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**Microbac Laboratories, Inc.**

**Venice Division**

115 Corporation Way, Unit F

Venice, FL 34285

(941) 484-6508

**Research Project for Zimek Technologies, LLC**

Main Office: Roy Beckett, Chairman and CEO, Zimek Technologies Management Company, 13537 Granville Avenue, Clermont, FL 34711.

Branch Office: David Sparks, Executive Vice President of Research and Development, Zimek Technologies Management Company, 12713 Thonotosassa Road, Thonotosassa, FL 33592.

General Counsel: Kurt Grosman, General Counsel and CFO, Zimek Technologies Management Company, c/o The Grosman Practice, 5043 Winwood Way, Orlando FL 32819

Initiation of Project: March 6, 2006

Report Date: March 13, 2006

**Background Information:**

Mr. David Sparks, Executive Vice President of Research and Development for Zimek Technologies Management Company, contacted Microbac Laboratories, with an interest in determining, scientifically, the effectiveness of the micro environmental sanitation machine produced by Zimek Technologies, LLC (“Zimek”) which produces negatively charged micro particles in delivering the microbicidal Zimek QD Disinfectant wherever air flows. Zimek Technologies Management Company is the Manager of Zimek Technologies, LLC. The initial study was done in the Microbac Laboratory February 21, 2006 and is reported March 1, 2006. The second study was done in the Microbac Laboratory February 21, 2006 and was reported March 8, 2006. This third research project is a study of the effectiveness of the Zimek QD fungicide when sprayed directly on mold cultures.

Field technicians of Zimek will be using micro particle generators to eliminate mold spores, invisible to the eye, from the air and from horizontal and vertical surfaces. Visible mold colonies will be saturated with spray from a hand sprayer. Standard sprays of 240 microns fall ten feet in six seconds and do not create a fog to kill spores in the air and all vertical and horizontal surface as do the micro particles of 1 micron size.<sup>1</sup> Standard sprays work very well for direct application to visible mold colonies.

**Preparation of Fungal Specimens:**

Concentrated spores in vials were obtained from the Microbac Laboratory – Knoxville Division:

*Aspergillus niger* ATCC 16888 –  $5.0 \times 10^8$  spores per mL.

*Penicillium citrinum* FRR 1841 –  $1.34 \times 10^9$  spores per mL.

*Stachybotrys chartarum* VAMH 6417 –  $2.15 \times 10^8$  spores per mL.

A one mL aliquot of each spore concentrate was diluted into a vial containing 99 mL of sterilized deionized water. A 0.5 mL aliquot was transferred to each of four petri dishes, with each petri dish containing Malt Extract Agar. A sterile, plastic spreader “hockey stick” was used to spread the spores evenly across each petri dish. The petri dishes were incubated for five days in the environmental chamber at 30° C and 97% relative humidity. Using sterile techniques, the spores were collected with sterile deionized water into a sterile 125 mL flask. Glass beads inside the flask were used to break up the clumped spores on the vortexing machine. The spores were washed with sterile deionized water and centrifuged three times. Following the last centrifugation (10 minutes at 2,000 rpm) the wash water was removed. 10 mL of sterile deionized water was then pipetted into each centrifuge tube.

An aliquot of 0.3 mL was removed from the *Aspergillus* spore suspension. 0.1 mL was placed in each of three petri dishes containing Malt Extract Agar and spread across the entire surface.

An aliquot of 0.3 mL was removed from the *Penicillium* spore suspension. 0.1 mL was placed in each of three petri dishes containing Malt Extract Agar and spread across the entire surface.

An aliquot of 0.3 mL was removed from the *Stachybotrys* spore suspension. 0.1 mL was placed in each of three petri dishes containing Malt Extract Agar and spread across the entire surface.

The nine petri dishes were incubated in the environmental chamber for 4 days at 30° C and 97% relative humidity.

### **Test Run:**

Two uncovered petri dishes of *Aspergillus* culture were placed on the antiseptically treated counter top. The first culture was given two sprays with Zimek QD. The second culture was saturated with 14 sprays of Zimek QD. The third, covered, petri dish culture was used as the control to obtain the initial spore count.

Two uncovered petri dishes of *Penicillium* culture were placed on the antiseptically treated counter top. The first culture was given two sprays with Zimek QD. The second culture was saturated with 14 sprays of Zimek QD. The third, covered, petri dish culture was used as the control to obtain the initial spore count.

Two uncovered petri dishes of *Stachybotrys* culture were placed on the antiseptically treated counter top. The first culture was given two sprays with Zimek QD. The second

culture was saturated with 14 sprays of Zimek QD. The third, covered, petri dish culture was used as the control to obtain the initial spore count.

Each of the sprayed petri dish cultures was allowed to sit 40 minutes before the collection of spores for the viable space spore count testing. Standard methods of dilution and plating were used to determine the viable mold spore counts.<sup>2&3</sup>

### **Test Results:**

Standard approved procedures established by AOAC and FDA/BAM were used in the collection of mold spores and bacteria and in their dilution into petri dishes with appropriate media and cultivation.<sup>2&3</sup> The mold spores germinated and grew into colonies in an environment chamber at 30° and 97% relative humidity. The bacteria grew into colonies in the 35° incubator.

The *Aspergillus* control had a count of 390,000,000 viable spores per petri dish. The culture receiving two sprays had a count of 150,000,000 viable spores per petri dish (a reduction of 61.5%). The culture being saturated with 14 sprays had a count of 620,000 viable spores per petri dish (a reduction of 99.84%).

The *Penicillium* control had a count of 45,000,000 viable spores per petri dish. The culture receiving two sprays had a count of 12,000,000 viable spores per petri dish (a reduction of 73.3%). The culture being saturated with 14 sprays had a count of 24,000 viable spores per petri dish (a reduction of 99.95%).

The *Stachybotrys* control had a count of 38,000,000 viable spores per petri dish. The culture receiving two sprays had a count of 18,000,000 viable spores per petri dish (a reduction of 52.6%). The culture being saturated with 14 sprays had a count of 160,000 viable spores per petri dish (a reduction of 99.58%).

### **Conclusion:**

*Aspergillus*, *Penicillium* and *Stachybotrys* cultures, in petri dishes with Malt Extract Agar nutrient, were sprayed to the saturation point with Zimek QD fungicide. The Zimek QD was effective in killing 99.58 to 99.95% of mold spores tested after a 40 minute dwell time. The cultures receiving only two sprays showed spore count reductions of 52.6% to 73.3% with *Stachybotrys* being the most resistant of the three molds tested.

### **Reference Material:**

1. Selecting the Correct Nozzle to Reduce Spray Drift, Iowa State University, IPM, and July 2001.
2. American Association of Analytical Chemists (AOAC) Official Method 966.23.
3. Federal Department of Agriculture/Bacteriological Analytical Manual (FDA/BAM) Chapter 3.



Respectfully submitted by:

A handwritten signature in black ink, appearing to read "Kayse M. Hasiak".

Kayse M. Hasiak  
Laboratory Director  
Microbac Laboratories, Inc. – Venice Division  
115 Corporation Way, Unit F  
Venice, Florida 34285