REPORT

Contact person RISE
Therese Andersson
Division Materials and Production
+46 10 516 59 29
therese.andersson@ri.se

Date: 2021-06-24
Reference: O100152-157423

Hållbar Teknik Sverige AB
Att: Andreas Bodén
Kungsgatan 62
903 26 Umeå

Measurement of antibacterial activity on one test item type using a method based on ISO 22196:2011

1 Assignment and objects

Measurement of antibacterial activity on one test item type using a method based on ISO 22196:2011, "Measurement of antibacterial activity on plastic and other non-porous surfaces".

See table 1 for information about the test item.

Date of test items arrival at RISE: 2021-06-14

Date of analysis: 2021-06-15 – 2021-06-23

Table 1. Information about test item 157423:1

RISE identity:	**157423:1**
Test item name or code:	Recoat AM Protector / Stay Safe Surface
Test item reference number:	586331-70
Test item batch/lot number:	2042160023
Test item expiration date:	Not given
Intended purpose of test item:	Anti-microbial surface protector, for use on public touch points to prevent the spread of bacteria and viruses.
Test item composition:	CAS: 34590-94-8 (0.5-2.5 %), CAS: 6846-50-0 (0.5-2.5 %) Classification of the substance or mixture. Classification according to Regulation (EC) No 1272/2008. The product is not classified, according to CLP regulation.
Date of manufacture of test item:	October 20th 2020
Method of manufacture of test item:	Not given
Manufacture location of test item:	Schaafdries 12 5371 NJ Ravenstein Netherlands
Physical description:	Pre-treated PVC material cut into test items. Upper side (grey) treated Lower side (beige) untreated

Surface area per test item (cm^2):	25 cm^2
Dimensions, thickness (mm):	50 mm x 50 mm, Thickness: ~1 mm
Test item sterility:	Not sterile
Cleaning procedure:	Test item was carefully wiped with 70 % ethanol before testing.
Test item preparation:	Test item was tested intact
Size of cover film	40 x 40 mm
Test area of cover film	16 cm^2
Material in cover film	Polypropylene

Method

Preparation of test specimens

Three test items and six specimens of untreated material were prepared for each bacterial strain and included in the testing. Three of the untreated specimens were used to measure viable bacteria immediately after inoculation and the other were used to measure viable bacteria after 24 h incubation.

Test items, untreated specimens and cover films were prepared as stated in table 1 above and wiped with 70 % Ethanol before testing.

Preparation of test inoculum

A few colonies of bacteria, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739, were transferred with a sterile loop to tubes containing 10 ml NB medium and incubated overnight at 250 rpm at 35°C. At the end of the incubation the tubes were centrifuged for 10 minutes at 2500 x g, 20°C. The supernatants were removed and the pellets re-suspended in 1 ml NB diluted 500 times in sterile H_2O (NB-500) to master bacterial suspensions.

Optical density (OD) at 600 nm was used to measure the bacterial content of the suspensions (Epoch plate reader). The bacteria suspensions were then diluted to final density of approx. 6×10^5 colony forming units (CFU)/ml (test inoculum). Number of bacteria in test inoculum was determined by viable count, the suspensions were diluted in tenfold steps in phosphate buffered saline (PBS) and 50 µl from each dilution was spread on horse blood agar plates. The plates were incubated at 35°C overnight and then the number of CFUs was counted.

Inoculation of test specimens

All test items and untreated specimens were placed in sterile petri dishes (one per dish, test surface facing upwards) and then 400 µl test inoculum was pipetted on each test item and untreated specimen. A cover film was applied by gentle pressing in order to spread out the test inoculum and then the lid was applied onto the dish.

The three test items and three of the untreated specimens, per bacteria strain, were then incubated at 35°C and relative humidity of 90 % for 24 h.

Recovery of bacteria from test items and untreated specimens

Untreated specimens immediately after inoculation (0 h)

Immediately after inoculation, bacteria on three of the untreated specimens were harvested by adding 10 ml of SCDLP broth into the petri dishes containing the specimens. The specimens were carefully washed by pipetting the SCDLP broth up and down at least four times. These

untreated specimens were used to determine recovery rate of the bacteria by counting the viable bacteria present in the SCDLP broth suspensions.

Test items and untreated specimens after 24 h incubation

After the 24 hour incubation the test items and remaining untreated specimens were treated as described above.

Determine the viable bacteria count

The SCDLP broth suspensions were diluted in tenfold steps in PBS buffer and 50 µl from each dilution was spread (in duplicates) on horse blood agar plates. The plates were incubated at 35°C overnight and the number of CFUs was counted.

Results

The number of bacteria in test inoculum suspension, the number of bacteria recovered immediately after inoculation (0 h) and also the number of bacteria recovered from untreated specimens after the 24 h incubation for the two strains are presented in table 2.

Table 2. Viable bacteria counts for untreated specimens presented as CFU/ml or CFU/cm^2.

Strain	Test inoculum (CFU/ml)	Mean value of viable bacteria recovered immediately after inoculation (n=3, 0 h) (CFU/cm^2)	Mean value of viable bacteria recovered from untreated specimens (n=3) after 24h incubation (CFU/cm^2)
Escherichia coli ATCC 8739	1.8 x 10^{6*}	5.3 x 10^{4**}	2.6 x 10^5
Staphylococcus aureus ATCC 6538	7.4 x 10^5	1.9 x 10^4	1.4 x 10^4

*Slightly higher than recommended in the standard (2.5 x 10^5 to 1x 10^6 CFU/ml)
** Slightly higher than recommended in the standard (6.2 x 10^3 to 2.5 x 10^4 CFU/cm^2)

No bacteria were recovered from the test items after 24 h incubation. According to ISO 22196:2011 the number of colonies recovered should be recorded as < 1 if there are no colonies recovered in any of the agar plates in the dilution series. The number of bacteria in test inoculum suspension and the number of bacteria recovered from test items after the 24 h incubation for the two strains are presented in table 3.

Table 3. Viable bacteria counts for test item 157423:1 presented as CFU/ml or CFU/cm^2.

Strain	Test inoculum (CFU/ml)	Mean value of viable bacteria recovered from test items (n=3) after 24h incubation (CFU/cm^2)
Escherichia coli ATCC 8739	1.8 x 10^{6*}	< 12.5
Staphylococcus aureus ATCC 6538	7.4 x 10^5	< 12.5

*Slightly higher than recommended in the standard (2.5 x 10^5 to 1x 10^6 CFU/ml)

Antibacterial activity was calculated using the equation below. Calculated values for each strain are presented in table 4.

$R = (U_t-U_0) – (A_t-U_0) = U_t - A_t$

where

R = Antibacterial activity

U_0 = the average common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the untreated specimens immediately after inoculation (0 h)

U_t = the average common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the untreated specimens after 24 h incubation

A = the average common logarithm of the number of viable bacteria, in CFU/cm^2, recovered from the test items after 24 h incubation.

Table 4. Calculated antibacterial activity for test item 157423:1.

Strain	**A_t**	**U_0**	**U_t**	**R**	**Reduction in %**
Escherichia coli ATCC 8739	< 1.1	4.7	5.4	> 4.3	> 99.99
Staphylococcus aureus ATCC 6538	< 1.1	4.3	4.1	> 3.0	> 99.90

Conclusion

Apart from the slightly elevated number of bacteria in the *Escherichia coli* test inoculum, the study meets all the acceptance criteria stated in ISO 22196:2011.

Under the conditions of this study, test item “Recoat AM Protector / Stay Safe Surface” was found to have an antibacterial activity of R > 3.0 for *Staphylococcus aureus* and R > 4.3 for *Escherichia coli*.

The result relates only to the test item evaluated. Any extrapolation of the result to other items is the responsibility of the Sponsor.

RISE Research Institutes of Sweden AB
Chemistry, Biomaterials and Textiles - Biological Function

Performed by

Signed by: Therese Andersson
Reason: I am the author of this document
Date & Time: 2021-06-24 08:27:04 +02:00

Therese Andersson

Examined by

Signed by: Sara Bogren
Reason: I have reviewed this document
Date & Time: 2021-06-24 13:57:09 +02:00

Sara Bogren

Performed by

Signed by: Karin Agrenius
Reason: I am the author of this document
Date & Time: 2021-06-24 12:22:38 +02:00

Karin Agrenius