# PROVEN UNDER THE MICROSCOPE IN THE LABORATORY

An investigation on how to visualize and quantify the antimicrobial effect of BioCote's additives on polymers via molecular dyes and microscopy.

Good performance to international standard test criteria can be considered the first step in taking an antimicrobial product to market.

Percentage reductions of bacteria numbers from this testing may require additional explanation and context when an understanding for the potential of antibacterial properties are made available to interested parties who perhaps do not have a scientific or technical background.

With this in mind, we investigated how we might demonstrate antibacterial performance via other means. In collaboration with Birmingham University Technology Hub Imaging Core, we determined that epi-fluorescent microscopy with a live/dead staining system could visualize microorganisms on BioCote® treated and untreated polymer.

The visual results of this imaging are contained within this report, as well as antibacterial efficacy data.

## Method

### Antibacterial analysis

BioCote® treated and control polymer was assessed for antibacterial properties via the international standard ISO22196:2011. Testing of the material was performed against three organisms, Escherichia coli, Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa.

Samples were inoculated with known quantities of test organism and incubated at 37°C for 24 hours. After incubation remaining cells were washed from the surface of the assessed material, diluted as appropriate and counted. Results were then expressed as a percentage.

### Fluorescence microscopy imaging

Small plaques of BioCote® treated and control material was prepared and test bacteria applied and incubated for 3 hours at 37°C. Bacteria were then colored with molecular dyes which stained the bacteria (**red** if dead and **green** if alive). Images were then obtained via microscopy. Test bacteria were Pseudomonas aeruginosa, E. coli and Salmonella spp.

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# RESULTS

**Antibacterial ISO22196:2011**

A summary of the results of antibacterial analysis are shown in Table 1. Excellent antibacterial effectiveness was demonstrated for all three organisms. The table displays percentage reductions against control (reduction when compared with organisms recovered from non-treated control material) and initial (reduction of the total number of organisms loaded onto the material).

**TABLE 1:**

| Species | % Reduction (control) | % Reduction (initial) |
|---|---|---|
| P. aeruginosa | 99.67% | 97.46% |
| E. coli | ≥ 99.99% | ≥ 99.91% |
| S. aureus (MRSA) | ≥ 99.89% | ≥ 99.93% |

**Fluorescence microscopy imaging**

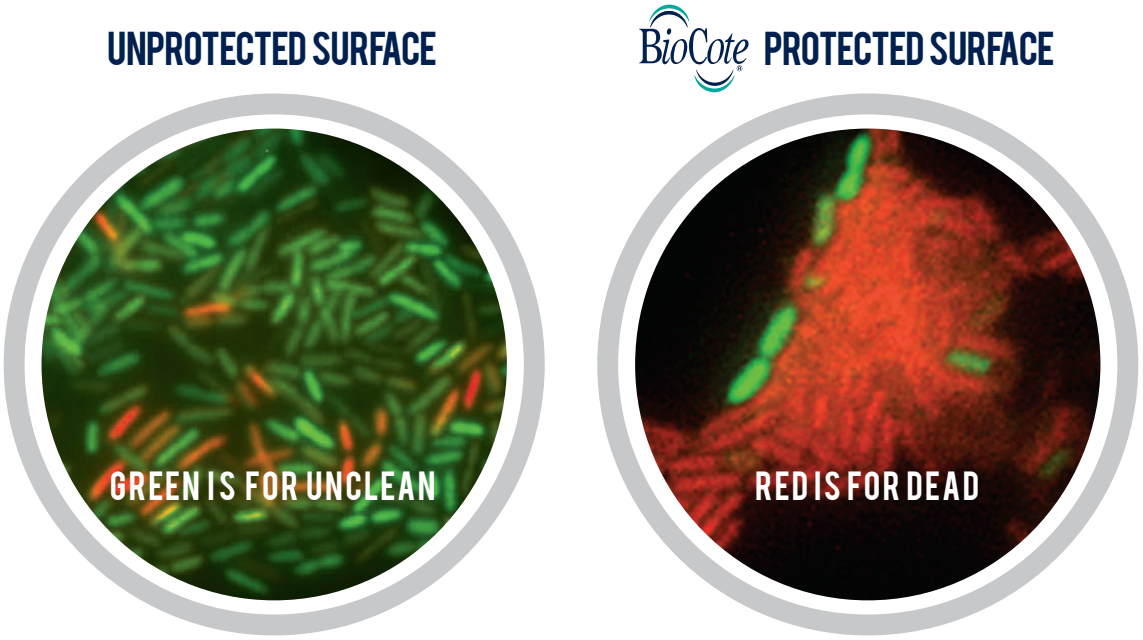
The results of the imaging of Pseudomonas aeruginosa are displayed below, in Figure 1.

Green indicates living cells, stained with the Syto9 dye, whilst red shows dead, stained with propidium Iodide dye.

Of the three organisms tested, Pseudomonas was able to adhere to the surface sufficiently for imaging in the 3 hour time frame.

Via computer based imaging tools we approximated 37% of cells to be dead in the field for the control material. In contrast, the treated sample displayed approximately 94% dead cells within the visual field.

**FIGURE 1:** **The results of the imaging of Pseudomonas aeruginosa on control (left) and silver ion containing (right) polyethylene plaques. Green indicates living cells, whilst red shows dead cells.**




**Imaging performed by Dr. Robert K. Shaw, Imaging Specialist, Technology Hub Imaging Core, College of Medical and Dental Sciences, University of Birmingham.**

# DISCUSSION

Pseudomonas aeruginosa was able to adhere sufficiently to the polymer surface in the allotted time, allowing imaging. After three hours of growth the organism had initiated biofilm formation, which aided or possibly allowed adherence to the necessary levels for visualization.

To achieve good a representative image a balance was sought between contact or incubation time of test organism and the polymer, and the desire to demonstrate a timely bactericidal action. The three hour incubation time of the test organism and material has presumably allowed P. aeruginosa time to adhere prior to accumulating sufficient cellular damage via the biocidal action of silver, which resulted in the death of the cell and accumulation of propidium Iodide stain.

We were able to successfully demonstrate visually the antibacterial properties of BioCote® treated polymer.

Significant differences between BioCote® treated and control material were observed, demonstrating excellent efficacy comparable levels of to ISO22196:2011 results.

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