

Scientific Services

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LABORATORY REPORT

- SOURCE:** Biotechnics Ltd.
- ITEMS:** AntiBak Residual.
- TESTS:** To determine the effectiveness of AntiBak Residual against *Aspergillus niger* over 1 7day period, when applied by 'Monster 60' steam cleaner
- METHOD:** AntiBak Residual was diluted 10ml: 190ml water and used as the 'sanitising' solution in the steam cleaner. The steam cleaner was brought up to temperature as per instruction manual, and then steamed/ sanitised for 2 minutes prior to usage, no attachments were used to direct the steam flow. Three 150mm x 150mm ceramic tiles were steam cleaned (Eight passes - back and forth is counted as one pass). These were inoculated with 0.5ml of *Aspergillus niger* ATCC 16404 suspension in ¼ strength Ringers solution at 10mins, 2 days and 7 days after steam cleaning (room temp storage) respectively. After a 15 minute contact period, the surviving organisms were recovered into a neutraliser broth and plated onto Rose Bengal Agar, incubated at 25 C for 7 days. An untreated tile was used as a control at each inoculation point.
- RESULTS:** See attached tables



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Recoveries of Aspergillus niger after 15 minutes contact

Time	A.niger/tile	
	Control	Treated
10 minutes	1.2x10 E6	9.7x10 E2
2 days	7.5x10 E5	4.3x10 E3
7 days	8.1x10 E5	4.0x10 E3

Overview –

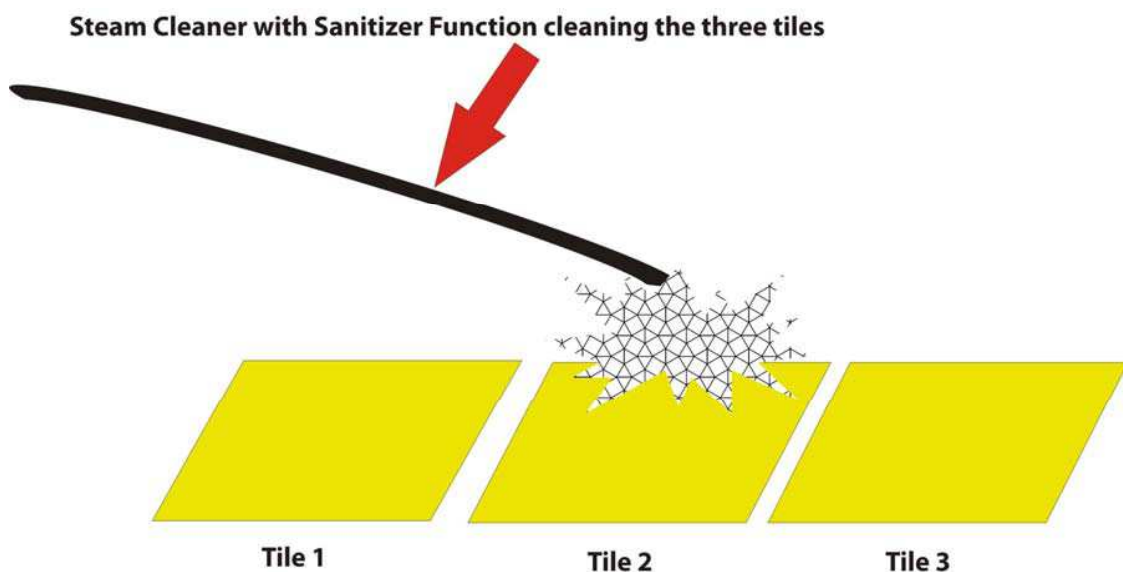
There are numerous European Standards designed to test a disinfectant's effectiveness against bacteria (EN1276) and fungi (EN1650). AntiBak Residual passes both of these tests. However, one problem with the standard tests is that they are laboratory conditions and not necessarily real world applications – i.e. a test tube (lab) versus use with a steam cleaner (real world).

There are many variables such as how the product will react with the steam, flow rate and how well it will be dispersed. To take into consideration these conditions, a test was designed specifically for AntiBak Residual's use through the Steam Cleaner.

Where possible, methodology was taken from the European Standard EN1650 standard (such as organism being tested against, contact time, organic material, etc.)

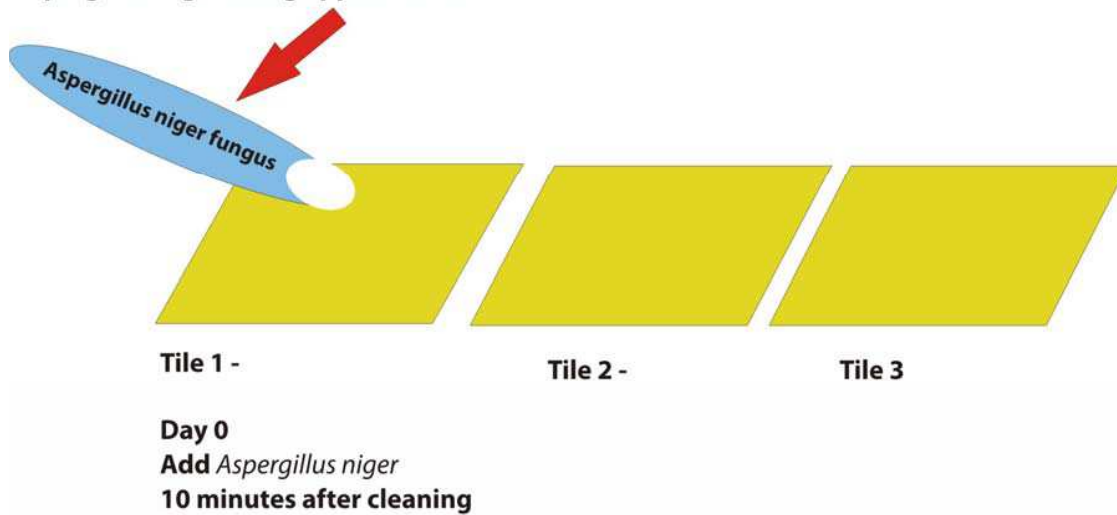
Testing Procedure –

Three blank ceramic tiles were used for the test. On Day 0, all 3 tiles were cleaned using the steam cleaner with the sanitizer (AntiBak Residual) option applied.

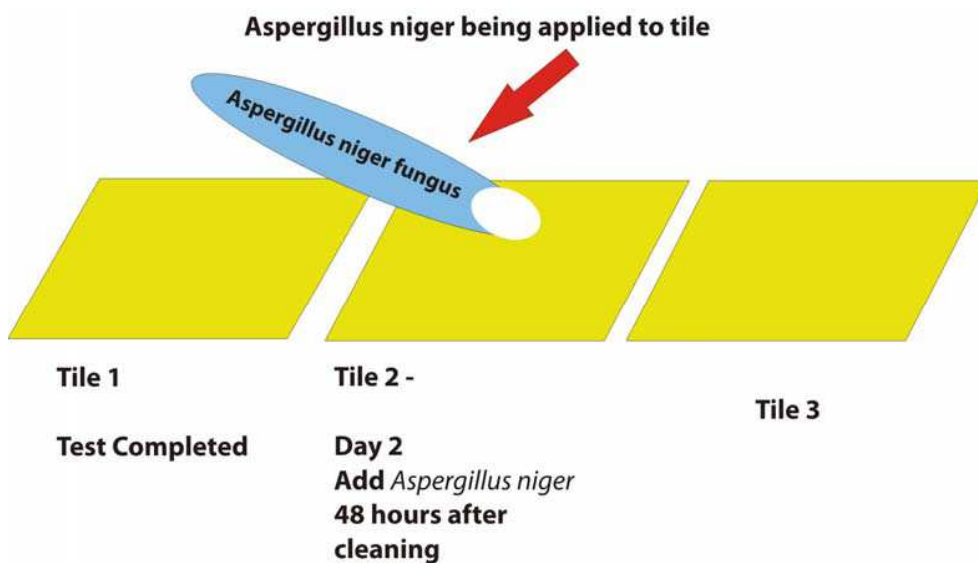


Ten minutes after Steam Cleaning + sanitised, **Tile 1** had *Aspergillus niger* applied to it. Fifteen minutes after the application of the fungus (25 minutes after it had been cleaned) the tile was sampled to determine how many fungi survived. This result is listed on the report under the column '**Treated**'.

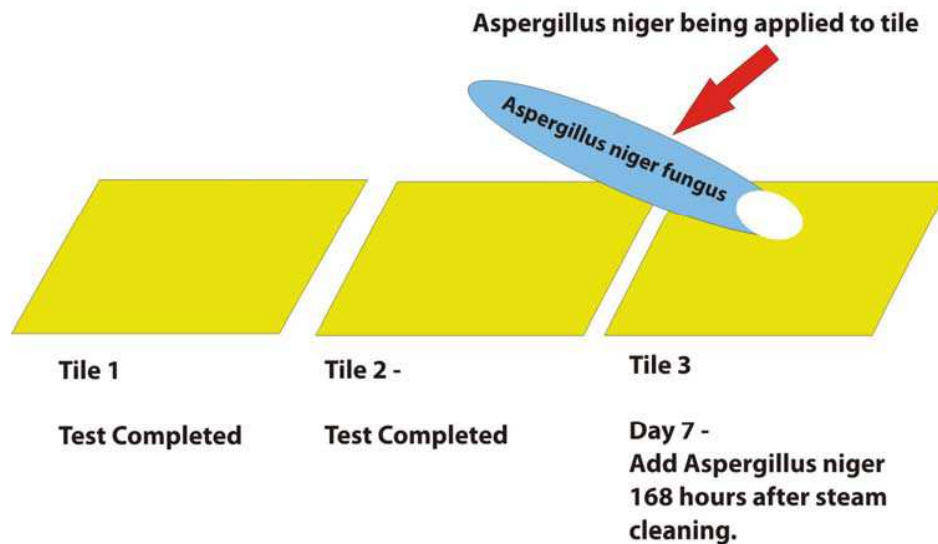
Aspergillus niger being applied to tile



48 hours after having been steam cleaned + sanitised, **Tile 2** had *Aspergillus niger* applied. Fifteen minutes after the introduction of *Aspergillus niger*, the tile was sampled to determine how many fungi survived. This result is listed on the report under the column '**Treated**'.



Seven days after having been steam cleaned + sanitised, **Tile 3** had *Aspergillus niger* applied. Fifteen minutes after the introduction of *Aspergillus niger*, the tile was sampled to determine how many fungi survived. This result is listed on the report under the column '**Treated**'.



Reading the Test Report -

The results of the testing are detailed on the second page of the report. It's divided into two columns:

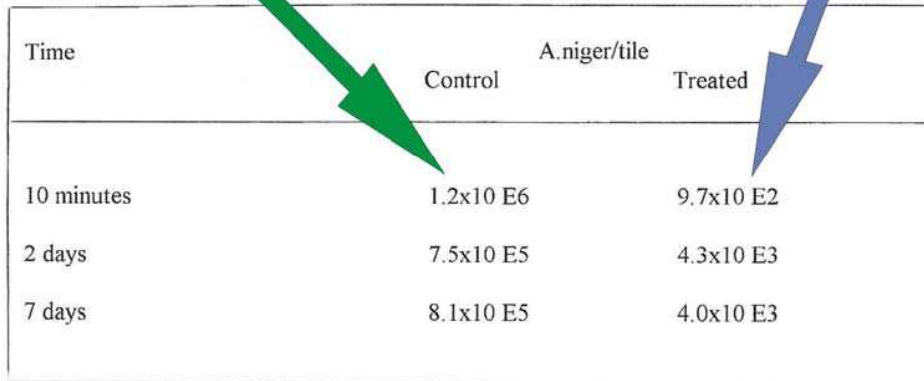
- ❖ **Control** column - how many *Aspergillus niger* were added to the tile initially
- ❖ **Treated** column - how many *Aspergillus niger* were not killed

Number of fungi added to tile -
Starting point

Number of fungi after 15 minutes -

(In other words: Number of fungi that were
not killed)

Recoveries of *Aspergillus niger* after 15 minutes contact



Time	A. niger/tile	
	Control	Treated
10 minutes	1.2x10 E6	9.7x10 E2
2 days	7.5x10 E5	4.3x10 E3
7 days	8.1x10 E5	4.0x10 E3

To determine how many fungi were killed, a **Reduction** is calculated. The **Control** number is divided by the **Treated** number. An example for 10 minutes is as follows:

$$1.2 \times 10^6 / 9.7 \times 10^2 = 1.2 \times 10^3$$

or in long-hand

$$1200000 / 970 = 1237$$

(control) / (treated) = reduction

The reductions for all of the times are as follows:

10 minutes reduction would be 1.2x10 E3 (99.92% kill)

Day 2's reduction would be 1.7x10 E2 (99.4% kill)

Day 7's reduction would be 2.0x10 E2 (99.5% kill)