



Certificate of Microbiological Analysis for Samples Submitted by:

Mr. Braden Schrum  
Beco Concrete  
St. Louis, MO

Client Project: **"Seal-it"**  
Report Date: 9/14/2009

*BIOBELT REPORT: 9198*

Project Name: “Seal-it” sealant  
Client Email: qcmail@becoconcreteprod.com  
Phone: 314-892-7400  
Fax: 314-446-5820

Report Date: September 14, 2008

Condition of Sample(s) Upon Receipt: Acceptable

Laboratory Receipt: Friday, July 24, 2009: Received by client delivery, request to participate the “Seal-it” liquid antimicrobial product for in-vitro study of application to concrete products using **ASTM method D4783-01** modified to be applicable to the liquid product and distinct concrete specimens

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### Study Principle

This method is designed to determine the resistance of liquid adhesive preparations to microbial attack in study containers by challenging the liquid specimens with known concentration of bacteria, yeast and mold. This method may be modified to suit the specific applications of the product for different commercial applications. Specifically, this method has been modified from assessing the antimicrobial qualities of the liquid samples testing in glass culturing containers to addressing the antimicrobial qualities of the liquid product when applied to the surfaces of concrete products and determining the viability of applied microorganisms after a series of exposure times to the liquid product.

Exposure times tested are determined according to method after exposure to the treated concrete product at 4hrs, 24hrs, 48hrs, 72hrs and after 7 days. At each time interval a sterile swab is pre-moistened with sterile distilled water and swabbed across the surface of the treated concrete to collect any viable microorganisms. The size of the area being tested on the concrete cylinder is 3 square inches for each test interval. This swab is then streaked to the appropriate selective growth media depending on the species being tested for. In addition to one concrete product being tested for viable growth of the bacterial blend and one concrete product being tested for viable fungal growth, a third concrete product will remain untreated by the liquid product as well as being unchallenged by any additional microbial suspension. This will represent a control concrete product to be used as a comparison where typical microorganisms already present (if applicable) on the concrete product will be evaluated.

### Test microorganisms

The following microorganisms were selected for use in this study based on those suggested in the ASTM method vs. the use of the concrete products when used in commercial settings and the possibility of exposure of listed microorganisms to the concrete products. The results obtained when using these procedures given in this method apply only to the species which are used for the testing.

Two microbial suspensions were created for testing against the liquid product. One suspension is a blend of two bacteria commonly found in the environment and therefore a possible contaminate to attack the concrete product in the environment in a commercial situation. Included in the bacterial blend are:

***Pseudomonas aeruginosa* (ATCC 10145)**. An opportunistic pathogen common in environmental soil and groundwater.

***Proteus vulgaris* (ATCC 9920)**. A bacteria of the coliform group, present in the environment but also common in sewage as a result of fecal matter of warm blooded animals.

In addition a fungal blend of microorganisms was created consisting of:

***Candida tropicalis* (ATCC 750)**. A yeast type of fungi, part of normal human microflora but also recognized as an opportunistic pathogen.

***Aspergillus niger* (ATCC 9642)**. A filamentous fungi common in the environment and opportunistic pathogen.

### Concrete Product Sampling Schedule and Summary of Testing Analysis

Three distinct challenges of microbial attack on the concrete products over a period of 5-6 weeks are evaluated. For each distinct challenge, a known concentration of bacteria blend and separately, a fungal blend are applied to the concrete product and the test for viable microorganisms collected and cultured. Following the reception of the “**Seal-it**” product, delivery of concrete cylinders and reception of purchased authenticated microorganisms, the product was applied to the cylinders on July 7, 2009 and allowed to incubate at room temperature for 8 days before the first challenge was conducted.

First Challenge setup 8/4/2009				
Initial Microbial Concentration (cfu/mL)		Bacteria Blend	2.5 x 10 <sup>8</sup>	
		Fungal Blend	1.2 x 10 <sup>7</sup>	
Exposure Time	Viable Bacteria	Viable Fungi	Control Concrete Cylinder (untreated)	
4hrs	No Growth	Growth	Growth	Bacteria and fungi present
24hrs	No Growth	Growth	Growth	Bacteria and fungi present
48hrs	No Growth	Growth	Growth	Bacteria and fungi present
72hrs	No Growth	Growth	Growth	Bacteria and fungi present
7 days	No Growth	Growth	Growth	Bacteria and fungi present

### First Challenge Summary

The results of the first microbial challenge show that after an adequate level of microorganisms for each blend were applied to the test concrete cylinders, The liquid product was effective at killing the bacteria applied to the concrete product at each of the time intervals being tested and can be determined to be **resistant to microbial attack on the cylinder**. The treated cylinder was able to return to sterility within the first 4hr time frame.

The liquid product was not effective at killing the fungal blend of microorganisms applied to the cylinder at any time interval tested therefore, the liquid product can be evaluated as **not resistant to attack by the applied fungal blend**.

The control concrete cylinder which was untreated with the “**Seal-it**” product and not inoculated with any deliberate microbial blend exhibited the presence of indigenous, common environmental fungi and bacteria already present on the cylinder.

Second Challenge setup 8/18/2009				
Initial Microbial Concentration (cfu/mL)		Bacteria Blend	2.1 x 10 <sup>8</sup>	
		Fungal Blend	1.3 x 10 <sup>7</sup>	
Exposure Time	Viable Bacteria	Viable Fungi	Control Concrete Cylinder (untreated)	
4hrs	No Growth	Growth	Growth	Bacteria and fungi present
24hrs	No Growth	Growth	Growth	Bacteria and fungi present
48hrs	No Growth	Growth	Growth	Bacteria and fungi present
72hrs	No Growth	Growth	Growth	Bacteria and fungi present
7 days	No Growth	Growth	Growth	Bacteria and fungi present



**Second Challenge Summary**

As was determined in the first challenge, the results of the second microbial challenge show that after an adequate level of microorganisms for each blend were applied to the test concrete cylinders, The liquid product was effective at killing the bacteria applied to the concrete product at each of the time intervals being tested and can be determined to be **resistant to microbial attack on the cylinder**. The treated cylinder was able to return to sterility within the first 4hr time frame.

The liquid product was not effective at killing the fungal blend of microorganisms applied to the cylinder at any time interval tested therefore, the liquid product can be evaluated as **not resistant to attack by the applied fungal blend**.

The control concrete cylinder which was untreated with the “Seal-it” product and not inoculated with any deliberate microbial blend exhibited the presence of indigenous, common environmental fungi and bacteria still residing on the cylinder.

<b>Third Challenge setup 9/1/2009</b>			
Initial Microbial Concentration (cfu/mL)		Bacteria Blend	1.8 x 10 <sup>8</sup>
		Fungal Blend	6.1 x 10 <sup>7</sup>
<b>Exposure Time</b>	<b>Viable Bacteria</b>	<b>Viable Fungi</b>	<b>Control Concrete Cylinder (untreated)</b>
4hrs	No Growth	Growth	Growth Bacteria and fungi present
24hrs	No Growth	Growth	Growth Bacteria and fungi present
48hrs	No Growth	Growth	Growth Bacteria and fungi present
72hrs	No Growth	Growth	Growth Bacteria and fungi present
7 days	No Growth	Growth	Growth Bacteria and fungi present

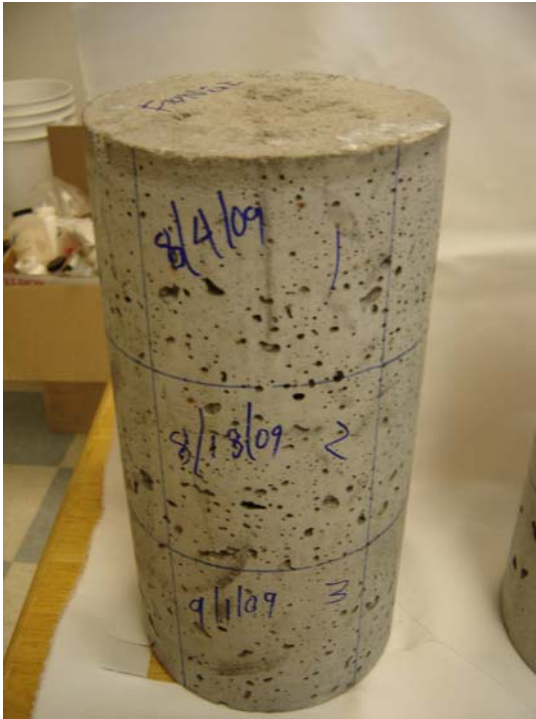
**Third Challenge Summary**

As was determined in the first and second challenge, the results of the second microbial challenge show that after an adequate level of microorganisms for each blend were applied to the test concrete cylinders, The liquid product was effective at killing the bacteria applied to the concrete product at each of the time intervals being tested and can be determined to be **resistant to microbial attack on the cylinder**. The treated cylinder was able to return to sterility within the first 4hr time frame.

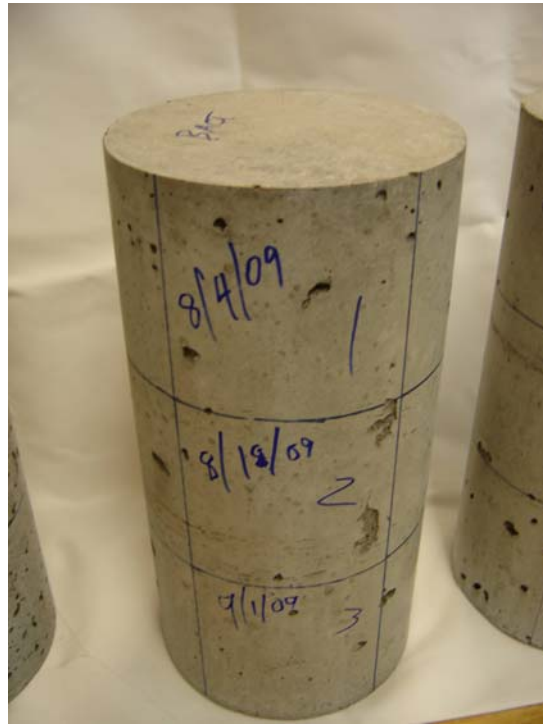
The liquid product was not effective at killing the fungal blend of microorganisms applied to the cylinder at any time interval tested therefore, the liquid product can be evaluated as **not resistant to attack by the applied fungal blend**.

The control concrete cylinder which was untreated with the “Seal-it” product and not inoculated with any deliberate microbial blend exhibited the presence of indigenous, common environmental fungi and bacteria still residing on the cylinder.

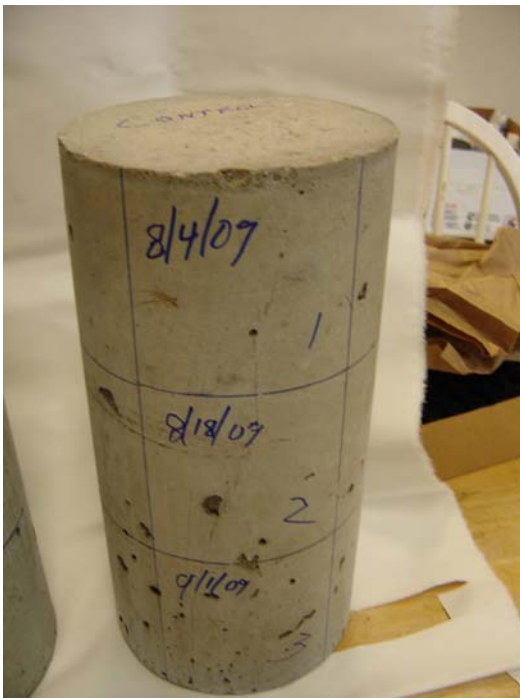
Confirmations of acceptable results were represented by acceptable control standards of sterility for diluents, neutralizers and control suspensions.



**Fungal Test Cylinder**



**Bacteria Test Cylinder**



**Control Test Cylinder**



From the staff at Biobelt Laboratories, we would like to thank you for the opportunity to assist you with your microbiological testing needs.

A handwritten signature in black ink, which appears to read "Kirk Hartwein".

Kirk Hartwein, B.S.  
Laboratory Director