INNOVATION PARTNER

RESISTANCE OF *C. DIFFICILE* ON A FLOOR COVERING

CRIQ File No. 640-PX46721

Technical Report

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June 6, 2013

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Québec

PRINCIPAL FINANCIAL PARTNER OF CRIQ

RESISTANCE OF *C. DIFFICILE* ON A FLOOR COVERING

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1. MANDATE

The objective of this project was to determine the bactericidal and bacteriostatic efficiency of a flocked carpet (Flotex) against the *Clostridium difficile* bacterium according to three different test methods:

- a qualitative evaluation of bactericidal or bacteriostatic efficiency (TNO seed layer method);
- a quantitative evaluation of bactericidal efficiency according to AATCC 100 Test Method *Antibacterial Finishes on Textile Materials: Assessment of;*
- an evaluation of the contact time necessary for the Flotex covering to be in contact with the bacterium to determine bactericidal or bacteriostatic activity (in vivo simulations method).

2. DESCRIPTION OF THE WORK

A Flotex sample was received at CRIQ on April 4, 2013 and was registered under CRIQ No. 50883. All tests were carried out in May 2013. Each test was performed on test specimens as received and on test specimens who had been submitted to one hour of leaching in tap water and then dried in ambient air.

For all tests, *Reinforced Clostridium Medium* broth or agar were used with 48 hours cultures of *Clostridium difficile* ATCC 9689.

2.1 PREPARATION OF THE SAMPLES

Before proceeding with each test, the samples were cut to the required shape and dimensions.

Half of the test specimens were then submitted to one hour of leaching in tap water. For this purpose, they were placed in a shallow container and a sufficient quantity of water was added to cover them. The containers were placed on an agitating plate adjusted to a high enough speed to allow a weak movement of the water over the surface of the specimens. After one hour, they were removed from the solution, drained and left to dry on a stainless steel grille for one hour at room temperature.

2.2 QUALITATIVE EVALUATION OF THE BACTERICIDAL OR BACTERIOSTATIC EFFICIENCY ACCORDING TO THE TNO SEED LAYER METHOD

The principle of this evaluation is to put a bacterial suspension of known concentration in liquefied agar and transfer a small quantity of this suspension to the surface of the test specimen. During the incubation period, the samples are inspected periodically to determine whether or not there is bacterial growth.

A *Clostridium difficile* culture was produced by inoculating 10 ml of Reinforced Clostridium Medium broth and incubating under anaerobic conditions at 35°C for 48 hours. This culture then was diluted in a liquefied agar containing the same culture medium to obtain a final concentration of about 10^5 CFU (colony forming units) per milliliter.

Two Teflon disks 1 cm in diameter and 0.5 cm thick were then placed on a 5 cm by 5 cm Flotex sample and each one was filled with 0.5 ml of inoculated agar. After solidification of the agar, the samples were placed in 100 x 15 mm Petri dishes and then incubated for 4 days at 35°C under anaerobic conditions. A control sample was also prepared by transferring the same quantity of inoculated agar to a disk placed at the bottom of a sterile Petri dish and was incubated under the same conditions, as well as controlling the sterility of the agar before its inoculation.

A count was also performed on the freshly inoculated gelose by making successive dilutions with sterile diluent and filtering a known volume of the dilution on a hydrophobic grid-membrane filter. The membrane was placed on the surface of Reinforced Clostridium Medium agar and incubated for 72 hours at 35°C under anaerobic conditions. At the end of the incubation period, the count was performed.

The bacteriostatic activity was assessed daily by examining the presence or absence of growth on the samples. The absence of growth confirms bacteriostatic or bactericidal activity and, conversely, growth shows that this activity is not recorded.

2.3 QUANTITATIVE EVALUATION OF BACTERICIDAL EFFICIENCY ACCORDING TO AATCC 100

The American Association of Textile Chemists and Colorists offers a method for quantitative evaluation of the bactericidal activity of a surface on which a known quantity of bacteria is added. This technique was developed to test fabrics but is also applicable to a floor covering.

A *Clostridium difficile* suspension at a concentration of about 10^5 CFU per 0.1 ml was prepared in a Reinforced Clostridium Medium broth. Flotex specimens 5 cm x 5 cm were placed individually at the bottom of sterile 500 ml plastic bottles. The samples were inoculated with 0.1 of the suspension and a count was performed on half of them by adding 100 ml of a neutralizing solution containing 0.1 % peptone and 0.1 % polysorbate 80 and by filtering 1 ml of this solution on a filtering membrane as described in the preceding test. Control samples moistened with 0.1 ml of sterile water were also prepared.

The other half of the samples was placed in the incubator at 35 °C, after hermetically sealing the containers, for 24 hours. A bottle containing only 0.1 ml of inoculum without sample was also tested to validate the inoculum's viability. After the incubation period, a new count was performed by adding 100 ml of neutralizing solution to each container and filtering 1 ml of the solution.

2.4 EVALUATION OF THE TIME NECESSARY FOR THE FLOTEX COATING TO BE IN CONTACT WITH THE BACTERIUM TO DETERMINE BACTERICIDAL OR BACTERIOSTATIC ACTIVITY (IN VIVO SIMULATIONS METHOD)

This evaluation consists in determining the contact time required between a piece of Flotex and the bacterium, either to prevent growth and multiplication, or to eliminate it.

The *Clostridium difficile* suspension at a concentration of about 10^5 CFU per 0.1 ml was prepared in Reinforced Clostridium Medium broth. A 0.1 ml volume of this suspension was spread with a curved glass rod on the surface of 100 x 15 mm Petri dish containing agar and left to dry for 15 minutes under a laminar flow hood.

The 1 cm by 1 cm test specimens were placed on the agar, face down and incubated at 35°C under anaerobic conditions. Test specimens were removed after 1, 2, 4, 6 and 24 hours. After each removal, the Petri dishes, now deprived from their samples, were put back in the incubator under the same conditions and examined every 24 hours up to 72 hours. Totally inhibited growth at the end of 72 hours indicates a bactericidal or bacteriostatic effect, while partial inhibition of growth in the first 48 hours followed by growth after 72 hours instead shows bacteriostatic activity. The method is validated by the growth of bacteria on the surface of an inoculated agar which was not put in contact with the test specimens and by the insertion of an uninoculated dish on which no growth must be observed.

3. RESULTS

3.1 RESULT OF THE QUALITATIVE EVALUATION OF EFFICIENCY MEASURED ACCORDING TO THE TNO SEED LAYER METHOD

The count performed on the inoculum used to perform this test was 1.9 x 10^5 CFU/ml. After one day of incubation, we noticed that the agar was drying out on the test specimens, probably because water was absorbed by the sample.

The test was rerun using 1.5 ml of inoculated agar in the Teflon disk instead 0.5 ml. The same phenomenon was observed after 48 hours of incubation.

Table I presents the results obtained after 24 hours of incubation. The plus (+) symbol indicates that there was growth, while the minus (–) symbol represents the absence of growth of the *Clostridium difficile* bacterium.

TABLE I: GROWTH OBSERVED AFTER 24 HOURS OF INCUBATION (*TNO SEED LAYER METHOD*)

SAMPLE	GROWTH OBSERVED
Flotex as is	-
Flotex soaked for one hour	-
Positive control (inoculated agar)	+
Sterility control	-

The result obtained indicates that the Flotex coating shows a bacteriostatic activity and a potentially bactericidal activity against *Clostridium difficile.*

3.2 RESULT OF THE QUANTITATIVE EVALUATION OF BACTERICIDAL EFFICIENCY ACCORDING TO AATCC 100

The inoculum used for this evaluation contained 6.2×10^5 CFU/0.1 ml. Table II presents the results obtained of the counts performed immediately after inoculation and after 24 hours of contact between the bacterium and the samples at 35°C.

Efficiency is calculated as follows:

$R = 100 (B - A)/B$

Where R = % reduction after 24 hours of contact
A = count of the inoculated sample after 24 hours of contact
B = count of the inoculated sample after inoculation

TABLE II: BACTERICIDAL EFFICIENCY

SAMPLE	COUNT (CFU/SURFACE)		% EFFICIENCY
	UPON INOCULATION	AFTER 24 HOURS OF CONTACT	
Flotex as is (inoculated)	1.1×10^5	< 100	> 99.9
Flotex as is (uninoculated)	< 100	< 100	---
Flotex soaked for one hour (inoculated)	2.7×10^5	< 100	> 99.9
Flotex soaked for one hour (uninoculated)	< 100	< 100	---
Inoculum	6.2×10^5	1.0×10^5	---

The lower quantity of bacteria in the inoculum after 24 hours of incubation is probably caused by natural mortality of *C. difficile* and the lack of nutrients in the environment. The count of the uninoculated test specimens is less than 100 and shows that they were not contaminated during handling. The results also confirm that Flotex is not an appropriate medium that allows the growth of bacteria.

3.3 RESULTS OF THE CONTACT TIME EVALUATION (IN VIVO SIMULATIONS METHOD)

The following table presents the results obtained after 72 hours of incubation following different contact times between the sample and the inoculum. The plus (+) symbol indicates that there was growth while the minus (–) symbol represents the absence of growth of the *Clostridium difficile* bacterium. The inoculum used for this test contained 1.9×10^6 living cells of *Clostridium difficile* at the beginning of the test.

TABLE III: GROWTH OBSERVED ACCORDING TO THE CONTACT TIME

SAMPLE	GROWTH OBSERVED				
	T = 1 HOUR	T = 2 HOURS	T = 4 HOURS	T = 6 HOURS	T = 24 HOURS
Flotex as is	-	-	-	-	-
Flotex soaked for one hour	-	-	-	-	-
Positive control (inoculated gelose)	+				
Sterility control	-				

Since, no growth was recorded after incubation of the Petri dishes once the specimens were removed, Flotex thus seems to be bactericidal against *Clostridium difficile* and is bacteriostatic towards this bacteria.

4. CONCLUSION

Flotex flocked carpet shows bacteriostatic and bactericidal properties against the *Clostridium difficile* ATCC 9689 strain. Also, soaking the sample in water for one hour does not seem to alter its antibacterial properties. Thus, it can be concluded that:

- Flotex shows a probable bacteriostatic efficiency against *Clostridium difficile* when tested according to the TNO Seed layer Method but the test method is not appropriate for this kind of sample*;*
- Flotex possesses bactericidal activity exceeding 99.9 % against *Clostridium difficile* when tested according to the AATCC 100 method;
- After one hour of contact with *Clostridium difficile*, Flotex shows its bacteriostatic activity when tested according to the in vivo simulations method, and is probably bactericidal.

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