Testing Antimicrobials Against Biofilm Bacteria

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There is a need for standard laboratory methods for assessing the efficacy of antimicrobial agents when applied to biofilm bacteria. Existing standard suspension tests and dried surface tests tend to show much greater efficacy than will occur when the agent is applied to biofilms. The greater resistance of biofilm bacteria to antimicrobial agents can be attributed to a number of interacting factors including, reaction and diffusion processes that limit an agent's accessibility to bacteria, phenotypic changes in biofilm bacteria due to stress, and adaptation of the bacteria. Because biofilm systems are so diverse, a variety of biofilm tests will ultimately be required. The new biofilm test methods will necessarily have features that differ in important ways from existing tests. For example, the biofilm test apparatus may include a pump and a continuous flow stirred tank reactor. This paper provides a overview of biofilm testing and suggests a strategy for creating standard test methods.

Biofilms are formed when bacterial cells in a flowing or moist environment attach to an available surface (1,2). The cells multiply and move across the surface, eventually aggregating into small colonies. The colonies grow into an irregular, three-dimensional structure held together by a protective slime, a spaghetti-like mesh of proteins and exopolysaccharide substances (EPS) produced by the bacteria. When the mature community gets overcrowded, chunks of the biofilm break off and float away. Individual cells are also continually eroding from biofilms, and may establish new biofilm communities on downstream surfaces. Bacteria are not the only inhabitants of biofilms (3). Fungi may establish their own territory, as may algae. Protozoans that consume bacteria may feed on biofilms (4). Protozoan (oo)cysts and virus particles can become entrapped in a biofilm and detach later to return to the environment (5).

Recent advances in molecular biology and laboratory instrumentation have provided the tools to show that a biofilm is not just a random collection of cells stuck to a surface, but rather, it is a dynamic, organized, cooperative community (6-8). Each bacterium possesses a genetic plasticity that allows it to alter its phenotypic state in response to its immediate environment (9-11). As a result, bacteria in biofilms are of very different phenotypes than free-floating bacteria (12,13).

In the real world, the vast majority of bacterial activity does not occur in liquid suspensions of dispersed planktonic organisms – it occurs in biofilms (14). Of all bacterial activity in an open ecosystem, 99% is in biofilms on surfaces (15). The importance of biofilm processes is now well-established in environmental microbiology (4,15-18). Biofilms can have detrimental effects. Of particular importance are biofilms occurring in environments that have been altered or built by man. For example, biofilms can clog and corrode pipes and filters, contaminate food-processing equipment, foul the surfaces of computer chips, coat the surfaces of

drinking water distribution systems, and contaminate swimming pools (19). Biofilms can be costly; e.g., an 18% difference in power consumption was observed in trials to determine the effect of biofilm removal from ship hulls (20). Worldwide, industries spend $7 billion (U.S.) annually on chemicals for controlling bacterial biofilms (21).

Biofilms are increasingly cited as sources of infection and disease in humans (22,23). Dental plaque, respiratory infections, stomach ulcers, arteriosclerosis, kidney stones, ear infections, prostatitis, and numerous other microbial-caused ailments have been associated with biofilms (6). Artificial joints, or other devices placed in the human body, are particularly susceptible to bacterial colonization. Roughly 5% of the patients who annually receive catheters and stents develop serious infections from biofilms growing on the devices (21). Biofilms can lead to either local infection or infection at other anatomical sites due to slime-coated clumps of bacteria that detach from the original biofilm. It has been estimated that the majority of the human bacterial diseases treated in the past few years are actually biofilm infections (23) and that treatment of these biofilm-based infections in the US costs more than $1 billion annually (24).

Food, water, and medical industries are greatly concerned about the potential of biofilms to act as pathogen reservoirs (25). Biofilms can increase the infectivity of the microbes because the immune system cannot easily destroy slime-protected clumps (6). For example, respiratory disease, such as Legionnaire's disease, can occur when people inhale aerosols containing microscopic clumps of detached biofilm bacteria (22). If you are interested in reading more about biofilms, a list of recent books is available on the web at http://www.biofilmsonline.com/VIEWS/MediaReviews/.

To help prevent bacteria-caused diseases, a wide variety of germicides and antibiotics have been produced. Most of the products were created to kill either planktonic bacteria, which are

disassociated cells suspended in a liquid, or dried surface bacteria, which are disassociated cells bound in a thin dried organic film on an inanimate surface. Unfortunately, chemicals that kill planktonic bacteria or surface-dried bacteria may well be ineffective against biofilm bacteria. The recent realization that biofilms significantly affect health, coupled with research advances that show how biofilms form and survive, has led to novel treatments created specifically to control biofilm bacteria (1). Scientists and engineers are now engaged in a major research effort to determine which treatments are effective at controlling biofilm bacteria in various important medical and environmental systems. The products of their research will eventually be presented to regulatory authorities and consumers. Convincing efficacy data will be a critical component of the presentation. The data will be convincing if they are based on accepted, reliable laboratory methods. Although there has been substantial progress toward the development of biofilm tests (17,26), no standard biofilm test methods exist at present (27,28).

The purposes of this article are to explain why biofilm testing is difficult, but feasible, and to present a general strategy for developing biofilm tests. The focus will be on quantitative tests (29). Biofilm tests comprise four basic components: growing a biofilm, treating the biofilm, sampling, and analysis. This article reviews the following special problems that arise when testing biofilms: (i) biofilms are resistant to treatment, (ii) biofilms may require special measurements and novel sampling plans, (iii) laboratory biofilm systems utilize engineering, as well as microbiological, concepts, and (iv) it is challenging to devise a laboratory test that is both relevant and feasible.

Antimicrobial Resistance of Bacteria in Biofilms

The basic antimicrobial test systems include: planktonic assays that emulate treatment of recreation water, drinking water, etc.; dried surface assays that emulate the treatment of a liquid

spilled and dried on a non-porous surface such as a floor or counter top; and various biofilm assays that emulate treatment to prevent biofilm formation, treatment to remove an existing biofilm, or treatment to kill microbes in a biofilm. In general, a germicide will be most effective in the planktonic assay, less effective in the surface-dried assay, and least effective in the biofilm assay (30,31). Microbes in biofilms are remarkably resistant to all kinds of antibacterial challenges (24,32,33)}. It can take 100 times, occasionally a 1000 times, the concentration of an antibiotic or germicide to achieve the same kill of biofilm bacteria as observed for planktonic bacteria (14).

Biofilms typically comprise many layers of bacteria, and those layers can contribute to the resistance of biofilms. For example, reactive antimicrobials (consider chlorine in the form of sodium hypochlorite) can be partially neutralized by reaction with the EPS and the outside layers of bacteria. Such neutralization effects are unlikely to affect suspension tests because there is little EPS to react with the chlorine and the glassware is pretreated to eliminate potential chlorine demand. Therefore, the suspended bacteria are attacked by the full chlorine concentration.

The structure of the biofilms, may contribute to resistance by inhibiting diffusion of the antimicrobial into them (34). Diffusion is not a consideration in suspension tests and diffusion is of little concern in surface-dried tests where the bacteria are sparsely distributed in a monolayer on the surface.

Reaction and diffusion effects may also act on nutrients required by the bacteria. Consequently, bacteria in the middle of microcolonies may not have ready access to nutrients, and they may exhibit a stress-induced phenotypic change that makes them resistant to antimicrobials (35). Stressed bacteria in biofilms may evade antibiotics by changing up to 40% of the proteins that make up their cell walls (21).

Biofilm bacteria may exhibit resistance because they have adapted to the antimicrobial, especially if the bacteria are located at protected positions where they receive prolonged exposure to sublethal concentrations (27,28). Compared to free-floating bacteria, bacteria in biofilms can much more readily exchange plasmids. This exchange of genetic material is stimulated in biofilms by the quorum-sensing signaling molecules, and it can lead to rapid spread of antimicrobial resistance throughout a biofilm community (14). To view figures that illustrate various resistance mechanisms, see the web site http://www.erc.montana.edu/CBEssentials-SW/research/Antimicrobials/.

Choosing the Response Measurement

Measuring Treatment Efficacy

Conventional antibacterial tests assess the ability of the treatment to kill bacteria. The conventional efficacy measure is based on comparing the viable cell counts after administering the antibacterial to the viable cell counts before treatment. However, biofilm control strategies may be directed at objectives other than killing the bacteria. Unlike suspension tests and dried surface tests, there are many different quantities that could be meaningful responses in a biofilm assay. Consider these alternative strategies for controlling biofilm bacteria: (i) pretreating surfaces so they resist biofilm formation, (ii) treating the bulk water to make the planktonic bacteria incapable of attaching to surfaces and building biofilms, (iii) removing established biofilm, and (iv) killing biofilm bacteria (i.e., disinfecting surfaces in the system). "Antibiofilm" is a generic expression that pertains to any or all of these treatment goals. For each of the four treatment strategies, a representative response measure will now be presented.

Consider strategies (i) and (ii). Pretreated, colonization-resistant surfaces have been constructed by impregnating the surface material with chemicals, such as active antibacterial

agents, metal ions, or cell-cell signaling molecules. For example, vascular catheters impregnated with antibiotics show reduced bacterial adherence and biofilm formation (36). There is evidence that signaling molecules, or analogues of such molecules, can be used to prevent surface colonization in marine systems (37). Alternatively, chemicals may be added to the bulk water to kill or affect the bacteria so they are less capable of forming biofilms in the system. For example, the bulk water concentrations of biocides commonly used in drinking water distribution systems and swimming pools can reduce the rate of biofilm growth, although biofilms do occur. When assessing a treatment's effectiveness at inhibiting biofilm formation, the response measurement could be the number of attached bacteria per surface area at a fixed time point. Such counts are often based on microscope images of the surface using a level of magnification that clearly shows individual bacteria (38). One could record the counts at multiple time points and use the biofilm growth rate as the response (39). Other measures, such as total protein per cm^2, may be both appropriate and less expensive (40).

For treatment strategy (iii), chemicals are applied to cause established biofilms to detach; that is, it cleans the surface by removing biofilms so they can be flushed out of the system (40). Removal treatments are used, for example, to improve fluid flow in a pipeline, to improve heat transfer in a water cooling tower, or to clean the surfaces of dental unit water lines. The appropriate quantitative response may be nonbiological; e.g., measurement of the improvement in fluid flow or heat transfer attributable to the treatment (41,42). On the other hand, it may be relevant and efficient to measure a biological quantity such as the total biomass density on the surface.

For treatment strategy (iv), germicides or antibiotics are administered for the purpose of disinfecting the surface. The most commonly used quantitative response is viable cell counts per

surface area. The counts can be obtained in various ways; e.g., using conventional methods where the biofilm bacteria are extracted from the surface and counted by conventional techniques (43) or using new *in situ* methods where the bacteria are stained or labeled and computer image analysis software extracts relevant counts from a microscopic image (44-46). When a biofilm has been knocked down by a short-term or shock treatment, it may be important to observe the rate of biofilm regrowth and record how long before the treatment has to be administered again.

No matter what the immediate goal of the antibiofilm treatment, the practical value may be in reducing the number of viable cells in the bulk water. If so, then the appropriate quantitative response may well be the density of viable bacteria in the bulk water. For example, the efficacy of antimicrobials in drinking water, swimming pools, and hot tubs is measured by viable cell densities in the bulk water (47). A related goal is treatment to prevent aerosolization of bacteria clumps, thereby reducing the risk of respiratory disease. For this goal, the response will be based on enumeration of bacterial particulate in air samples (48).

For the remainder of this article, I will focus on surface disinfection where the response is the density of viable cells (count per cm^2) on the surface. However, the general principles under discussion apply to any treatment strategy for which there is an appropriate quantitative response.

Sampling and Data Analysis

Biofilms can exhibit important temporal and spatial heterogeneity. The biofilm structure is elastic. It expands and contracts in response to mechanical forces such the shear stress induced by the flow of water over the biofilm. The biofilm may be immobilized on the surface, or it may slowly flow along the surface exhibiting the characteristics of a viscous material (49,50). Bacterial colonies, encased in EPS, can grow into mushroom-shaped structures (e.g., colonies of width equal to a few hundred cell diameters), perhaps separated by areas of little or no biofilm. A

colony can suddenly detach and leave a hole where there was once a colony (51). To see movies of biofilm dynamics, visit the Biofilm Movies web site http://www.erc.montana.edu/.

The typical biofilm system operating under a stable set of conditions will reach a "steady state;" that is, when averaged over the whole biofilm, the measurement of interest changes little over time. Steady state occurs when the immigration plus growth rates are equal to the death plus detachment rates. It may take days, weeks, or months for a laboratory biofilm to reach steady state, depending on the bacterial species and growth conditions (43,52). For many purposes, the treated and control biofilms can be compared at steady state. Experimentation during development of the testing protocol should be able to establish the appropriate time to sample.

The spatial heterogeneity of a biofilm diminishes as one views larger and larger areas. In order to gather a statistically representative sample of the biofilm, it is necessary to sample a large enough surface area and to sample at more than one location. For measurements such as viable cell density (cfu cm^{-2}) or total cell density (number cm^{-2}), two or three samples of approximately one cm^2 each may be sufficient (43,53). For each new growth protocol, a sampling experiment should be conducted to determine the appropriate sample area and number of samples.

One popular method of sampling is based on coupons that are embedded in the surface, or otherwise placed at appropriate locations in the biofilm reactor, before the experiment begins. At the designated observation times, coupons are extracted and the biofilm on each coupon surface is analyzed. This approach is very similar to the use of carriers when conducting dried surface tests.

There will be some random measurement error affecting the analysis of a single coupon and it will be necessary to decide how much effort should be directed at minimizing the measurement variance. One conventional microbiology method for viable cell determination

involves removing the biofilm from the coupon, disaggregating the bacteria in a well-mixed suspension, creating parallel dilution series, making up spread plates at each dilution, incubating the plates, and counting the colonies on each plate at the appropriate dilution. Two plates per dilution in a single dilution series usually provides sufficiently precise measurement of a coupon.

The biofilm growth protocol is of little value as part of a standard testing protocol unless it provides repeatable results. The development work must include complete repeats of the growth experiment. The replicate experiments should be conducted in a way that allows for estimation of the components of variance for the most important biofilm measurement. For example, one should be able to partition the total variance among repeats into the variance between days, the variance between reactors on the same day, the variance between coupons within the same reactor, and the variance between spread plates for the same coupon. The final protocol can then be chosen to minimize the total variance without increasing the cost of the test.

Differences between reactors is a major source of variability (52). If so, the protocol should require multiple reactors and expend less effort on measurements within each reactor, rather than requiring just one reactor that is measured intensely (many coupons with many dilutions series and spread plates per coupon).

Laboratory Biofilm Test Apparatus

Many of the sampling and analysis issues reviewed in the previous section are similar to those encountered in suspension or dried-surface tests. The novel aspect of antibiofilm testing is the biofilm reactor system. The batch systems used to grow populations of planktonic bacteria for use in suspension tests and dried surface tests have limited relevance to the biofilm world. In the field, biofilm bacteria have continuous or periodic access to nutrients. Many new antibiofilm efficacy tests will use a continuous-flow stirred tank reactor (CSTR) chemostat as the relevant

environment for growing biofilms. As conventionally used, a CSTR is a beaker (one liter, say) with continuous inflow and a drain constructed so that the well-mixed liquid volume is kept constant (at 500 ml, say). The inflow contains nutrients for supporting bacterial population growth. The reactor is inoculated with bacteria at the start of the experiment. Because the environment in which the biofilm grows can be rather harsh compared to usual culture conditions, it may be important to acclimate the bacteria to that environment before they are inoculated into the reactor. The reactor may sit in batch mode for a short period of time (24 hours, say), then flow commences.

The system should be operated aseptically to prevent contamination. In the typical system, all inflow liquids are sterile. However, the species being tested can be added to the inflow, thereby creating a periodic or continuous inoculum. The rate at which the nutrients are added to the reactor is referred to as the dilution rate. The dilution rate controls the rate of bacterial growth (54). A biofilm will form on surfaces within the liquid, including coupons that have been placed in the reactor. Above a critical dilution rate, when nutrients are flowing through the reactor faster than the bacteria can divide, suspended bacteria are flushed out of the reactor. Operating a chemostat at a high dilution rate therefore selects for biofilm growth. The CSTR setup requires at least one pump and a stirring mechanism. It may be necessary to place a flow break on the inflow line to keep biofilms from growing up that line where it could contaminate the nutrient source.

The research literature contains many novel biofilm growth systems other than the CSTR that may be suitable for use in a standard testing method. For example, the Calgary Biofilm Device, which is a microtiter plate version of a CSTR where the stirring is accomplished by placing the plate on a shaker (55,56), the flow cell (51), the artificial biofilm system (57), the

colony biofilm system (58), and the drip flow reactor (59). The biofilms in these systems exhibit some of the same resistance to disinfection as seen in natural biofilms. In general, the simpler the laboratory system, the less it is relevant for efficacy testing, although it may have great potential for in-house screening.

Relevance of a Laboratory Biofilm Test

In order for the reactor to be a reasonable surrogate for the targeted real world system, it should be a properly scaled down version of the real system. Because factors, such as surface to volume ratio and shear stress from fluid flow, should be calculated and controlled, the design of a biofilm reactor system involves engineering principles as well as microbiology. For all these reasons, one can expect that an antibiofilm test will be more expensive and challenging to run than suspension or dried surface tests.

Laboratory biofilms used in efficacy testing must exhibit approximately the same types and degrees of resistance as exist for real-world biofilms. In all likelihood, the targeted application will require a special biofilm reactor system and growth protocol. Consequently, the test development process may require some preliminary field studies to produce critical information about real-world biofilm characteristics such as species, surface materials, nutrient sources, nutrient concentrations, growth conditions, water chemistry, biofilm growth rate, steady-state biofilm thickness, and antimicrobial resistance.

The most challenging aspect of methods development is balancing the conflicting constraints of relevancy and feasibility. It is not feasible to create an exact real-world environment in the laboratory. The laboratory system needs to emulate only the critical factors. The determination of those factors is guided by expert knowledge and experience, and a series of well-designed experiments. The final laboratory protocol should be relatively easy, inexpensive,

and not require rare equipment or highly specialized technicians. Biofilm growth and treatment in the laboratory should be accelerated relative to the real world. For most applications, it should be possible to create an efficient test that is sufficiently relevant to the real world system. Relevancy, however, cannot rely simply on expert judgment and good intentions; it must be checked. The key criterion for relevancy is that the lab and field experiments produce similar concentration-response curves. For example, in a study of the efficacy of chlorine as a biofilm growth inhibitor in toilet bowls, parallel experiments were conducted in the lab and in the field (52,53). Because at two chlorine levels the log reduction values observed in the laboratory CSTR reactor were essentially identical to the values observed in real toilets, the relevance of the laboratory test was not discredited.

A Strategy for Developing an Antibiofilm Test

The process of developing an antibiofilm test begins with a description of the real world application for which the test is being developed. Next, the investigators gather all available data on that application. If there is a scarcity of information, the investigators may need to perform their own field study. Then the laboratory work can begin. The development team uses the literature and some imagination to select promising protocols for (i) growing the biofilm, (ii) treating the biofilm, (iii) sampling the biofilm, and (iv) analyzing the samples. The test must exhibit the qualities of any good experiment, including appropriate controls, adequate replication, and the use of randomization and blind analysis whenever there is potential for bias.

A major milestone is reached when the investigators produce an efficient, relevant protocol for growing a repeatable biofilm. Many preliminary experiments may be required to arrive at that protocol. A systematic approach is to start with a highly controlled, perhaps over-designed, laboratory system. The more characteristics that are controlled to match the real world,

the more relevant the lab system should be, and the more costly. Then the laboratory system is systematically simplified until the biofilm is affected in some important manner. The precise point where the protocol can be simplified no further is determined via a combination of expert judgment and experimental data. After the investigators settle on an efficient protocol, replicate experiments are conducted to show that the key biofilm characteristic (e.g., viable cell density or average biofilm thickness or total biomass density) has an acceptably small standard deviation across independent trials, thereby establishing repeatability.

The antibiofilm treatment must be applied in the lab test in a way relevant to the field application. If one is studying pretreated surfaces, then the test coupons in the reactor should be pretreated and the control coupons should be identical to the test coupons except for the pretreatment. If the treatment is applied continuously to the bulk water to prevent biofilm growth, then there must be treated reactors and untreated reactors. If the treatment is to be applied to an existing biofilm, then the biofilm should be grown in the reactor until it reaches the state where treatment is specified. The treatment could be applied to the whole reactor, or just to coupons extracted from the reactor, whichever is more relevant. In some cases, the time of exposure to the treatment needs to be controlled, in which case some effective neutralization is required. The neutralization step can pose a difficult problem because the neutralizer may not be able to penetrate biofilms immediately. Another problem can arise if the treatment affects the water chemistry (e.g., pH) more in the small reactor system than would occur in the real system. In this case, some appropriate adjustment of the chemistry (e.g., the addition of a buffering agent) may be required.

Sampling and analysis have been discussed above. There are two problematic issues to emphasize, how to remove biofilms from coupons or other surfaces and how to disaggregate the

biofilms prior to applying conventional microbiological assay techniques. These steps require special evaluation to be sure that they are not introducing any bias. The bias evaluations during development of the test may require sophisticated microscopy or specialized equipment (23). The purpose for testing will dictate whether routine checks by such expensive techniques need to be part of the standard protocol.

A statistical analysis of the data generated during development of the laboratory test is required to provide convincing results . When the measurement is based on viable cell densities or on total cell densities, the log reduction is an appropriate summary statistic. To calculate the log reduction, I recommend the mean of log densities approach (43,60,61). The efficacy measure should be accompanied by some measure of uncertainty such as a standard error or confidence interval. It is imperative that the uncertainty measure include all relevant sources of variability, especially the variability among independent repeats of the test. A recent literature review showed that the median repeatability standard deviations ranged from 0.2 to 1.2, with a median of 0.5, for a selection of standard germicide tests (62). One antibiofilm test method has produced a repeatability standard deviation that is less than 0.7, thereby being acceptably repeatable compared to other germicide tests (43,52).

The test should be applied both to different concentrations of an established antibiofilm treatment (positive control) and to some inactive treatments (negative controls). It is imperative that the test method possesses enough sensitivity to detect an important concentration-response relationship and sufficient specificity that the negative controls are not falsely shown to be effective.

After the complete test protocol has been written up and nominated as a standard, there will be a period of peer review and evaluation. It will be necessary to conduct a collaborative

study to establish both the reproducibility of the test results for some standard antibiofilm treatments and the sensitivity to detect a concentration-response relationship. If the log reduction in viable cell density is the chosen efficacy measure, then a reproducibility standard deviation around 1.0 should be acceptably small (62). In addition, it will be necessary to conduct parallel laboratory and field experiments to check the relevancy of the protocol.

The careful development of a standard efficacy testing method requires more than a few person-months of effort. Such development is best done as a supported and focused research task, the opportunity of success being reduced if it is treated as a "spare time" project.

It may not be feasible to arrive at as tight a protocol for an antibiofilm test as is expected for suspension tests or dried surface tests. There may be steps that one should calibrate to each specific field condition; e.g., choosing the appropriate shear stress to match the real world system. Ideally, experimentation would show that the adjustments do not degrade the repeatability and relevancy of the test. Ruggedness testing during development, in particular, should elucidate the effects of anticipated variations in the test conditions and parameters. Although it would be wasteful to perform an exhaustive set of experiments for each small adjustment to the protocol, some check on the effects of the adjustment is prudent.

Applying a New Standard Method

Surveillance Database for Standard Methods

It would be wise for all parties interested in standard antibiofilm tests to cooperate in establishing a dynamic surveillance database. For each use of a standard test, the user would enter into the database all operating parameters, any alterations to the basic protocol, and all nonproprietary results. The results would include the statistical characteristics of controls and of associated nonproprietary treatments, which are often used as positive controls. Periodic queries

of the database, and the appropriate statistical analyses, would yield updated information on the repeatability, reproducibility, and relevance of the protocol. Because one can use the internet for entering data and submitting queries, a dynamic surveillance database is entirely feasible.

Performance Standards

In an effort to protect the public from infectious disease, governmental agencies have promulgated rules and regulations for treating and testing drinking water, air conditioning systems, etc. A new treatment must pass specific laboratory test performance standards in order to gain official acceptance. Most of the existing policies were based on expert opinion or precedent, not on scientific studies or quantitative risk assessment. In the future, one can expect that performance standards will depend increasingly on microbial risk assessment for which a body of literature is evolving (63-68).

Because there are not yet any standard biofilm tests, there are no performance standards for antibiofilm agents. The quantitative microbial risk assessment literature completely ignores biofilms. Consequently, a major research effort will be required to develop a rigorous regulatory framework of standard tests and performance standards for antibiofilm treatments.

Conclusion

A biofilm is a complex, dynamic, self-organized microbial community. Medical and environmental studies show that biofilms have significant detrimental effects on health and productivity. Antibiofilm agents are being developed but researchers do not have an array of accepted laboratory tools to use for testing the agents. There is an obvious necessity for standard antibiofilm test methods and accompanying scientifically defensible performance standards. It is feasible to devise tests that have sufficient sensitivity and specificity for practical use. In order to achieve the same repeatability and reproducibility as existing suspension and dried surface tests, the new antibiofilm tests will be more expensive in both time and materials. A major research initiative is required to provide a quantitative microbial risk assessment basis for performance standards in antibiofilm testing.

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