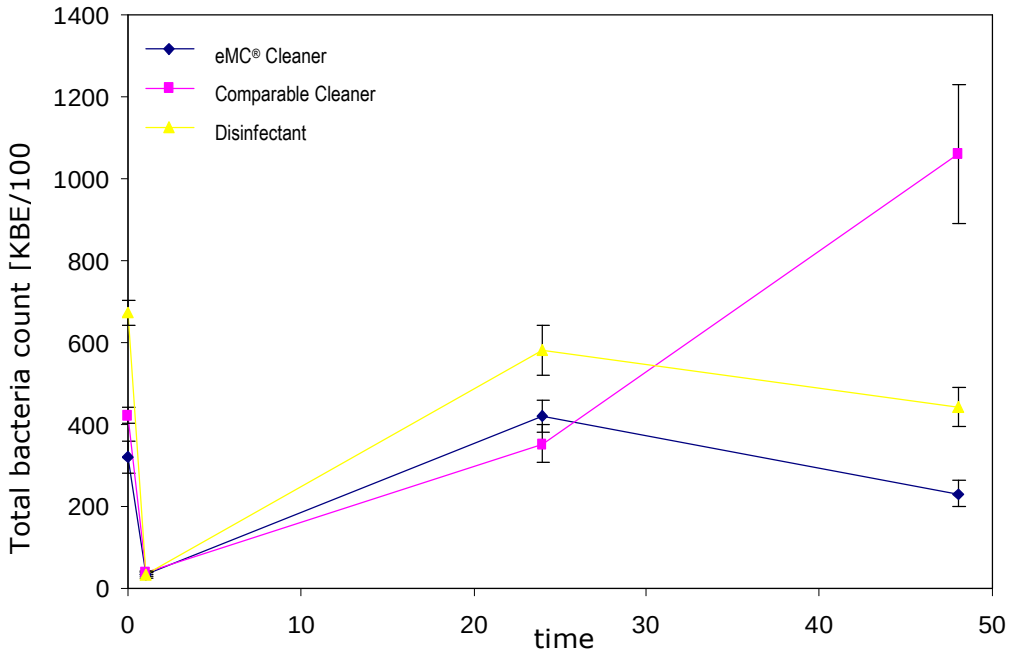

Fig.40 Test surface 6 – temporal KBE development

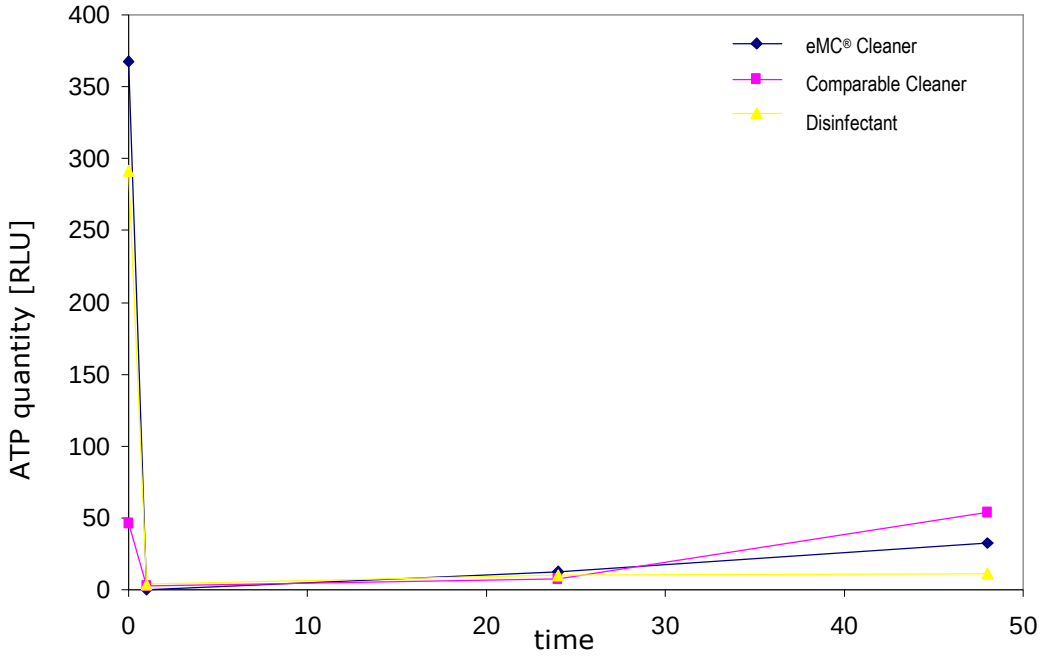

Fig.41 Test surface 6 - temporal ATP development

Test surface 7	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	624	176	644	139	495	86
1	5	1	4	0	13	3
24	443	3	502	4	491	11
48	212	145	239	12	302	43

Table 27 Phase 3_2 – atrium

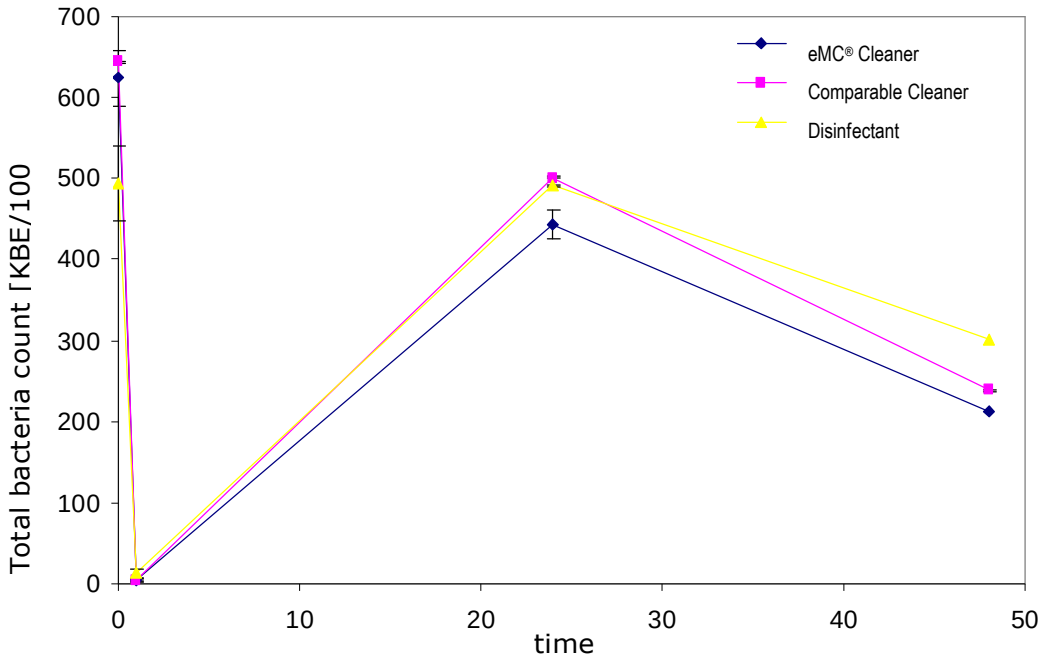

Fig.42 Test surface 7 – temporal KBE development

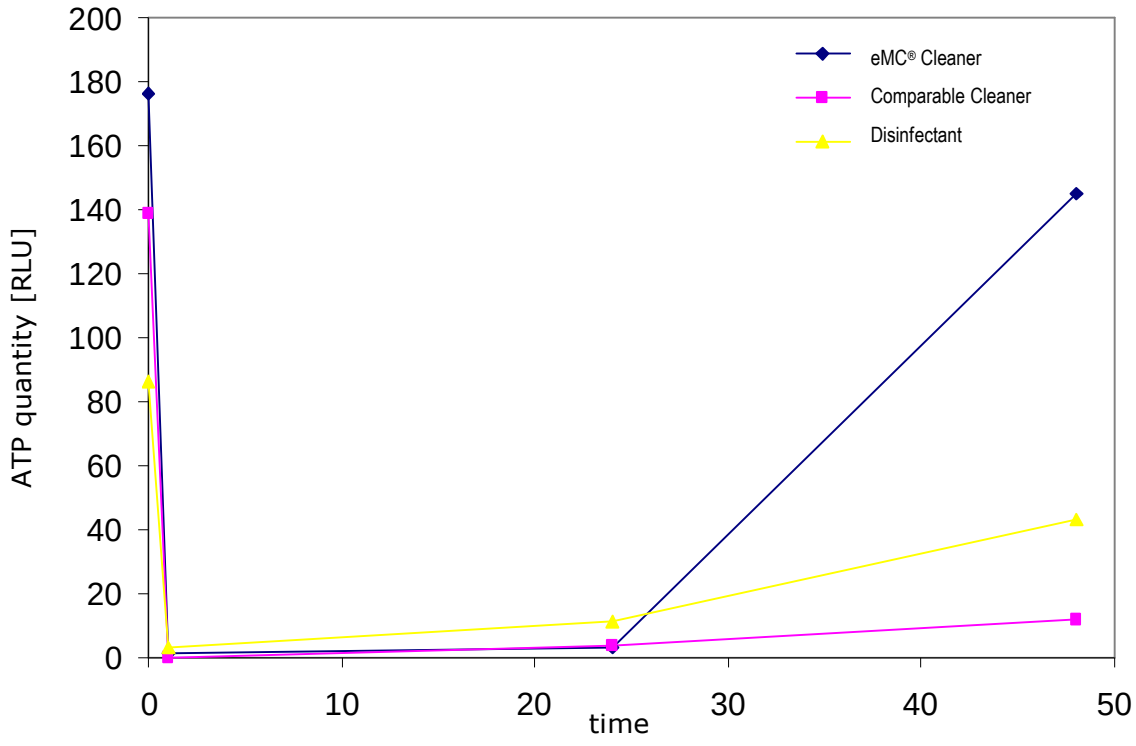

Fig.43 Test surface 7 - temporal ATP development

Test surface 8	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	303	3	181	37	202	25
1	6	1	15	2	4	2
24	297	8	170	7	330	23
48	174	24	109	29	341	20

Table 28 Phase 3_2 – PVA 2

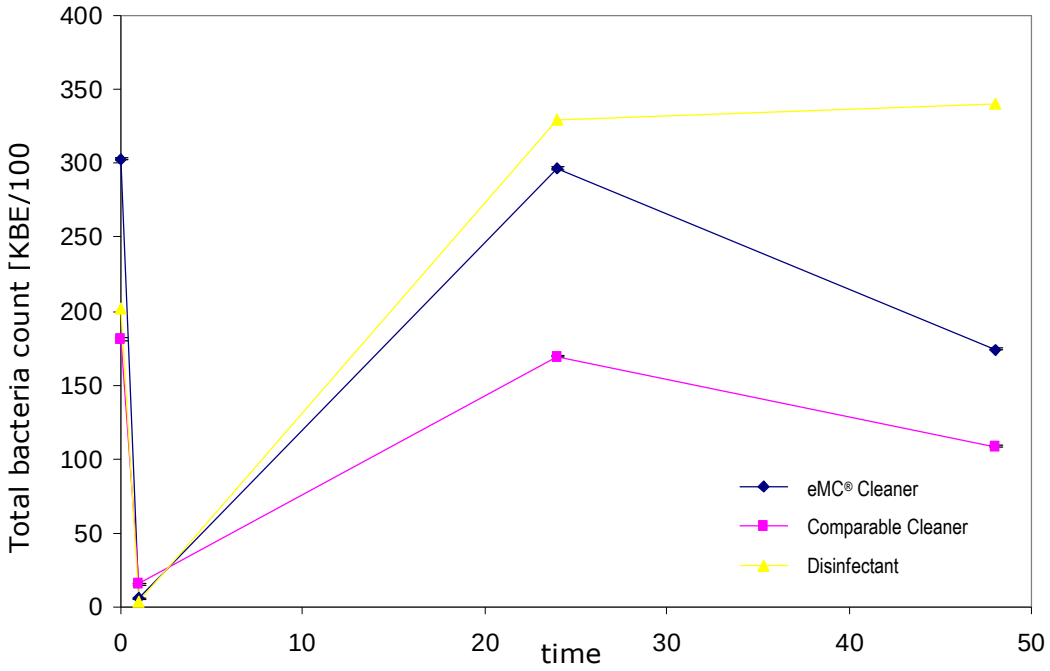

Fig.44 Test surface 8 – temporal KBE development

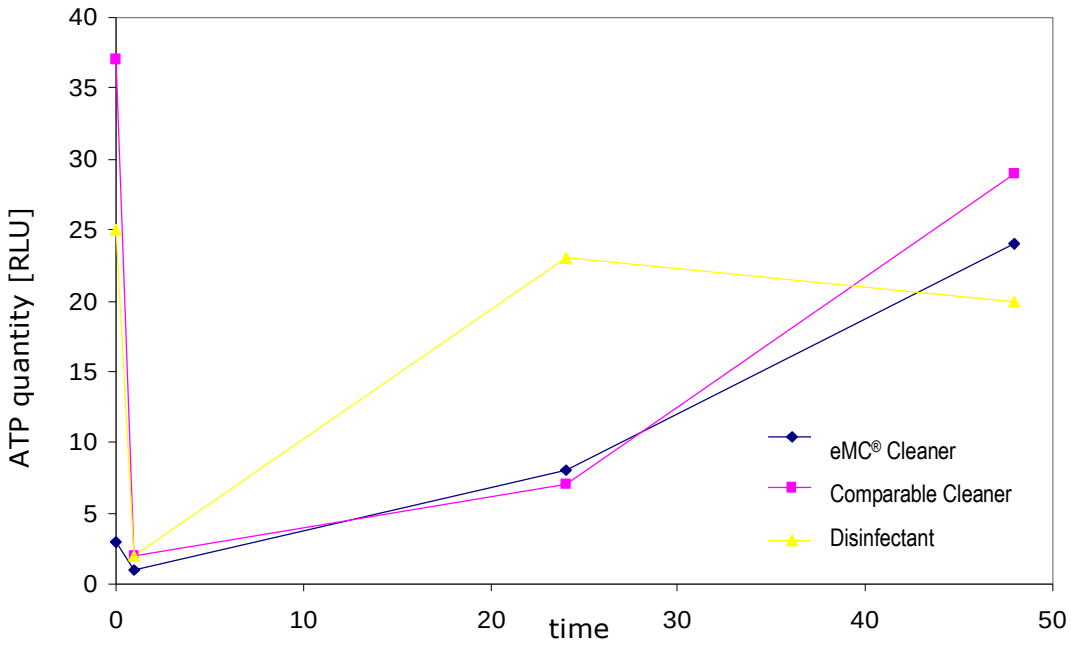

Fig.45 Test surface 8 - temporal ATP development

Test surface 9	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	246	19	306	26	299	31
1	3	0	5	8	10	1
24	145	10	418	3	368	6
48	138	12	337	37	353	10

Table 29 Phase 3_2 – Parkhotel

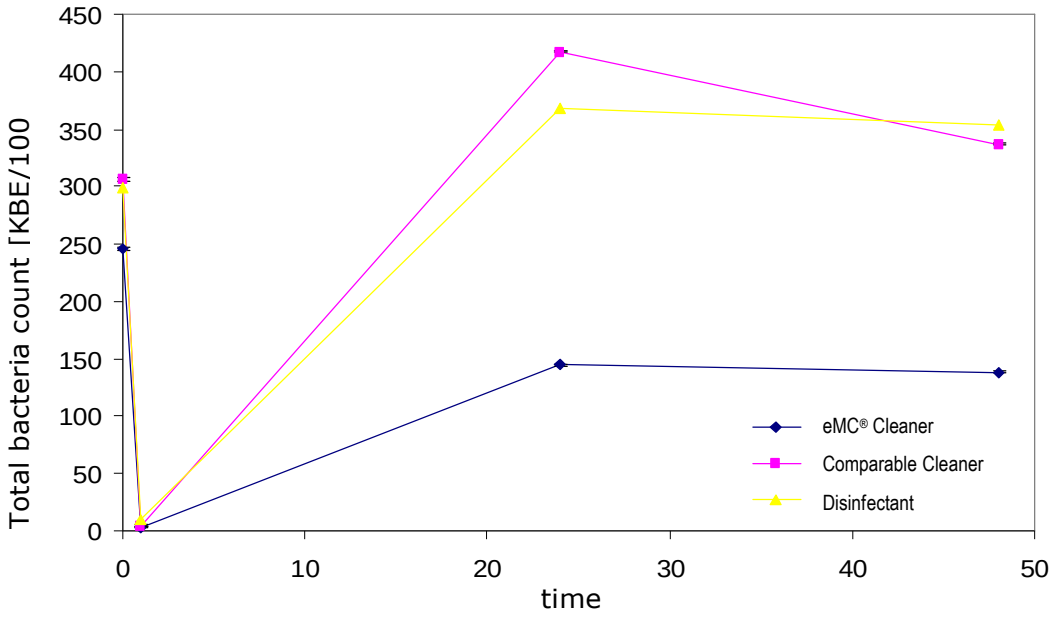

Fig.46 Test surface 9 – temporal KBE development

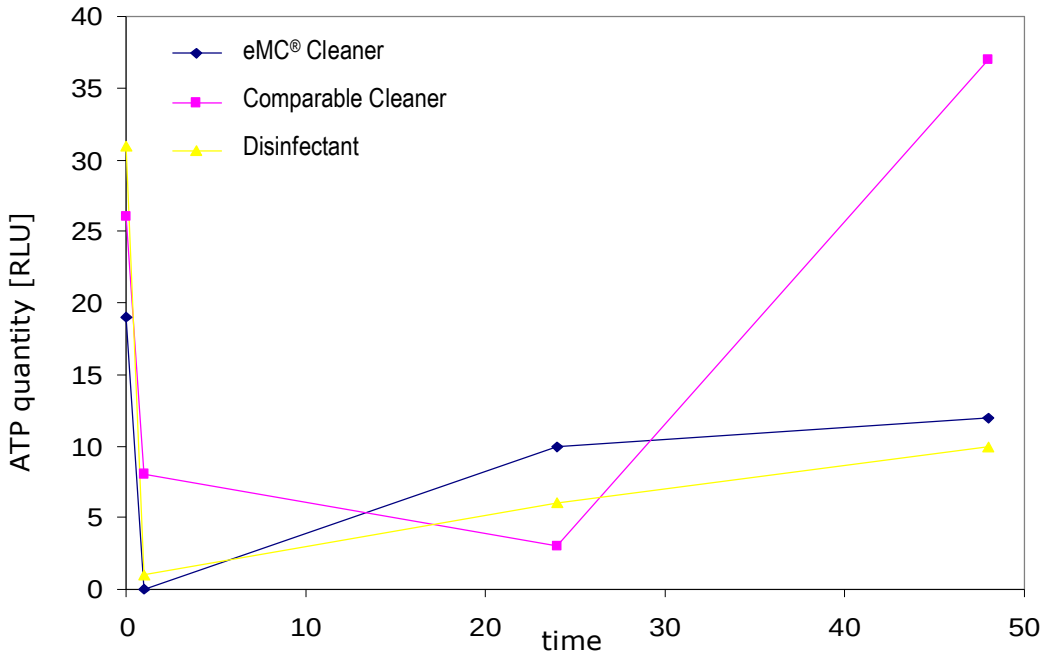

Fig.47 Test surface 9 - temporal ATP development

Test surface 10	EM		Disinfectant	
Time [h]	KBE/100 cm²	RLU	KBE/100 cm²	RLU
0	49	17	58	1
1	6	2	7	6
24	110	1	86	8
48	31	2	47	3

Table 30 Phase 3_2 – Wels Hospital/Pathology II

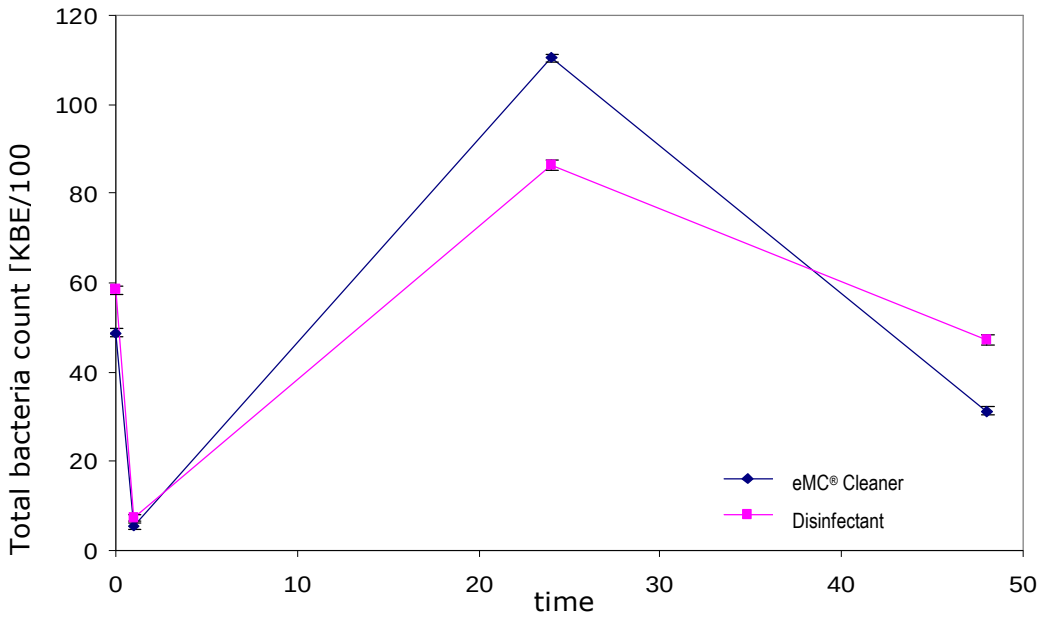

Fig.48 Test surface 10 – temporal KBE development

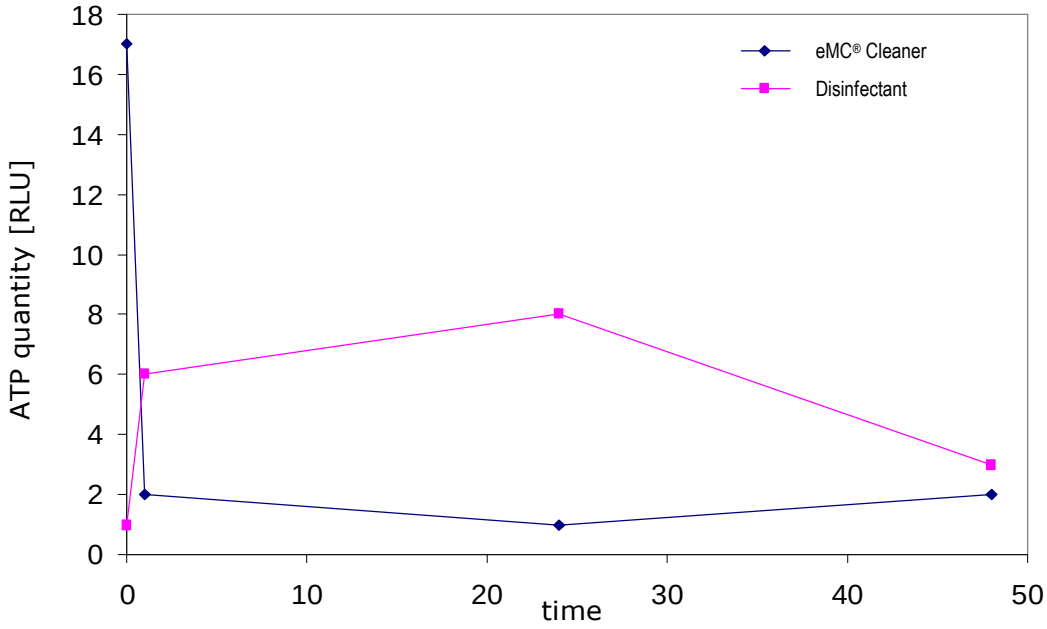

Fig.49 Test surface 10 - temporal ATP development

4.3.3. Phase 3_3

Figs 50-59 show how temporal KBE and ATP development progressed. Prior to cleaning, the impact range extended from "very small" to "moderate". After cleaning, the coliform levels on all surfaces were "very small" or undetectable (coliform key: see page 20, Table 6). Moulds accounted for 30-90% of the total bacteria count at all the testing times.

Test surface 6	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	219	11	124	10	410	34
1	10	0	6	0	7	3
24	116	22	112	24	142	15
48	141	2	227	19	149	8

Table 31 Phase 3_3 – PVA1

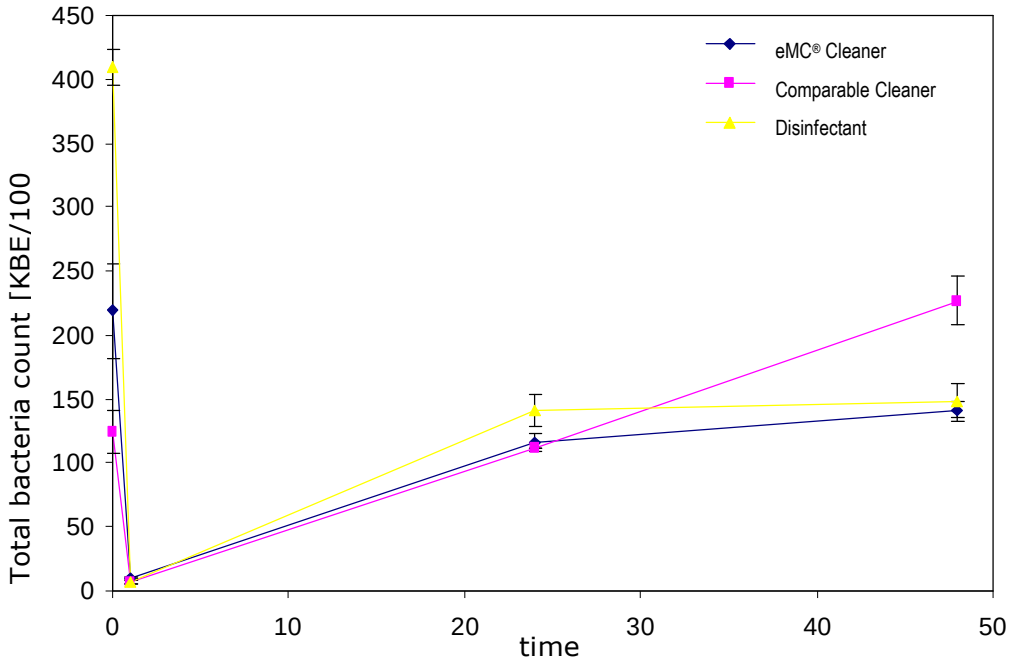

Fig.50 Test surface 6 – temporal KBE development

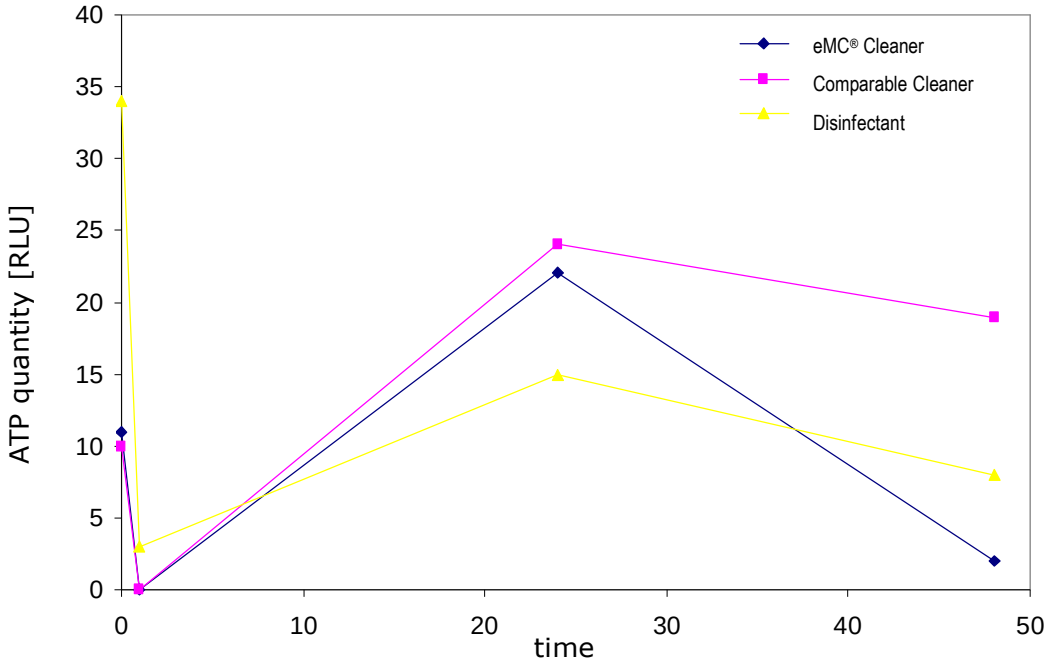

Fig.51 Test surface 6 - temporal ATP development

Test surface 7	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	152	13	259	34	46	5
1	1	2	2	5	2	1
24	206	4	251	5	232	4
48	176	16	281	29	240	22

Table 32 Phase 3_3 – atrium

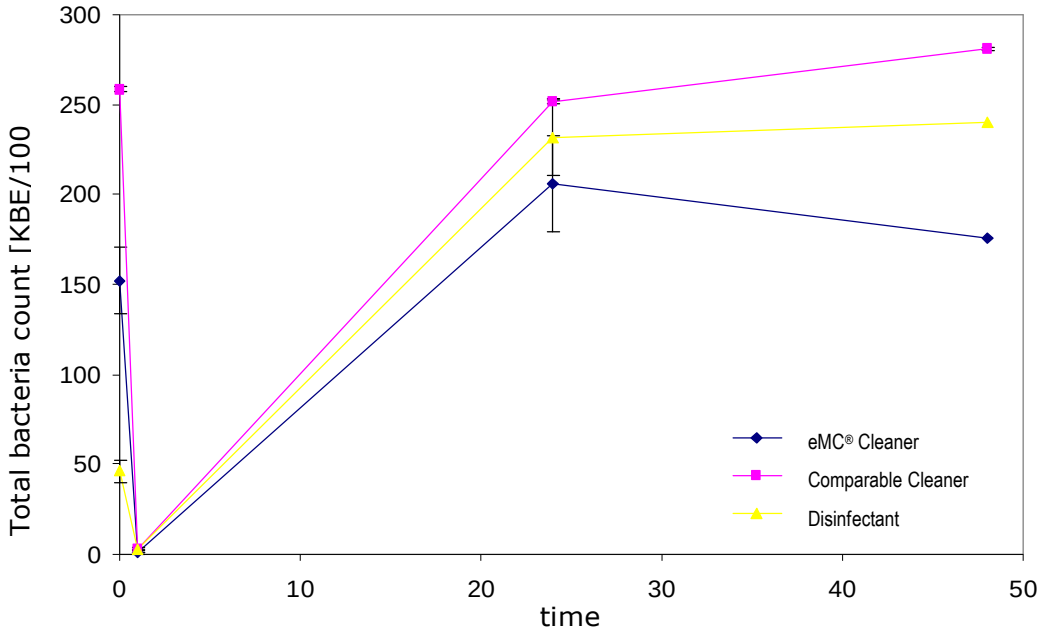

Fig.52 Test surface 7 – temporal KBE development

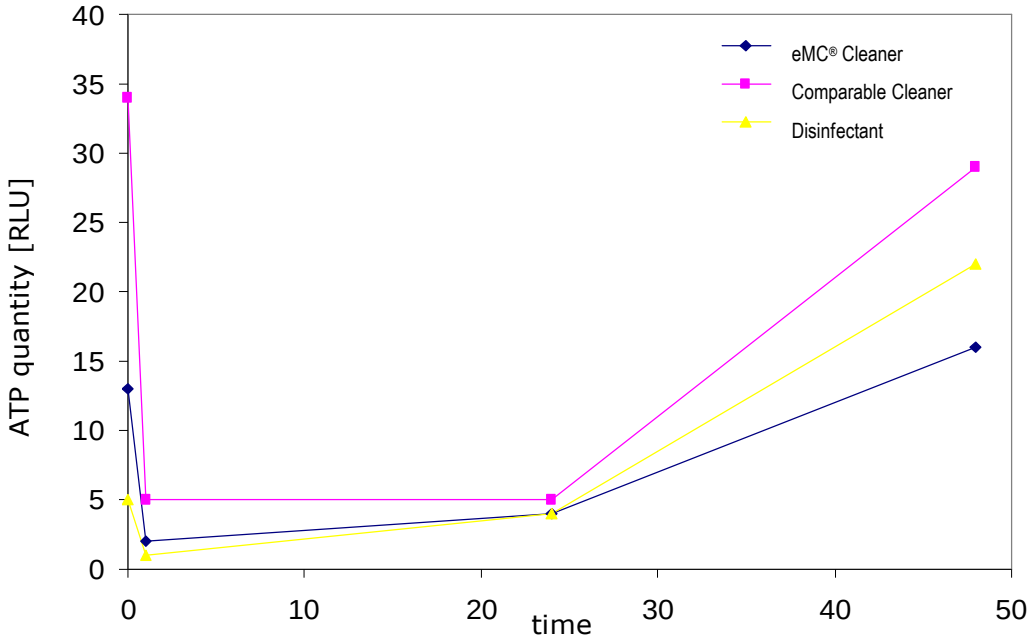

Fig.53 Test surface 7 – temporal ATP development

Test surface 8	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	106	29	123	43	82	6
1	0	3	13	11	3	7
24	113	4	106	13	82	7
48	135	16	139	17	102	4

Table 33 Phase 3_3 – PVA 2

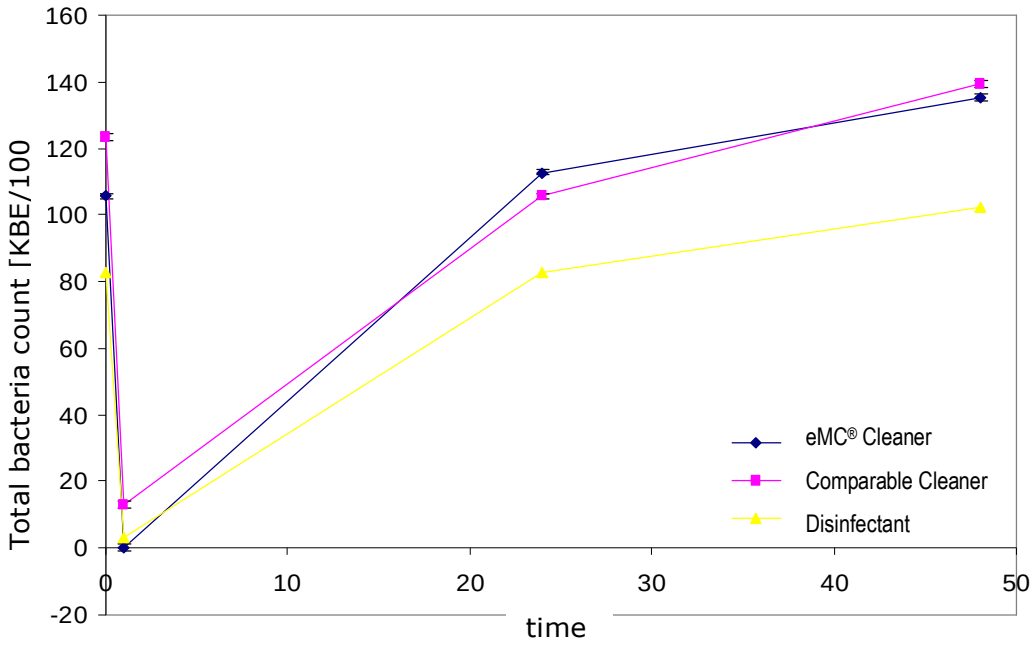

Fig.54 Test surface 8 – temporal KBE development

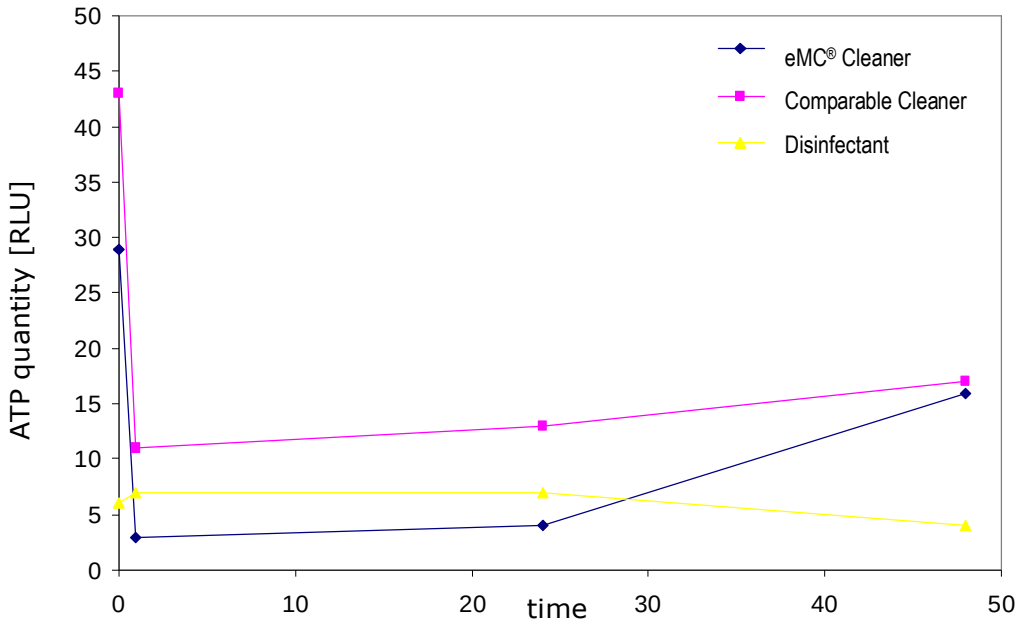

Fig.55 Test surface 8 - temporal ATP development

Test surface 9	EM		Comp. cleaner		Disinfectant	
Time [h]	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU	KBE/ 100cm²	RLU
0	298	23	298	12	278	14
1	7	1	3	0	4	0
24	169	6	126	7	141	10
48	231	4	287	18	183	12

Table 34 Phase 3_3 – Parkhotel

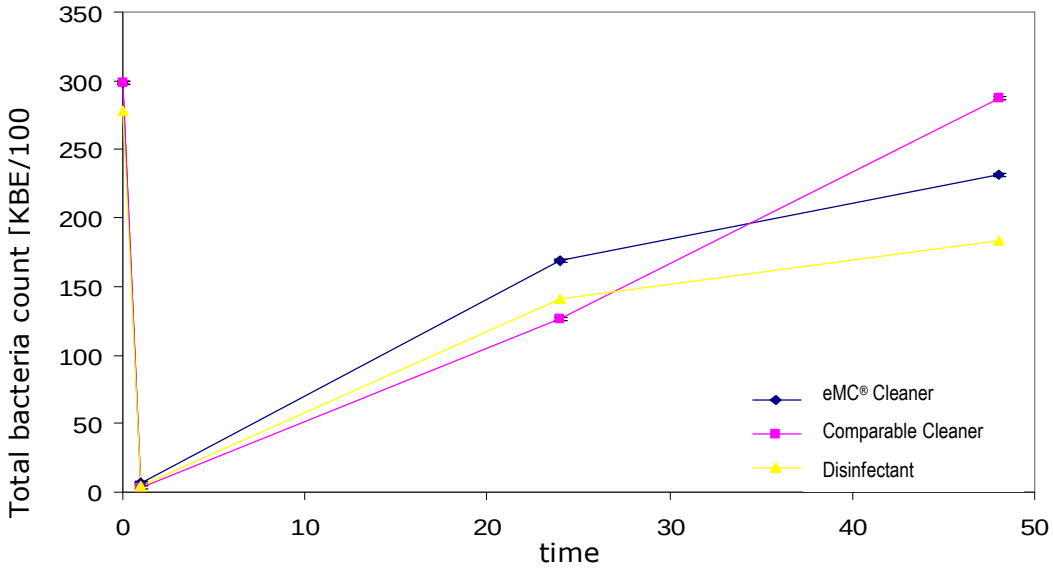

Fig.56 Test surface 9 – temporal KBE development

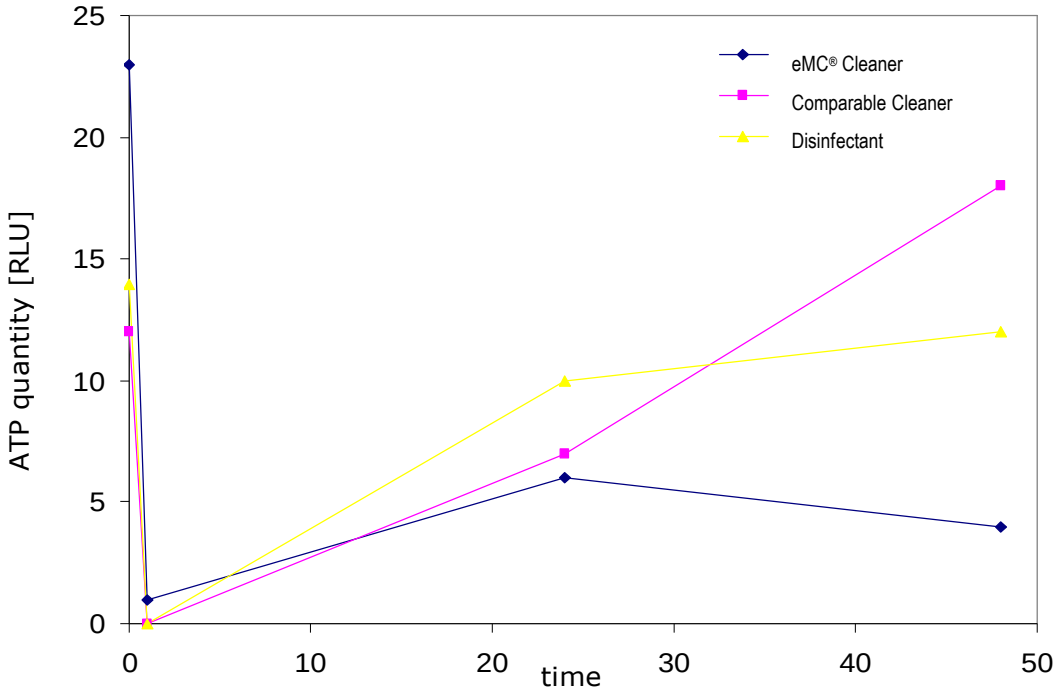

Fig.57 Test surface 9 - temporal ATP development

Test	EM	Disinfectant

surface 10				
Time [h]	KBE/100 cm²	RLU	KBE/100 cm²	RLU
0	71	9	88	4
1	2	1	6	4
24	54	12	66	4
48	44	6	54	1

Table 35 Phase 3_3 – Wels Hospital/Pathology II

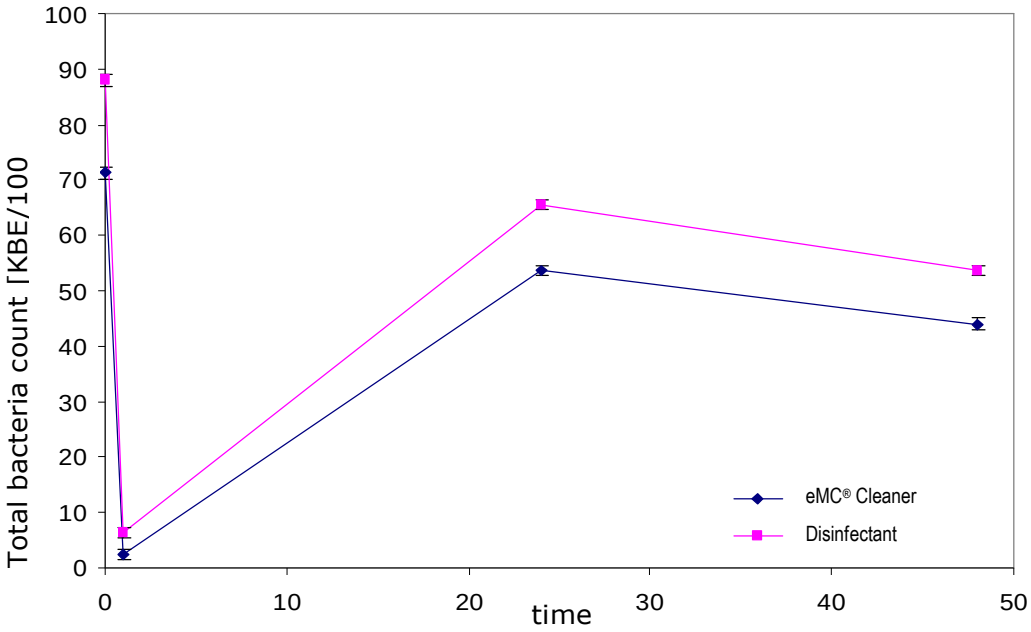

Fig.58 Test surface 10 – temporal KBE development

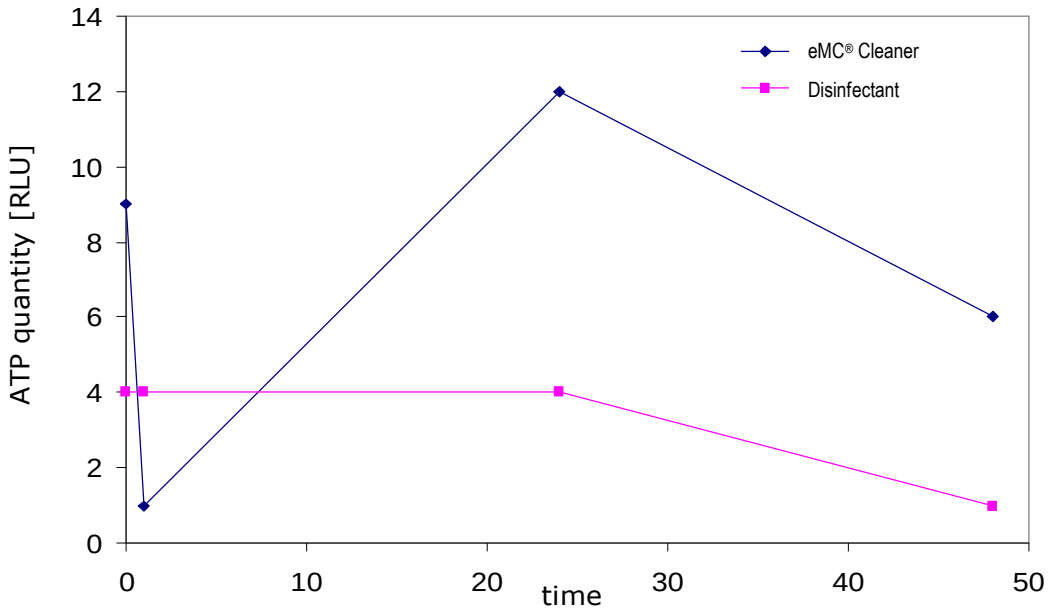

Fig.59 Test surface 10 - temporal ATP development

5. Discussion

The first phase showed that the cleaning effect of the eMC® Cleaner and the comp. cleaner ❶ were virtually identical (t_1). In addition, it was shown that at the time points t_1 to t_4, *Multikraft* eMC® Cleaner reduced the repeated soling of the surfaces (Figs 12,13 and 16-21).

Fig.14 and 15 show the comp. cleaner to be superior, as here the comp. cleaner❶ was used in a 1:3 dilution, as previously the 1:100 dilution was apparently ineffective (Fig. 12 and 13). It should also be noted that no one would use the comp. cleaner❶ in a 1:3 dilution, as in this ratio it demonstrates a gel-like consistency.

Phase 2 confirmed the conclusions of Phase 1 with regard to the reduction in the recontamination of the surfaces.

As a result of the increased appearance of moulds in the third phase, it can be assumed that bacteria growth was nonetheless inhibited, despite the disinhibition agent in the *Rodac TVC Plates*. However, at the pints in time of greatest significance for the evaluation (t_1 und t_2) the bacteria inhibiting effects of the moulds would appear to have not played a role, as the growth density on the *Rodac plates* was low.

As in Phase 1, the eMC® and comp, cleaners showed virtually identical cleaning effects with regard to the evaluation of the bacteria groups (Fig. 27) after cleaning. Equally, the results indicate that at the point in time (t_0), the disinfectant worked slightly better than the comp. and eMC® Cleaners. However, if one studies the statistical evaluation at the time points t_2 and t_3, it can be seen that the cleaning effect of the eMC cleaner was longer lasting than that of the comp. cleaner (Fig.28 and 29). Moreover, in comparison with the disinfectant at the t_2 and t_3 points in

time, the eMC® Cleaner showed a lower frequency of high number bacteria groups and thus a superior result.

6. Notes

6.1. Illustration

6.2. Tables

7. Literature

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