LONG LASTING ANTIMICROBIAL SURFACE PROTECTION

AEGIS® IN SPORTS

The Benefits of AEGIS® in Sports Facilities and Related Studies

Safe | Durable | Effective

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Contents

Overview

For over 45 years, AEGIS® (AEM 5700) has been the world's most widely used enduring antimicrobial surface protectant and coating. AEGIS® products are proven safe for their intended end use, durability, and efficacious.

Backed by more than 45 years of testing, AEGIS® technology has a long history of safe use. AEGIS Microbe Shield® is a durable and effective way to control a wide range of microbes, including bacteria, mould, mildew, fungi, yeast and algae on surfaces found in transportation, commercial, and other public settings.

1 Benefits of AEGIS® in Sports Facilities

AEGIS® has been used extensively in the Sports and Entertainment Industries most notably in sport locker rooms and training facilities where teams spend most of their time together. There are several benefits to using AEGIS Microbe Shield® in their facilities:

1. **Enhanced Hygiene and Safety**: AEGIS® can help reduce the presence of harmful microbes, including bacteria, mould, and fungi. This can create a safer and healthier environment for athletes, reducing the risk of infections and illnesses that can spread in communal areas.
2. **Odour Control**: Locker rooms and training facilities can often develop unpleasant odours due to sweat and bacteria. The antimicrobial properties of AEGIS® can help control and eliminate these odours, maintaining a fresher environment.
3. **Increased Athlete Performance**: A cleaner environment can contribute to better overall health and well-being for athletes. By minimizing the risk of infections and illnesses, athletes can maintain peak performance and avoid downtime due to sickness.
4. **Extended Lifespan of Equipment**: The use of AEGIS® can protect equipment and surfaces from microbial growth, which can cause deterioration. This can extend the lifespan of expensive sports equipment and facilities, saving money in the long run.
5. **Improved Team Morale**: A well-maintained and hygienic training environment can positively impact team morale. Athletes are more likely to feel valued and taken care of, which can translate into better performance and team cohesion.
6. **Compliance with Health Standards**: Using antimicrobial treatments like AEGIS® can help teams meet or exceed health and safety standards required by leagues, organisations, or governing bodies, ensuring that they remain compliant with regulations.
7. **Public Relations and Image**: Demonstrating a commitment to the health and safety of athletes can enhance a team's public image. It shows that the organization is proactive about protecting its players and staff, which can be a positive message for fans, sponsors, and the broader community.
8. **Cost-Effective Solution**: Investing in antimicrobial treatments can be a cost-effective solution to managing cleanliness and hygiene over time, reducing the need for frequent deep cleanings and potentially lowering medical expenses related to infections and illnesses.

Using AEGIS Microbe Shield® can be a strategic move for sports teams to ensure a high standard of hygiene and safety in their facilities, promoting the overall well-being and performance of their athletes.

Below is a list of sports teams, colleges and sports facilities, under their respective leagues, that have used AEGIS® in their facilities:

NHL (National Hockey League)

1. Edmonton Oilers - Edmonton, Alberta, Canada
2. Winnipeg Jets - Winnipeg, Manitoba, Canada

3. Detroit Red Wings - Detroit, Michigan, USA
4. Atlanta Thrashers - Atlanta, Georgia, USA

CFL (Canadian Football League)

1. Edmonton Eskimos - Edmonton, Alberta, Canada (now Edmonton Elks)
2. Montreal Alouettes - Montreal, Quebec, Canada

NFL (National Football League)

1. Minnesota Vikings - Minneapolis, Minnesota, USA
2. Cleveland Browns - Cleveland, Ohio, USA
3. Washington Redskins (now Washington Commanders) - Washington, D.C., USA

NBA (National Basketball Association)

1. Miami Heat - Miami, Florida, USA
2. Atlanta Hawks - Atlanta, Georgia, USA
3. Milwaukee Bucks - Milwaukee, Wisconsin, USA
4. Detroit Pistons - Detroit, Michigan, USA
5. New Jersey Nets (now Brooklyn Nets) - Brooklyn, New York, USA
6. Washington Wizards - Washington, D.C., USA

NCAA (National Collegiate Athletic Association)

1. Ohio State University - Columbus, Ohio, USA
2. University of Illinois - Urbana-Champaign, Illinois, USA
3. Southern Illinois University - Carbondale, Illinois, USA
4. University of Tulsa - Tulsa, Oklahoma, USA
5. Southeast Missouri State University - Cape Girardeau, Missouri, USA
6. Virginia Tech - Blacksburg, Virginia, USA
7. University of South Carolina - Columbia, South Carolina, USA
8. Cal State Bakersfield - Bakersfield, California, USA
9. Swarthmore College - Swarthmore, Pennsylvania, USA
10. Thiel College - Greenville, Pennsylvania, USA
11. Winthrop University - Rock Hill, South Carolina, USA

Other

1. Colleton River Plantation: Nicklaus and Dye Clubhouses - Bluffton, South Carolina, USA
2. Highlands Country Club - Highlands, North Carolina, USA
3. Warwick Hills Country Club - Grand Blanc, Michigan, USA

2 Related Studies on AEGIS®

Skin Irritation or Skin Sensitivity

Various sock materials (wool, cotton, nylon, ORLON and SPANDEX) were treated with AEM 5700 Antimicrobial at 0.35% owf (on weight of fibre) for safety evaluation of potential hazard to skin under normal wear conditions. The antimicrobial (AEM 5700) treated socks were evaluated for skin-irritating and skin sensitizing properties on 44 young male subjects for an entire football season (approximately 90 continuous days). The results of the investigation showed the male subjects to be free from any observable skin irritation or skin sensitization at the end of the test period.

Control of Athlete's Foot Fungus by Articles Treated with the AEGIS Microbe Shield®

Athlete's foot is caused by microorganisms isolated in dark moist areas of the feet. Shoes and socks in direct contact with these areas are potential harbours of refuge for the attacking organisms. The ÆGIS Microbe Shield® Program provides antimicrobial protection from the growth of fungi and bacteria on these treated articles.

The AEGIS antimicrobial agent acts by specifically rupturing the cell membrane of these organisms and has been shown to be fully effective against fungi, bacteria and algae. This study contains data using the ASTM E2149-01 antimicrobial test method demonstrating the antibacterial activity against the bacteria responsible for secondary and more severe forms of Athlete's foot infection. For each bacteria tested, the commercially sold sock demonstrated excellent activity with over 99.9% bacterial reduction within the 1 hour of contact time.

In Vitro Evaluation of the Bio-Activity of Different Fabrics for Underwear Against Lactobacillus Acidophilus, Staphylococcus Epidermidis, Staphylococcus Aureus and Candida Albicans

This study has been included to support the fact the AEGIS Microbe Shield® is not a leaching biocide. It permanently bonds to treated surfaces. This is unlike all other products studied which exert an antibacterial effect through a leaching process. Leaching biocides can have a negative effect on staff, and patients and can contribute to the growth of resistance.

The only material that showed to exert its maximal antimicrobial activity in a short time (within the first 60 minutes) was Dermasilk treated with AEGIS AEM 5772/5. All fabrics evaluated had a microbe-killing capacity when in strict contact with the microorganisms in a warm and humid environment. This killing activity was released in various degrees in the incubation medium by all of the fabrics but Dermasilk showed no release into the environment.

Use of DermaSilk® Briefs in Recurrent Vulvovaginal Candidosis: Safety and Effectiveness

AEGIS Microbe Shield® was applied to intimate undergarments with the goal of testing whether it would have an impact on the growth of microbes. Of special interest was the fact that no study participant experienced any collateral effects of wearing undergarments during very aggressive and active infection presence. The AEGIS Microbe Shield® is non-leaching and in this study demonstrated no effect other than inhibition of fungal growth to study participants

No patient reported any collateral effects. Both groups showed a similar reduction of symptoms and objective signs during the first month, but after 3 months a difference began to appear in favour of the DermaSilk group with regards to itching, burning and erythema.

Enhanced Filtration Performance with AEM 5700 Antimicrobial Treatments: Laboratory and Field Studies

Air filters are crucial in managing microbial issues in modern buildings. Enhancing air filters with a durable, safe, and effective antimicrobial treatment can prevent abnormal fungal growth and improve air quality. By choosing the right antimicrobial agent for air filtration media, you can inhibit microbial growth on filter surfaces, reduce microbial cells in the air stream, and improve indoor air quality.

This paper presents laboratory test data on air filtration products treated with AEGIS Microbe Shield Technology, examining microbial retrieval from surfaces and flow-through. Filters treated with this technology reduced microbial activity by 99.99% in the filter matrix and downstream. Additionally, two real-life field evaluations are discussed, demonstrating the effectiveness and suitability of AEGIS Microbe Shield Technology in air filtration systems for reducing microbial growth and enhancing air quality.

3 Appendix

Skin Irritation or Skin Sensitivity

Year:

2012

Authors:

Matt Richter (Director, Regulatory and Environmental Affairs)

By:

Microban

Location:

AEGIS Environmental Management Inc.,

Huntersville,

North Carolina,

USA

Skin Irritation or Skin Sensitivity

AEM 5700 Antimicrobial

32 Day Human Wear Test for Treated Socks:
Various sock materials (wool, cotton, nylon, ORLON and SPANDEX) were treated with AEM 5700 Antimicrobial at 0.35% owf (on weight of fiber) for safety evaluation of potential hazard to skin under normal wear conditions. The antimicrobial (AEM 5700) treated socks were evaluated for skin irritating and skin sensitizing properties on 44 young male subjects for an entire football season (approximately 90 continuous days). The results of the investigation showed the male subjects to be free from any observable skin irritation or skin sensitization at the end of the test period.

Date: December 12, 2012

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Appendix

Control of Athlete's Foot Fungus by Articles Treated with the AEGIS Microbe Shield®

Year:

2008

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USA

Control of Athlete's Foot Fungus by Articles Treated with the ÆGIS Microbe Shield®

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Abstract

Athlete's foot is caused by microorganisms isolated in dark moist areas of the feet. Shoes and socks in direct contact with these areas are potential harbors of refuge for the attacking organisms. The ÆGIS Microbe Shield® Program provides antimicrobial protection from the growth of fungi and bacteria on these treated articles. This report documents the laboratory data that demonstrates the effectiveness of the durably bound antimicrobial against the specific organism involved in athlete's foot infection.

Introduction

Athlete's foot is a common skin infection in which most people will develop over the course of their lives. The medical term, *tinea pedis,* indicates a fungal infection of the feet and is seen as itchy, scaly, toe-web lesions to which athletic young adults are most prone. In the UK this past year, 17% of all adults indicated some form of athlete's foot infection. Athlete's foot is the result of the infection of the fungi *Trychopyton rubrum* and *Trychopyton mentagrophytes*. These fungal organisms colonate the dark and moist area between the toes. It is the moisture, sweating and lack of ventilation that provides the perfect environment for the fungus of Athletes foot to grow. Later and more severe cases of Athletes foot are the result of bacterial infections which colonate due to the stressed environment brought on by the fungal infection. The more severe infections result in redness, swelling, and are accompanied by foul foot odors.

In general, antifungal creams can prevent and cure the fungal infection of athlete's foot. If the more severe case of the infection is found, antibacterial agents are also needed. Daily practices are responsible for the infections of Athletes foot infections. Routine cleaning and thorough drying of the feet in a addition to the use of clean shoes and socks treated with a durable, broad spectrum antimicrobial agent will decrease the likelihood of infection.

The active ingredient found in the ÆGIS Microbe Shield antimicrobial product has been demonstrated for the past 30 years to be effective against a broad spectrum of microbial organisms. From bacteria, fungi, yeast, and algae, the ÆGIS antimicrobial agent physically and ionically kills the microorganism on contact. The presence of the ÆGIS microbe shield in the shoes and socks will significantly reduce the local concentration of organisms that contribute to the athlete's foot infection. Demonstrated durability will provide long lasting protection upon multiple washes.

Results and discussions

ÆGIS Environments and the Dow Corning Corporation have studied the effectiveness of the ÆGIS Microbe Shield product against the specific organism responsible for Athlete's foot. The treated and untreated sock fabrics consisted of Orlon/nylon, nylon, cotton/nylon, and commercially treated socks purchased from a major retailer, London. Each set of samples was tested using the test organism, *Trychopyton mentagrophytes*, using the industry standard antimicrobial test method, AATCC100. Letheen broth has been shown in the historical data base for AEM 5772 and other quats to be effective as a neutralize in a test system and was used in this system.

The fungal counts from the treated and untreated sock fabrics are presented in Table I (Nylon), Table II (Orlon/nylon), Table III (Cotton/nylon), and Table IV ("Fresh Feet"). The treated sock fabrics were capable of reducing significant levels of athlete's foot fungus, as compared to the untreated sock fabrics, through 10 laundering cycles. The activity diminished slowly after 10 launderings using this test method to determine efficacy. A 99.9% reduction or 3 log decrease in fungal CFU count on the treated test fabric versus the untreated control fabric indicates excellent activity against the test organism.

The antifungal activity of a commercially prepared sock sample was also tested for the activity against the athlete's foot fungus. The full antimicrobial activity was demonstrated using the industry standard test method ASTM E2149-01. These results are presented in Table IV. In this test method, fungal cultures are grown and before testing, conidia are separated from the Mycelia fragments. The resulting solution is used as the standard inoculum for the Dynamic portion of the test method. After 1 hour of agitation, the cells are re-isolated, grown in the incubator and counted. As an additional measure of control, the cells isolated after the test were further analyzed to ensure that they were the proper test organism. Each isolated organism re-grew the fungal Mycelia associated with *Trychopyton mentagrophytes*.

Athlete's foot infection is the result of the growth of the fungal organism *Trychopyton*. More severe infections occur after the fungi have conditioned the environment for

further growth. The secondary bacterial infections are much more severe than the original fungal infection. The organisms are typical skin type bacteria and include Gram + bacteria, *Staphylococcus aureus*, and Gram - bacteria, *Proteus* and *Pseudomonas*. Many antimicrobial agents are specific against either Fungi, Gram + or Gram - bacteria but usually are not effective against all these organisms. The ÆGIS antimicrobial agent acts by specifically rupturing the cell membrane of these organisms and has been shown to be fully effective against fungi, bacteria and algae. Table V contains data using the ASTM E2149-01 antimicrobial test method demonstrating the antibacterial activity against the bacteria responsible for secondary and more severe forms of Athletes foot infection. For each bacteria tested, the commercially sold sock demonstrated excellent activity with over 99.9% bacterial reduction within the 1 hour of contact time.

Tables:

#	**Description** Antimicrobial Fabrics	**Microbiological Analysis**		**Pass/Fail***
		Fungal CFU/sample 18 hours	**Fungal (% Reduction) Versus untreated control**	
1	Untreated	9.7×10^5	0	**Fail**
2	Treated	$< 10^2$	99.99	**Pass**
3	Untreated 5 wash	1.01×10^6	0	**Fail**
4	Treated 5 wash	$< 10^2$	99.9	**Pass**
5	Untreated 10 wash	9.0×10^5	0	**Fail**
6	Treated 10 wash	3.15×10^2	99.97	**Pass**
7	Untreated 20 wash	8.6×10^5	0	**Fail**
8	Treated 20 wash	2.13×10^4	97.52	**Pass**
9	Untreated 25 wash	9.5×10^5	0	**Fail**
10	Treated 25 wash	9.6×10^4	89.89	**Pass**

Table I. Antifungal activity of treated Nylon fabrics against Trichophyton mentagrophytes.
Test Fungus: Trichophyton mentagrophytes ATCC 9533. Test methods AATCC 100 antimicrobial

#	**Description** Antimicrobial Fabrics	**Microbiological Analysis**		**Pass/Fail***
		Fungal CFU/sample 18 hours	**Fungal (% Reduction) Versus untreated control**	
1	Untreated	2.91×10^6	0	**Fail**
2	Treated	$< 10^2$	99.99	**Pass**
3	Untreated 5 wash	2.03×10^6	0	**Fail**
4	Treated 5 wash	1.11×10^2	99.9	**Pass**
5	Untreated 10 wash	1.98×10^6	0	**Fail**
6	Treated 10 wash	1.27×10^3	99.94	**Pass**
7	Untreated 20 wash	2.20×10^6	0	**Fail**
8	Treated 20 wash	2.13×10^4	99.03	**Pass**
9	Untreated 25 wash	2.83×10^6	0	**Fail**
10	Treated 25 wash	1.93×10^5	93.18	**Pass**

Table II. Antifungal activity of treated Orlon/Nylon fabrics against Trichophyton mentagrophytes.
Test Fungus: Trichophyton mentagrophytes ATCC 9533. Test methods AATCC 100

#	Description Antimicrobial Fabrics	Microbiological Analysis		Pass/Fail*
		Fungal CFU/sample 18 hours	Fungal (% Reduction) Versus untreated control	
1	Untreated	1.89×10^6	0	**Fail**
2	Treated	$< 10^2$	99.99	**Pass**
3	Untreated 5 wash	2.36×10^6	0	**Fail**
4	Treated 5 wash	1.44×10^2	99.9	**Pass**
5	Untreated 10 wash	2.79×10^6	0	**Fail**
6	Treated 10 wash	2.33×10^3	99.95	**Pass**
7	Untreated 20 wash	2.41×10^6	0	**Fail**
8	Treated 20 wash	2.11×10^4	99.0	**Pass**
9	Untreated 25 wash	1.01×10^7	0	**Fail**
10	Treated 25 wash	1.99×10^5	90.4	**Pass**

Table 111. Antifungal activity of treated Cotton/Nylon fabrics against Trichophyton mentagrophytes.
Test Fungus: Trichophyton mentagrophytes ATCC9533. Test method: AATCC 100

#	Description Antimicrobial Fabrics	Microbiological Analysis		Pass/Fail*
		Fungal CFU/sample 1 hour	Fungal (% Reduction) Versus untreated control	
1	Untreated	9.7×10^5	0	**Fail**
2	Treated	$< 10^2$	99.99	**Pass**

Table IV. Antifungal activity of treated Marks & Spencer "Fresh Feet" socks against Trichophyton mentagrophytes.
Test Fungus: Trichophyton mentagrophytes ATCC 9533. Test method: ASTM E2149-01

#	Description Antimicrobial Fabrics	Microbiological Analysis		Pass/Fail*
		Fungal CFU/sample 1 hour	Bacterial (% Reduction) Versus untreated control	
Escherichia coli				
1	Untreated	1.2×10^5	0	**Fail**
2	Treated	$< 10^2$	99.99	**Pass**
Proteus mirabilis				
3	Untreated	5.2×10^5	0	**Fail**
4	Treated	$< 10^2$	99.99	**Pass**
Staphylococcus aureus				
5	Untreated	1.5×10^5	0	**Fail**
6	Treated	$< 10^2$	99.99	**Pass**
Pseudomonas aeruginosa				
7	Untreated	1.8×10^5	0	**Fail**
8	Treated	$< 10^2$	99.99	**Pass**
Serratia marcescens				
9	Untreated	1.9×10^5	0	**Fail**
10	Treated	$< 10^2$	99.99	**Pass**

Table V. Antibacterial activity of treated Marks & Spencer "Fresh Feet" socks against common skin and soil bacteria.
Test method: ASTM E2149-01

ÆGIS Environments

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Appendix

In Vitro Evaluation of the Bio-Activity of Different Fabrics for Underwear Against Lactobacillus Acidophilus, Staphylococcus Epidermidis, Staphylococcus Aureus and Candida Albicans

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In vitro evaluation of the bio-activity of different fabrics for underwear against *Lactobacillus acidophilus, Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans*

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Background: fabrics could exert antimicrobial activity against different micro organisms, depending on the addition of antimicrobial molecules to the textile. Silver ions, Triclosan Sanitized T99-19 are used. Recently the AEGIS AEM5772/5 has been linked to a silk fabric (Dermasilk), showing antimicrobial activity. The aim of this study was the, in vitro, comparative evaluation of the microbe-cidal capacity of 10 different fabrics (used in contact with the skin) against 4 different microbes that can be saprophytes or pathogens in the human epidermis.
Methods: the following micro organisms have been used: *Lactobacillus acidophilus* (ATCC11975), *Staphylococcus epidermidis* (ATCC 1228), *Staphylococcus aureus* (ATCC 700698 – methycillin resistant) and *Candida albicans* (ATCC 10261). All the isolates were grown in TSB medium and a final dilution of 1,0 x 108/ml was made in 0.2 M PBS. The antimicrobial activity was evaluated by using a modification of the "Dynamic shake flask test" methods as reported by the standard ASTM E2149-01 and expressed as percent reduction of the initial microbial load.
Results: the quantitative evaluation of the antimicrobial activity showed that all the fabrics studied exerted an anti microbe activity ranging from 18% to 100% over 24 hours of incubation. Most of the textiles showed a marked increase of the antimicrobial activity that correlated with the incubation time, reaching the highest reduction rate after a time variable between 6 and 22 hours of incubation. The only material that showed to exert its maximum antimicrobial activity in a short time (within the first 60 minutes) was Dermasilk. The evaluation of the antimicrobial activity released by fabrics after 24 hours of incubation in PBS, demonstrated that all the textiles released variable levels of antimicrobial molecules in the incubation medium, with the only exception of the pure cotton and Dermasilk (Pure silk,100% fibroin, lacking sericin, treated with AEGIS AEM5772/5).
Conclusion: all the fabrics evaluated had a microbe killing capacity when in strict contact with the micro organisms in a warm and humid environment. *This killing activity was released in various degree in the incubation medium by all the fabrics but Dermasilk, that showed no release in the environment. This fact could raise concern about the insurgence of cutaneous allergy or damage*. In addition Dermasilk's antimicrobial activity was very fast, being the highest level reached within 1 hour of incubation.

Aim of the study

The interaction between textile with antimicrobial properties and the microbes located onto the surface of skin and mucosa may results in a persistent modification of the normal microbial ecology of the body surfaces. This may be caused in particular by fabrics that can release antimicrobial molecules, giving raise to an accumulation of these antimicrobial substances. The aim of the present study was to evaluate and compare, in vitro, the microbe-cidal effect of 10 different fabrics against four different micro organisms: *Lactobacillus acidophilus, Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans.*

Table 1: Tested Bio-functional underwear available in the market

1. DERMASMART: polyester with Silver ions
2. PLATATEX: 50% cotton, 42% polyamide, 8% silver
3. PADYCARE: 82% polyamide, 18% lycra with, 20% Silver filaments
4. ENVICON: cotton with Silver filaments
5. SANITIZED T99-19: polyamide with T 99-19
6. CRABYON: chitosan and viscose
7. TRICLOSAN/SANITIZED: polyamide with TRICLOSAN (SANITIZED)
8. DERMASILK: pure silk (100% fibroine, without sericine) with AEM 5772/5
9. COTTON: 100% without antimicrobial treatment
10. ECZEMACLOTHING: 100% cotton with Silver ions

Conclusions and Comments

The results of this study demonstrated that different textile fabric could have a large variety of antimicrobial activity against germs that are commonly identified as saprophytic or pathogenic micro organisms onto skin and genital mucosa in both male and female, when evaluated following the ASTM-E2149-01 test (after 24 hours of incubation).

Quantitative evaluation of the antimicrobial activity of textiles. All the textile showed a medium high level of antimicrobial activity against *L. acidophilus, S.epidermidis, S.aureus* and *C. albicans* with the only exception of that identified with n. 9. This last textile fabric showed only a very low (3,38%) inhibitory effect against *L. acidophilus*.

Quantitative evaluation of the antimicrobial activity over time.
The antimicrobial activity of fabric textile is dependent upon time with some difference between the different material evaluated. In particular, most of the textiles showed a marked increase of the antimicrobial activity that correlated with the incubation time, reaching the highest reduction rate after a time variable between 6 and 22 hours of incubation.

The only material that showed to exert its maximum antimicrobial activity in a short time (i.e. within the first 60 minutes of incubation) was Dermasilk.

These differences in the antimicrobial activities over the time are likely due to a different structure of the materials evaluated. It is possible to speculate that these differences are due to a different mechanism of action: those materials that required a longer period of incubation are likely to release the antimicrobial in the medium. On the other hand, Dermasilk is likely to exert its activity only by getting in strict contact with the micro organisms, since the activity its self reached the highest level within 60 minutes of incubation, clearly suggesting that the this material has a minimal release of antimicrobial substance in the environment, under the experimental condition used.

Moreover a general consideration based on these findings is that the vast majority of the so called antimicrobial textiles, with the only exception of Dermasilk, exert their functions by releasing the active molecules in the environment where these substance can accumulate. This fact is likely to provoke a marked modification in the microbial ecology of the body district (e.g. the genital area). The consequent variation in the resident microbial flora may be the cause of additional infections, allergies and tissues damage.

Materials and methods

Strains used and growing conditions. In these experiments the following micro organisms have been used: *Lactobacillus acidophilus* (ATCC11975), *Staphylococcus epidermidis* (ATCC 1228), *Staphylococcus aureus* (ATCC 700698 – methycillin resistant) and *Candida albicans* (ATCC 10261). All the strains were thawed from a frozen stock (stored at -80°C in individual appropriate storage vials) and streaked onto 5% horse blood agar plates in order to ensure colonies isolation. After 48 hours of incubation under aerobic atmosphere at 37°C one individual colony was picked up with a sterile plastic loop and transferred to a tube containing 5 ml of Trypticase soy broth medium. Individual tubes were further incubated for additional 24 hours and the concentration of the micro organisms in each tube was checked by a standard McFarland density evaluation. A final dilution of each organism to 1,0 x 10^8/ml was made in 0.2 M phosphate buffered saline (PBS) (pH 7.2). This suspension was immediately used for the experiments described in this report. The tested fabric textiles used in this study are reported in Table 1 in the previous page .

Anti micro organism activity of fabric textile – Dynamic shake flask test (ASTM E2149-01). This test was performed in two different ways, as follows.

Quantitative evaluation of the antimicrobial activity of textiles.

The antimicrobial activity of each individual textile was evaluated as follows: 2 grams of each textile were cut in small pieces and the whole amount was inserted in sterile flasks containing 50 ml of PBS. Each flask was inoculated with a final concentration of 1-6 x 10^5 CFU/ml of each micro organism and 10 μliters of suspension was taken and used (see below for the counting procedure) to assess the initial load of micro organism (defined as T0 load and expressed as CFU/ml). Flasks were then incubated under gentle shaking aerobic conditions for 24 hours at 37°C. At the end of this incubation, 10 μliter of each individual micro organism suspension (defined as T24 load and expressed as CFU/ml) was plated onto agar plates as described above at point 1.1. After 48 hours of incubation the number of colonies present onto each individual plate was counted and annotated in order to asses the load of viable micro organisms in each suspension. As a control, for each individual micro organism evaluated, an identically prepared suspension lacking any textile fabric inside was incubated and counted as above reported. Each experiment was made in duplicate and the results are the mean of each individual evaluation.

Quantitative evaluation of the antimicrobial activity over time.

In order to evaluate the antimicrobial activity of selected textiles over the time the following experiments have been performed. The above reported dynamic shake flask test has been performed with the slight modification. Following the inoculum of the micro organism in each individual flask, a 10 microliter samples was immediately taken (T0).Subsequently identical additional samples were taken after 30, 60, 120 and then every 240 minutes up to 26 hours.

Individual samples were plates onto appropriate agar plates in order to asses the number of viable organism, as reported above. Results are showed in the graphs in the next page.

Antimicrobial activity. This parameter was calculated as follow for each individual textiles/micro organism:

(T0-T24)/T0 x100 = % reduction of viable micro organism load

Quantitative evaluation of the antimicrobial activity released by textiles after 24 hours of incubation in PBS.

In order to asses the release of antimicrobial molecule from textiles incubated in the presence of a “water-saline” physiological environment, 3 grams of each textile were cut and incubated in the presence of 75 ml of PBS under aerobic shaking conditions up to 26 hours at 37°C. At the end of the incubation time, 50 ml of each solution obtained from individual textile were removed and inoculated with 1 x 10^5/ml of each micro organism. Incubation and counting was performed as above reported.

Results are shown in the table showed below.

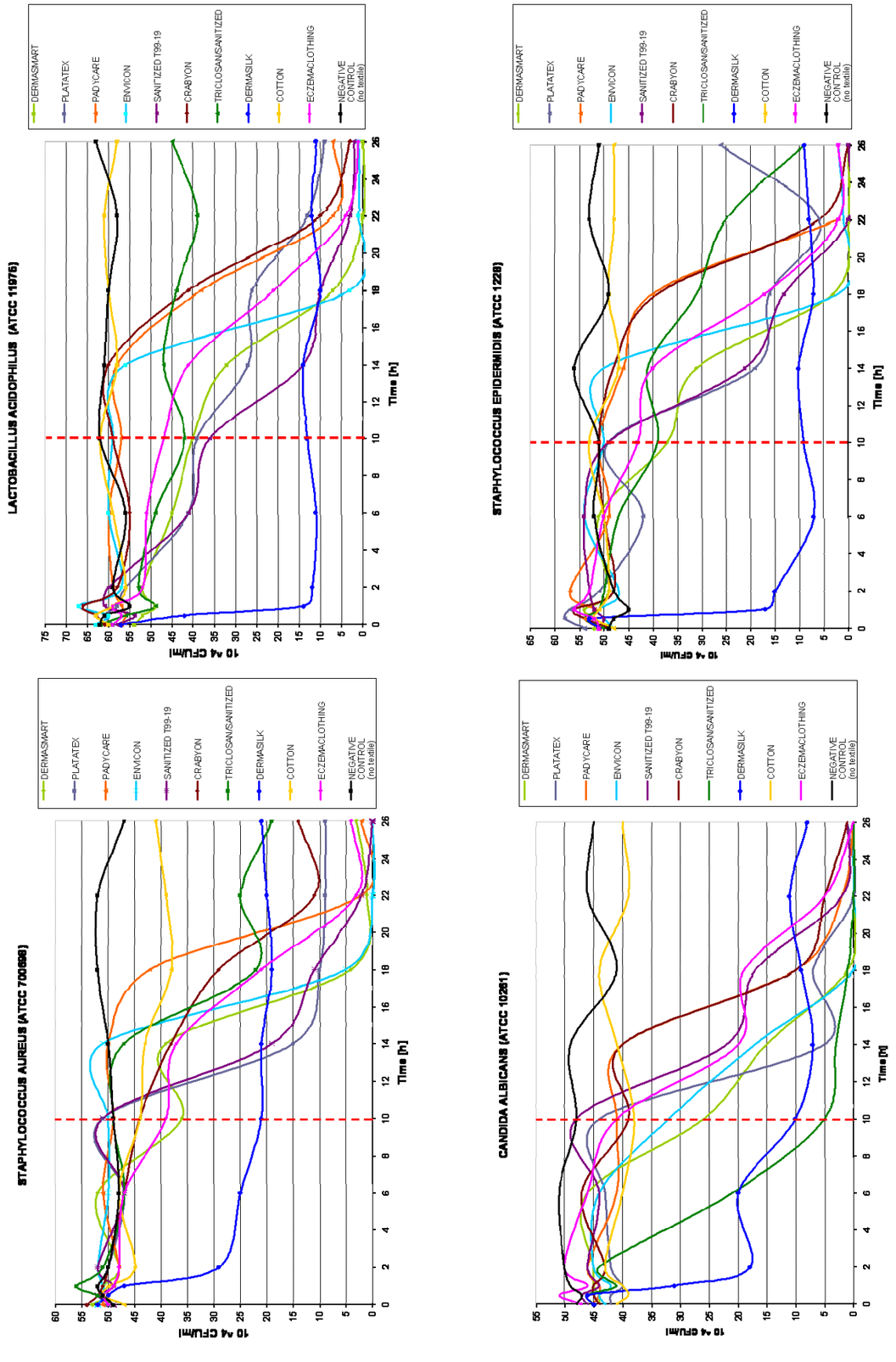
Antimicrobial activity released by textiles

Quantitive evaluation after 24 hours of incubation in PBS

Textiles	Antimicrobial agent	% reduction			
		Lactobacillus acidophilus (ATCC 11975)	Staphylococcus epidermidis (ATCC 1228)	Staphylococcus aureus (ATCC 700698)	Candida albicans (ATCC 10261)
DERMASMART	Ag^+ ions	100,0	98,11	100,0	100,0
ECZEMACLOTHING	Ag^+ ions	100,0	96,07	98,38	86,84
PLATATEX	Pure Ag	94,82	100,0	100,0	100,0
PADYCARE	Ag filaments	96,66	100,0	95,00	100,0
ENVICON	Ag fibers	100,0	100,0	100,0	100,0
SANITIZED T 99-19	T 99-19	96,72	98,0	96,36	56,09
TRICLOSAN/SANITIZED	Triclosan	96,36	98,07	100,0	17,64
CRABYON	Chitosan	98,33	100,0	100,0	34,37
DERMASILK	AEM 5772/5	1,69	0	0	0
COTTON	-	0	0	0	6,25
Negative control (no textile)	-	0	0	0	0

Results. The results of the study are reported in the graphs below.

Quantitative evaluation of the antimicrobial activity over time



Appendix

Use of DermaSilk® Briefs in Recurrent Vulvovaginal Candidosis: Safety and Effectiveness

Year:

2012

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Journal:

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Use of DermaSilk® briefs in recurrent vulvovaginal candidosis: safety and effectiveness

D'Antuono A., Baldi E., Bellavista S., Banzola N., Zauli S., Patrizi A.

OBJECTIVE OF THE STUDY
Compare DermaSilk® underwear in pure silk protein with placebo cotton underwear, to see whether the use of DermaSilk may be a useful additional tool in the treatment of Recurrent Vulvovaginal Thrush (RVVT).

- **Double blind randomised study DermaSilk briefs vs placebo briefs in pure cotton**
- Patients enrolled: **96 women** (mean age, 30.25 years) **suffering from Recurrent Vulvovaginal Thrush**, for 1 to 6 years, mean 2.4 years.
- **48 patients used DermaSilk briefs** (DS group)
- **48 patients used cotton briefs** (CT group)

The randomised double blind study involved 96 women (mean age 30.25) with a long history of RVVT (from 1 to 6 years, mean 2.4 years) who did not respond completely to the antimycotic therapy administered orally. In the six months prior to the start of the study they had already been treated with standard antimycotic therapy but continued to present relapses and vulvar irritation.

At the time of recruiting, the suitable patients presented an episode of acute VVT, a positive vaginal culture for Candida and a level 3 of severity of vaginitis as described by Sobel et al. This system for measuring the severity of the symptoms evaluates the symptoms (itching, irritation and burning) and the objective signs (erythema, oedema and skin wounds or fissuring).
The severity of each sign and symptom was classified on a scale from 0 (absent) to 3 (severe).
In each patient bacterial vaginosis was excluded thanks to a measurement of the pH and a microscope examination with Gram staining.

Abstract written by Alpretec Srl
Original: "Use of DermaSilk briefs in recurrent vulvovaginal candidosis: safety and effectiveness" (D'Antuono A., Baldi E., Bellavista S., Banzola N., Zauli S., Patrizi A.). MYCOSES, VOLUME 55, ISSUE 3 (e85-e89), may 2012.

The yeast cultures indicated Candida Albicans in 82 patients and Candida Non Albicans in 14 (6 in the DermaSilk Group and 8 in the Cotton Group).

The mean duration of recurrent VVT and the incidence of the symptoms and the objective signs were not statistically different between the two groups, even though burning and dyspareunia were slightly more frequent in the DermaSilk group.

One group was told to use cotton underwear (CT) and the other group DermaSilk® underwear (DS). Both groups were treated with Fluconazole (150 mg once a week) for six months.

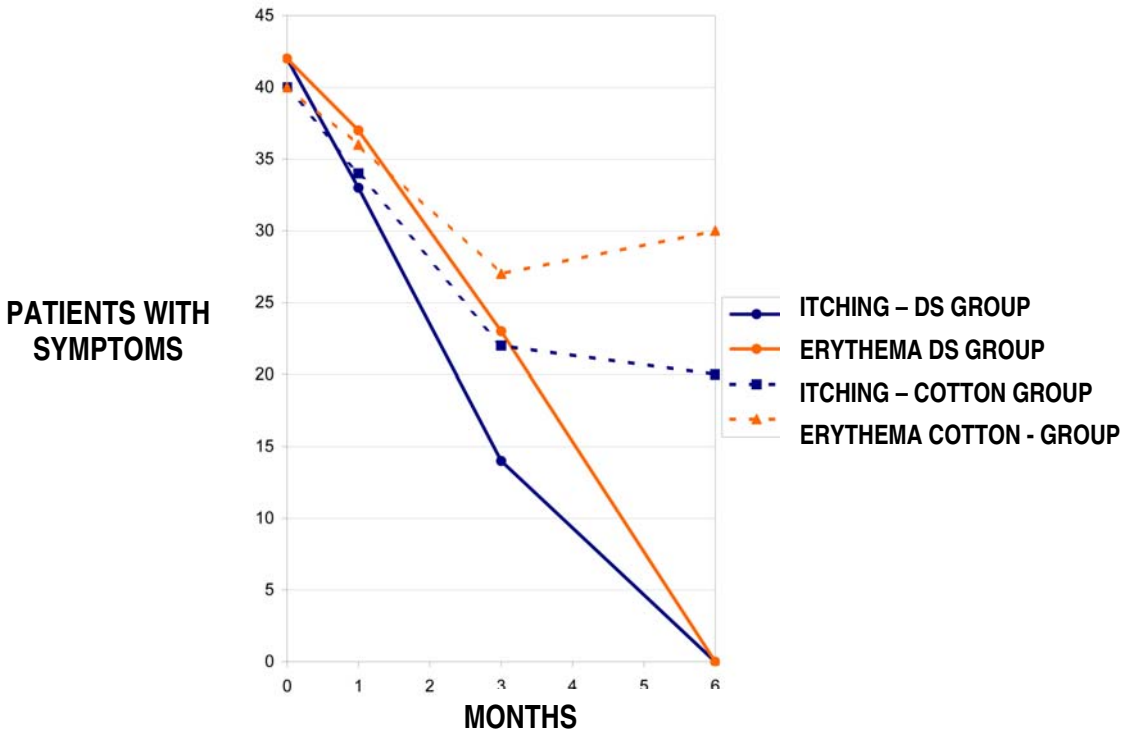
Each patient was given a sealed envelope containing 3 pairs of DermaSilk briefs or 3 pairs of cotton briefs. Each envelope was identified with a progressive number; the correspondence between the number on the envelope and its content was communicated by the manufacturer of the briefs only at the end of the study.

The patients were asked to wear these briefs, night and day, throughout the duration of the study. During check-ups, after 1, 3 and 6 months, the cultures for Candida were repeated and the measurements of the symptoms and of the objective signs. A score of 3 with a positive culture was considered a relapse.

RESULTS

No patient reported any collateral effects. Both groups showed a similar reduction of symptoms and of objective signs during the first month, **but after 3 months a difference began to appear in favour of the DermaSilk group as regards itching, burning and erythema.**

In the sixth month the patients in the DS group showed a significant decrease of itching, erythema (Fischer exact test p<0.0001) and burning (Fischer exact test p<0.05) in comparison with the patients in the CT group.



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Most of the patients in the DS group (32/48, 66.7%) had no relapses or had only one, whereas in the CT group most (29/48, 60.5%) confirmed 2 or more relapses, as before recruiting.

The results show that at the end of the study no patient in the DermaSilk group suffered any longer from either itching or erythema, while in the Cotton group 50% continued to suffer from itching and 75% from erythema.

At the end of the study in both groups no other symptoms (burning and vulvar irritation) or objective signs (oedema and fissuring) were present in any patient.

The number of relapses in the study is higher than in other reports, but it must be stressed that our patients suffered from a particularly persistent form of RVVT and had had 4 or more episodes of VVT during the previous year, treated with a long-term antimycotic therapy.

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Appendix

Enhanced Filtration Performance with AEM 5700 Antimicrobial Treatments: Laboratory and Field Studies

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Enhanced Filtration Performance with AEM 5700 Antimicrobial Treatments: Laboratory and Field Studies

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1. Abstract

Air filters are part of the front-line for dealing with microbial problems in our modern buildings. Upgrading efficiency of air filters by adding a durable, safe and effective antimicrobial treatment can protect the air filter from abnormal fungal growth and improve the air quality of our buildings. With the proper choice of an antimicrobial agent applied to air filtration media, you can prohibit the growth of microbes on filter surfaces, reduce the number of microbial cells in the air stream, and reduce microbes in the indoor environment.

This paper describes test data from laboratory experiments where air filtration products treated with the ÆGIS Microbe Shield Technology were studied for the level of retrieval from the surface and the flow through of microorganisms. Filters treated with the ÆGIS Microbe Shield Technology reduced microbial activity in the filter matrix and downstream of the filter by 99.99%. Besides laboratory data, this report covers two real life field evaluations. These studies show clearly the utility and appropriateness of the ÆGIS Microbe Shield Technology as part of air filtration systems for the reduction of microbial growth on filters and the added benefits to air quality.

2. Introduction

Microbiological pollutants, as sources of irritants, sensitizers, discomforting products, toxicants and as disease causing agents, have enormous consequences on human productivity, comfort, health, and general well being. This fact clearly links microbes to the cause, effect, and remedy equation needed for establishing and maintaining indoor environmental quality.

But, as important and necessary as antimicrobials are, they cannot be used without serious safety considerations. Many chemical agents can and do exhibit excellent biocidal and antimicrobial properties, yet often with dangerous side effects and severe human and animal toxicity problems. The antimicrobial agent must not introduce toxicants into the environment, lose its effectiveness by dissipating before filter life expires, adversely affect filter efficiencies or static pressure characteristics, or allow for microbial adaptation.

The ÆGIS Microbe Shield Technology addresses these criteria completely. This technology has an outstanding toxicological and safety profile. Due to the chemical bonding of the active ingredient, the ÆGIS Microbe Shield Technology assures long lasting effectiveness without migration of the active ingredient into the environment.

The ÆGIS Microbe Shield Technology provides a powerful tool on air filters for dealing with one key element in the battle for improved indoor environmental quality – minimizing microbial infiltration via the air handling system.

3. Indoor Environmental Quality and the ÆGIS Microbe Shield Technology

3.1. Introduction

The indoor environment in any building is a result of complex interactions between a wide variety of conditions and situations – the building site, the building system itself (original architectural design and modifications), the climate, building use, and mechanical equipment systems. There are literally thousands of potential contamination sources inherent in these interactions. In the broadest sense, these

sources are the building materials, furnishings, moisture, manufacturing processes, machinery and equipment, activities, occupants, and the outdoors. If the contaminant sources are strong or if contaminants are allowed to concentrate, serious Indoor Environmental Quality (IEQ) problems occur. How serious and how costly the problems become is entirely within the control of the building's owners and managers. Technology is available to eliminate or at least minimize most serious pollutants. ÆGIS Environments, through its ÆGIS Microbe Shield Program, effectively controls and helps prevent IEQ problems, which are related to microorganisms – by far the largest single pollutant segment. One of the most critical parts of this program is the ÆGIS Microbe Shield Technology – a comprehensive package of application technology, chemistry, and intellectual property rights that transform normal material surfaces into active antimicrobial sites.

The following data are intended to provide a very general introduction to the role of air filtration in the IEQ equation and to explain in more detail the role that can be played by the incorporation of the ÆGIS Microbe Shield Technology into air filtration systems.

3.2. The Air Handling System

Great attention has been focused on the air handling system as a source and dispersal vehicle for a variety of pollutants. The debate on the "fault" of design, materials of construction, operating conditions, and maintenance is both unending and, to a large extent, unsolvable.

The most important fact out of this debate is that a clear mandate comes to upgrade and better maintain the filtration components of the air handling system.

3.3. General Pollutants

Pollutants can be classified in many ways, but, in general, they are divided into gases, liquids, solids, and sensory impactors. A second critical classification is between biologicals and non-biologicals. Finally, from the standpoint of both human significance and remediation, source considerations, such as single event or regenerative, are important. Regenerative sources may be either continuous or cyclic.

3.4. Microbiological Consequences

The contribution of microbiological pollutants as sources of irritants, sensitizers, discomforting products, toxicants and disease causing agents surpasses all other types of pollutants in their affect on human productivity, comfort, health, and general well-being.

3.5. A Unique Antimicrobial System

3.5.1. The Antimicrobial Technology of Choice for Air Filtration Products

A significant body of data[1] has been developed which supports the usefulness of using the ÆGIS Microbe Shield Technology as the antimicrobial technology of choice for air filters. These data are embodied in a series of patents and publications from Dow Corning Corporation, Burlington Industries, Baxter Healthcare, ÆGIS Environments, other corporations and university test laboratories.

The extraordinary safety profile of this technology, its unique chemical bonding capability, its subsequent durability and, most importantly, its proven real world control of bacteria, fungi and algae (without concern for adaptation and mutation[2]) permits its use on surgical drapes, nurses uniforms, hosiery, carpeting and "Air Filtration Products."

ÆGIS Environments has developed and implemented highly controlled application procedures and a comprehensive quality control program to achieve efficient and effective integration of the antimicrobial chemistry into air filtration media. This, bolstered by participation of the best air filter and air filter media companies, assures the quality and efficacy of all products, which reach the marketplace as a part of the ÆGIS Microbe Shield Program.

3.5.2. A non migrating Silane-based antimicrobial

The ÆGIS Microbe Shield uses a "bound" antimicrobial that was conceived and produced by Dow Corning Corporation, the world's leading producer of silicones, in the early 1970s.

Dow Corning Corporation utilized its unmatched

silicone chemical technology to incorporate a standard antimicrobial substance (a quaternary amine) into a silane. The result was extraordinary: The world's first odorless, colorless, non-leaching, durable, broad-spectrum antimicrobial with an unheard of oral LD50 of 12.65 g/kg (ordinary table salt is 3 g/kg) (Fig.1). Today Dow Corning produces the antimicrobial material in an ISO 9002 certified plant, but does it exclusively for ÆGIS Environments.

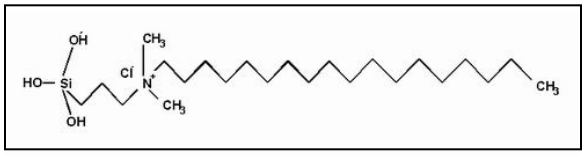

Fig.1. The chemistry

In contrast to most silicones, which are slippery, non-reactive materials, silanes are highly reactive materials which are used primarily as coupling agents and adhesion promoters and react with virtually all surfaces to alter the surface characteristics.

Applied in a single stage of the wet finish process, the attachment of this technology to surfaces is made even more durable by the silanol functionality, which enables them to homopolymerize. After they have coated the surface in this manner, they become virtually irremovable, even on surfaces with which they cannot react covalently.

The chemical bonding allows the treated surfaces to become antimicrobially active. Due to this bonding, the antimicrobial does not leach or volatilize from the treated surface.

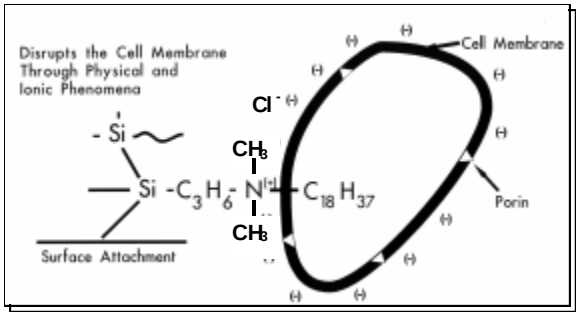

Fig. 2. Cell disruption.

3.5.3. The Antimicrobial Action

On direct contact with a microorganism the technology works by disrupting (or rupturing) the cell membrane. This interrupts the normal life processes and destroys the cell (Fig. 2). Two forces cause the interruption: the quaternized nitrogen acts as an electrocuting charge and the 18 carbon link chain acts as a sword. The chemical nature of the polymer consists of the mass of cationic character created by the quaternized nitrogens and the oleophilibed of 18 carbon link chains. This structure is ideal for taking advantage of the anionic nature and the lipoprotein (fat-like) composition of microbial membranes in a way that, on contact, causes their disruption and the death of the cells.

The unique bonding and killing capacity of the ÆGIS Microbe Shield technology, with its one-two punch, allows it to effectively control an extremely broad spectrum of bacteria, fungi (mold, mildew and yeast), algae, and other one-celled organisms.

Because the ÆGIS Microbe Shield acts only on the membrane and does not lose strength over time, it doesn't create the conditions which allow microorganisms to adapt to its presence or develop resistance.

3.6. The Solution Meets the Need

Air filters are part of the front-line for dealing with microbial problems in our modern buildings. Upgrading efficiency to ASHRAE (Association of Specialists in Heating, Refrigeration, and Air Conditioning Engineers) standards and practices, adding antimicrobial activity were considered critical elements for better indoor air quality. But, until the ÆGIS Microbe Shield Technology was developed, the existing technological, durability, and safety problems for antimicrobial treatments were unsolved.

An antimicrobial agent applied to air filtration media must minimize the growth of microbes on filter surfaces, reduce the chances for microbial grow-through, and should reduce the number of microbial cells in the air stream. Many chemical agents can and do exhibit excellent biocidal and antimicrobial properties, but the critical problem has always been the delivery of those desirable properties without dangerous side effects such as human and animal toxicity problems, minimal effective life, susceptibility to microbial adaptation and resistance, and degradation of filtration efficiency or static pressure characteristics. The ÆGIS Microbe Shield

Technology delivers antimicrobial capabilities and does so in a safe, long lasting way that enhances the filtration capabilities of many substrates.

4. Microbial Growth and Microbial Flow-Through on Air Filtration products

4.1. Introduction

A microbiologist views an air filter as a substrate that has the potential to support microbial growth and as a capture device to reduce the flow-through of microorganisms. The following discussions reflect on experimental observations and theoretical considerations appropriate to understanding microbial growth on untreated air filters vs. air filters treated with the ÆGIS Microbe Shield Technology and on a filter's ability to reduce microbial flow-through.

4.2. Microbial Growth on Air Filter Surfaces

Air filter surfaces, soiled or not, are perfect environments for the growth of many types of microorganisms. Microorganisms need moisture, receptive surfaces, nutrients, and proper temperatures for survival, growth, and reproduction.

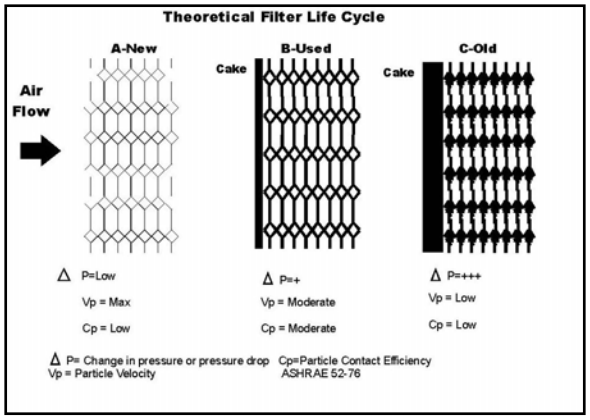

Fig. 3. Theoretical Filter Life Cycle

Moisture regain of various filter media, relative humidity of the air being filtered, the conditions of condensation, and the inevitable presence of the molecular water layer all provide the moisture necessary for fungal growth and, in extremes, provide for bacterial, yeast, and algal growth. Nutrients for growth are provided from finishing compounds, fiber lubricants, binding agents, surfactants, the filter medium itself, and dirt. Figure 3 is an illustration of a filter and provides us a view of how an air filter performs as it ages through use.

"A-NEW" shows a filter with very low pressure drop (P), a condition where particle velocity (Vp) is maximized reducing particle contract time. Filter efficiency is low and particle velocity is high. Since particle size, shape, texture and velocity dictate impact of particles such as microorganisms and their reproductive parts, the path of least resistance will rule these small particles and flow-through of microorganisms would be predicted, even in high efficiency filters.

"B-USED" illustrates a small build-up of dirt cake and a distribution of dirt within the matrix of the filter. Here the pressure drop begins to build, slowing down particles and increasing contact with the filter matrix fibers. As in "A-NEW", the microorganisms are ruled by the pathway of least resistance. This allows particles to move toward less soiled areas and pass through the filter. The filter matrix is loaded at this point, and, because nutrients and growth promoting conditions are present, microorganisms (particularly fungi) will grow on the cake and other soiled areas. This causes odors, staining and deterioration and produces metabolic products and reproductive parts that can cause irritation, sensitization, toxic response, or illness. No longer is the only source of microbes the recycled or make-up air. It is in a large part the filter itself.

"C-OLD" shows us a filter with a significant cake and a very considerable build-up of dirt within the filter matrix. The pressure drop (P) is now high and particle velocity is lowered, thus increasing particle contact. As microorganisms make their way through the cake, they are forced by the path of least resistance to the cleaner areas of the filter matrix and into greater certainty of fiber contact.

4.3. Microbial Flow-Through

Once we understand that filter surfaces, with the right nutrients, temperature and moisture, support microbial growth, we can consider the consequences of such growth and the benefits of the ÆGIS Microbe Shield Technology. The earlier discussion of Figure 3 is instructive when we consider the physical phenomena affecting

flow-through and filter fiber contact. The ability to show benefits centers on whether sufficient contact with treated fibers can be effected to kill the contacting organisms and whether growth rates on the filters can be minimized to reduce flow-through and grow-through from microorganisms.

5. Test Evidence – AFTL Reports

5.1. Filter Performance Tests

There were concerns that applying an antimicrobial to filters might degrade efficiencies, change filtration characteristics or generally adversely alter the original product Air Filter Testing Laboratories, Inc. (AFTL), a leading independent air filter testing facility in Crestwood, Kentucky, performed filter performance tests and biological tests. Filters used for this test series were 24"X24"X2", pleated cotton/polyester blend. Tests were all conducted on pairs of filter specimens and all results averaged for accuracy of the data base.

Table 1
ASHRAE 52-76 Testing

Test Parameters Filter	Treated Filter	Untreated
Initial Resistance	0.24" W.G. (water gauge)	0.33" W.G.
Initial Atmospheric Dust Spot Efficiency	12.7%	13.1%
Arrestance	N/A	N/A
Dust Fed to 1" W.G.	120 g	138 g

These ASHRAE tests, Table 1, have shown that the filters treated with the ÆGIS Microbe Shield Technology have a lower resistance to the air pressure in the standard water gauge (W.G.) test, have slightly less weight gain in the dust spot efficiency test exposed to dust (12.7%) than the untreated control (13.1%), did not affect the arrestance or trapping capabilities (N/A – no affect), and allowed for less dust required (120g versus 138g) to attain 1" W.G. The ÆGIS Microbe Shield Technology enhanced the filter products by adding value and problem solving features that provided excellent antimicrobial effect, making the surface more antistatic, cationic, and hydrophobic. This all shows no negative effects on filter performance because of the treatment.

5.2. Biological Testing: Microbial Flow-Through

The test filters above were placed in test tunnels with airflow of 2000 CFM (cubic feet per minute) for microbial challenge. A BGI Nebulizer® arosolized a culture of *Micrococcus luteus* to impact the test filters. The nebulizer delivered $1.3x10^8$ cells over the test period. Andersen Viable Particle Samplers were placed both upstream and downstream and were fitted with nine retrieval plates of nutrient agar and used to collect microbial samples. The upstream retrievals were done in a pre-trial to determine dose and viability on the test retrieval agar. All plates were incubated and Colony Forming Units (CFU) were counted on a Quebec Colony Counter after 48 hours of incubation. A percent bacterial removal efficiency was calculated comparing the average upstream counts to the average downstream counts.

This procedure was used on new, clean ÆGIS Microbe Shield Technology treated filters. Additional tests were performed on the same filters after they had been ASHRAE dust loaded to 2.5 cm W.G. and 120g dust holding capacity.

Calculation of the flow through of the dust loaded (2.5 cm W.G., 120 g) filter surface: The total filter area was 3600 cm^2, so each 1 cm^2 area of filter surface received 36,111 organisms (130,000,000 / 3600). Results in the AFTL test report show that 247 organisms were retrieved on nine post filter impact retrieval plates representing 706 cm^2 of filter surface area.

Projecting on this rate of retrieval to the total filter area one calculates 1259 organisms as flow-through. (247 organisms retrieved x 3600 / 706)

5.3. Filter Surface Testing

After the filters were challenged with bacteria, the surface was tested for the test bacteria by a sterile swab rinse. This was done to determine a ratio of retained viable organisms versus viable organisms that flowed through the test filters. A 6.25 cm^2 area of the filter surface was used and a serial dilution retrieval was performed on

Table 2
AFTL- Treated Filter Surface Test After Insult[1]

New. Clean filter - untreated	68 CFU/Swab (6.25cm^2)
New, Clean Filter – ÆGIS treated	14 CFU/Swab (6.25 cm^2)
Dust Loaded Filter – ÆGIS treated	16 CFU/Swab (6.25 cm^2)

1. Total insult of *Micrococcus luteus,* 1.3X10^8 colony forming units (CFU).

nutrient agar plates, incubated for 48 hours and counted on a Quebec Colony Counter.

As shown in Table 2, the sterile swab rinse retrieved 2,56 CFU/cm^2 (16/6.25 cm^2 test area) or 9216 CFU/Filter Face Surface Area (2.56), 14 CFU from the New, Clean Filter and 16 CFU from the Dust Loaded Filter. The untreated filter had 68 CFU retrieved.

5.4. Total Microbial Reduction of a Filter Treated with the ÆGIS Microbe Shield Technology

These data provide us a view of the organisms retrieved from the surface and the flow-through organisms [9216 CFU (surface retrieved) + 1259 CFU (flow through retrieved) equal 10,475 CFU(total retrieved)]. These 10,475 organisms are a 4 log reduction, or over 99.99% reduction of the original 130,000,000 organism insult (1.3X10^8/ test surface).

These test results allow for the following observations:

1. The *Micrococcus luteus* was able to travel through the filter media as evidenced by the retrievals, therefore minimizing the chance that the organisms were mechanically trapped. *Micrococcus luteus* averages between 0.9 and 1.8 microns in size.

2. Contact with the treated surfaces does occur and does kill the organisms as evidenced in literature and the baseline runs of this study.

3. Treated filters reduce microbial activity in the dust load, in the filter matrix and downstream of the filter by 99.99% of challenge in AFTL's test protocol.

6. Flow-Through Testing

6.1. Flow-Through Testing – Not Soiled

AFTL ran a test series using a filter treated with the ÆGIS Microbe Shield Technology without a soil load. Results from the treated and the untreated control are listed in Table 3 and data

Table 3
Filter Treated with the ÆGIS Microbe Shield Technology
Flow-Through Reduction of *Micrococcus luteus*[1]
Unsoiled Filter

Plate #	CFU[2] Upstream	CFU Downstream	% Efficiency
I	121	47	61.2
II	146	78	46.6
III	134	69	48.5
IV	154	83	46.1
V	153	94	38.6
VI	199	101	49.3
VII	175	99	43.4
VIII	182	104	42.9
IX	195	92	52.8

Average Bacterial Removal Efficiency 47.7%

1. Tests run by Air Filter Testing Laboratory, Inc., Crestwood, Kentucky
2. Colony Forming Units

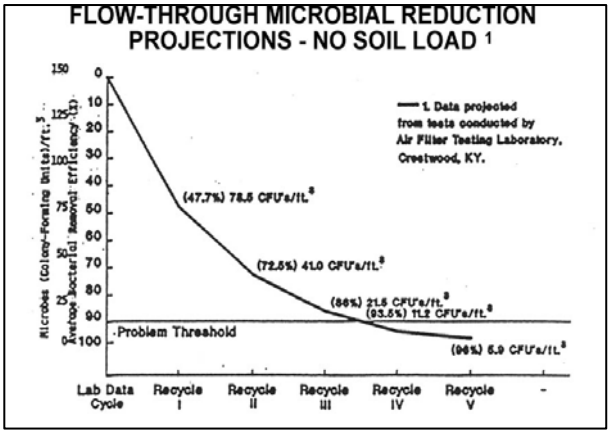

Fig. 4. Flow-through microbial reduction projections – no soil load.

Table 4

Filter Treated with the ÆGIS Microbe Shield Technology

Flow-Through Reduction of *Micrococcus luteus* Soil Loaded Filter

Plate #	CFU2 Upstream	CFU Downstream	% Efficiency
I	210	43	79.0
II	198	28	85.9
III	187	23	87.7
IV	213	20	90.6
V	208	25	88.0
VI	199	38	80.9
VII	203	29	85.7
VIII	221	23	89.6
IX	197	18	90.9

Average Bacterial Removal Efficiency 86.5 (%)
1. Test run by Air Filter Testing Laboratory, Inc., Crestwood, Kentucky
2. Colony Forming Units

projections are shown in Figure 4. The projection of the treated filter's 47.7% reduction to the recycling of air prevalent in most buildings, gives us an exciting view of performance.

In Figure 4 we have illustrated the laboratory test stand data obtained on the clean filter at AFTL. Assuming an initial dose of 150 CFU's/ft^3 of a contaminating organism, we can project further reductions as cycles of the air continue. Using this worst case scenario of microbial reduction, ultimate levels going through the filter are reduced below the 10 CFU's/ft^3 problems trigger level within only four cycles.

6.2. Flow-Through Testing - Soiled

Results of this study are listed in Table 4. The average bacterial removal efficiency on this soil loaded filter treated with the ÆGIS Microbe Shield Technology was 86.5%. The extremes for the nine sample sites were Plate I (79.5%) and Plate IX (90.0%).

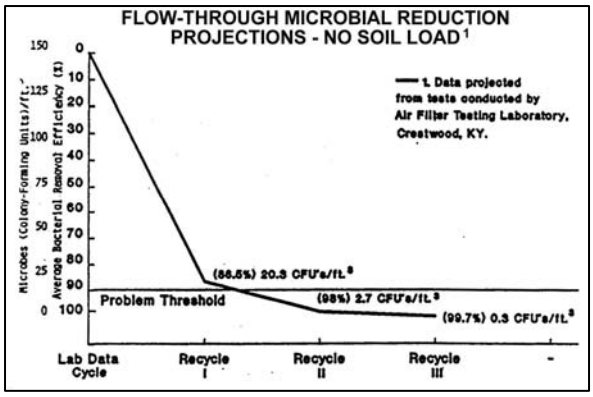

Fig. 5. Flow-through microbial reduction projections – no soil load.

The ability of this filter to reduce the test organism under soiled conditions supports the theoretical consideration of the flow-through of microorganisms seeking cleaner areas of the filter (due to the path of least resistance) where contact with the treated surfaces can occur. These data also support the performance of the antimicrobial as a chemical and physical entity. The radius of influence of the cationic and oleophilic ÆGIS Microbe Shield Technology favors contact with the anionic and lipid outer layer of microorganisms, hence contact and kill.

Figure 5 illustrates the projection for the soil loaded filter data in Table 4 (86.5% reduction in one cycle). Levels below the 10 CFU's/ft3 problem trigger level are reached in less than two cycles.

7. Field Testing

7.1. Phoenix Study

A study was undertaken by St. Luke's Medical Center, Phoenix, Arizona, to determine the effectiveness of the active ingredient of the ÆGIS Microbe Shield Technology when reacted to woven bag air filters against bacterial and fungal contaminants.

Table 5
Imprint Culture ÆGIS Microbe Shield Technology;
Treated and Untreated Bag Filters
St. Luke's Medical Center, Phoenix, Arizona

Sample	Blood Agar	Mueller-Hinton	Total
Treated Filter	9	7	16
Untreated Filter	21	35	56
% Reduction	57	80	71
# Times More Effective	2.3	5.0	3.5

A standardized mixed culture of *Staphylococcus epidermidis, Streptococcus viridens, Escherichia coli* and *Aspergillus niger* was applied to samples of treated and untreated filters. These samples were cut into 25X25 cm squares and incubated for eight hours at 37°C for 5-7 days, read, and the colonies counted. The eight hours was picked by the hospital as it represents a workday. The two agars were chosen to allow maximum growth of the full range of the test organisms.

This overall 71% reduction in a very heavy dose of bacterial and fungal contamination in nutritive carrier solution approaches levels of control seen in other studies. Note that in the growth of the retrieved cultures, none of the fungus (*Aspergillus niger*) was retrieved. A uniform reduction of the three test bacteria was observed. The results show absolute control of the test fungus and from 2.3-5.0 times more reduction of the test bacteria comparing treated to untreated filters.

7.2. Cancer Hospital Study

Two side-by-side mechanically identical air handlers in a 12 story Research and Cancer Hospital were chosen to evaluate the effectiveness at reducing fungal growth on air filter elements and reducing the risk of contamination reaching the service areas (See Fig.6). These systems provided air to the 11th and 12th floor laboratory complexes in a pattern of Air Handler (AHU) 9 providing for one zone on the 12th floor and two zones on the 11th floor; AHU 10 providing for one zone on the 11th floor and two zones on the 12th floor. These units were in operation for approximately 60 days during the commissioning of this facility before the test began. Soil was very light in both units and all physical appearances were the same.

The pre-filters (A), bag filters (B), and post-

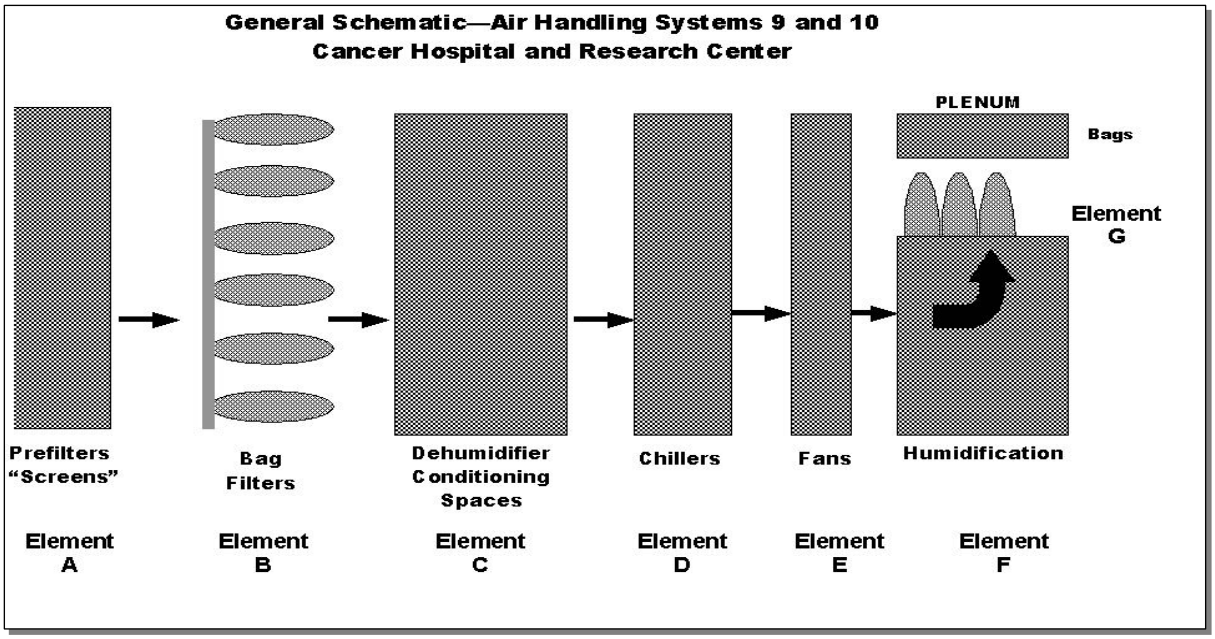

Fig 6. Air Handling Systems 9 and 10. Cancer Hospital and Research Center

Table 6
Cancer Hospital and Research Institute
Filter Treated with the ÆGIS Microbe Shield Technology and Untreated Systems
Observations 30 Days Post-Treatment
The pre-filters (A), bag filters (B), and post-chiller bag filters (G) were all treated with the active ingredient of the ÆGIS Microbe Shield Technology

Air Handling System Element	Observations Thirty Days Post-Treatment	
	Treated	Untreated
A – Screen	No Difference	No Difference
B – Bag Filters	No Growth	Slight Growth
C – Dehumidifier	No Difference	No Difference
D – Chillers	No Difference	No Difference
E – Fans	No Difference	No Difference
F – Humidifier	No Difference	No Difference
G – Bag Filters	Slight Growth	Significant Growth
Post G Pressure	No Change	Increase 0.5" W.G.
Post G Particles	196/m^3	808/ml^3

chiller bag filters (G) were all treated with the active ingredient of the ÆGIS Microbe Shield Technology to the quality control standards of ÆGIS Microbe Shield Quality Control Program. At the end of thirty days, the following observations were made: (Table 6).

The fiberglass filter screens appeared somewhat soiled but no differences between the treated and untreated systems were observed.

The dehumidifier, chillers, fans, and humidifier units showed no differences between the control and the treated systems.

The final bag filters just beyond the humidifiers had noticeable soiling, showed slight growth on the treated bags and significant growth on the untreated systems.

Readings of static pressure showed a significant pressure drop on the untreated filter (0.5" W.G.) whereas the treated filter showed no change from initial readings.

Using a laser particle counter set at 1.0 micron sensitivity, it was determined that the treated filter passed fewer particles (196/m^3) compared to the untreated filter, which passed four times as many (808/m^3).

The post chiller bag filter had a particulate sluffage differential of 300% more particles coming through the untreated filter system than the treated filter system. This in spite of the fact that the untreated filter evidenced a pressure drop of 0.5" W.G. from initial readings; as compared to the treated filter that did not change its pressure drop characteristics. The increased cake and pressure build-up on the untreated system, along with the increase of one micron sized particles and the physical observations of fungal build-up on the untreated system, allows for mildew growth and allows spore sized particles to enter the supply air stream. This field study provides insights into both growth on ÆGIS Microbe Shield Technology filters and untreated filters and on the flow-through consequences of such growth.

8. Conclusions

Air filters in commercial buildings and residences are an essential component in establishing and maintaining indoor environmental quality. Air filters can be a barrier to microorganisms and, at the same time, can be an amplification site for microbial growth.

Test data from laboratory and field evaluations, as presented and referenced in this report, show clearly the utility and appropriateness of the ÆGIS Microbe Shield Technology as part of air filtration systems.

The efficacy of the ÆGIS Microbe Shield Technology has been clearly demonstrated in numerous patents, peer-reviewed publications and trade articles – all showing long-term, broad spectrum control of fungi, algae, and both Gram (+) and Gram (-) bacteria. The efficacy of the filter is greatly enhanced by the chemical bonding of the active ingredient – giving long-lasting effectiveness, and a better performance of the filter. Due to the covalent bonding of the active ingredient, microbiological adaptation becomes highly improbable.

The ÆGIS Microbe Shield Technology has an excellent toxicology and safety profile and has been marketed for years for healthcare products, consumer use products, and products used in commercial buildings. ÆGIS Environments has also established an operational capability at dealing with the broader sources of indoor environmental quality problems attributed to materials of construction, furnishings, equipment, and occupants.[3]

Treatment of a wide variety of substrates and products allows for a broad reduction of microbial habitats and transfer sites leading to greatly improved IEQ.

Data such as those cited above, and the properties of the ÆGIS Microbe Shield Technology allows ÆGIS Environments to bring to the Air Filter Industry – from the manufacturer to the end user – a powerful tool for dealing with one key element in the battle for improved IEQ.

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