Validation study:
Microbiological efficacy of UVC-disinfection with UV Smart D25

Report Date: 23-12-2019

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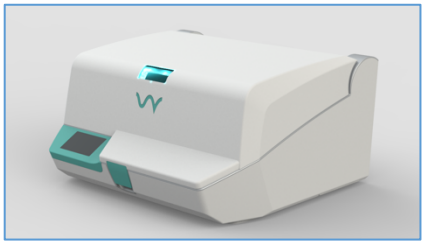
Testing dates:
-Mycobacteria tests were initiated in week 45
-Fungi tests were initiated in week 46
-Bacterial tests were performed in weeks 48 and 48
-Yeas tests were performed in week 50

BACKGROUND

UV Smart is developing a device, the D25, to be used for disinfection of small medical devices such as (dect) telephones, smartphones / tablets and stethoscopes. UV Smart aims to enable rapid disinfection of these small medical equipment, for example during the execution of hand hygiene, while reducing the use of chemicals and water.
The disinfecting efficacy of the D25 technology has already been demonstrated in similar studies performed in a microbiology laboratory setting (at Streeklab Haarlem) and in clinical practices (at two Academic Medical Centers: UMCG and Radboud).
The current tests with the D25 are specifically aimed to meet the European standard for chemical disinfectants and antiseptics of medical devices: the NEN-EN-14885: 2018.

MATERIALS

- D25 (made available by UVSmart)



- Clinical isolates of micro-organisms:
    - Micro-organisms as recommended from NEN-EN-14885:2018):

Micro-organism	Claim	Field of application			
		Surface		Instrument	
		Standard (Phase, Step)	Logarythmic-reduction	Standard (Phase, Step)	Logarythmic-reduction
Staphylococcus aureus (ATCC 6538)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0
Pseudomonas aeruginosa (ATCC 15442)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0
Enterococcus hirae (ATCC 10541)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0
Bacillus cereus (ATCC 12826)	Sporicidal	EN 13704 (2,1) [3]	≥ 3,0	EN 13704 (2,1)	≥ 3,0
Candida albicans (ATCC 10231)	Yeasticical	EN 13624 (2,1) EN 13697 (2,2) [2]	≥ 4,0 ≥ 3,0	EN 13624 (2,1) EN 14562 (2,2)	≥ 4,0 ≥ 4,0
	Fungicidal	EN 13624 (2,1) EN 13697 (2,2) [2]	≥ 4,0 ≥ 3,0	EN 13624 (2,1) EN 14562 (2,2)	≥ 4,0 ≥ 4,0
Aspergillus brasiliensis (ATCC 16404)	Fungicidal	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0
		EN 13697 (2,2)	≥ 3,0	EN 14562 (2,2)	≥ 4,0
Candida auris (DSM 21092)	Yeasticidal	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0
Mycobacterium avium (ATCC 15769)	Mycobactericidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1) EN 14563 (2,2)	≥ 4,0 ≥ 4,0
Mycobacterium terrae (ATCC 15755)	Mycobactericidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1) EN 14563 (2,2)	≥ 4,0 ≥ 4,0
	Tuberculocidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1) EN 14563 (2,2)	≥ 4,0 ≥ 4,0
Escheriachia coli ATCC 10536	Bactericidal	EN 13697a (2,1)	≥ 4,0	EN 13697a (2,1)	≥ 4,0

1 Based on Table 12 of EN 14885: 2018.
2 Based on Table 13 of EN 14885: 2018.
3 Based on Table 15 of EN 14885: 2018.

- Micro-organisms as tested in this study and rationale for changes:

As specified in NEN-EN-14885		Tested in this study		Rationale
Organisms	ATCC[a]	Organism [a]	ATCC	
Staphylococcus aureus	6538	Same	Same	No changes.
Pseudomonas aeruginosa	15442	Same	Same	No changes.
Escherichia coli	10563	Same	Same	No Changes.
Enterococcus hirae	10541	Enterococcus faecium	12952	Enterococcus faecium is one of the most clinically relevant enterococcus species to human, with identical properties to the less clinically relevant E. hirae[1]
Bacillus subtilis	6633	Bacillus cereus	12826	B. cereus possess similar properties as B. subtilis, but is more frequently isolated from clinical samples and patient environments[2].
Candida albicans	10231	Same	Same	No changes.
Candida auris	DSM 21092	Same	Same	Candida auris is considered a worldwide emerging pathogen, associated with nosocomial outbreaks[3].
Aspergillus brasiliensis	16404	Aspergillus niger	16404	Name change, see NEN-EN-14885
Mycobacterium avium	15769	Same	Same	No changes.
Mycobacterium terrae	15755	Same	Same	No changes.

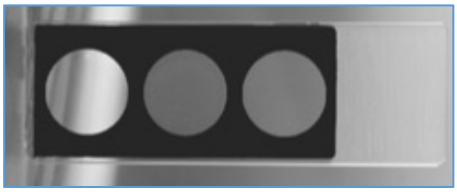
[1] García-Solache M, Rice LB. 2019. The enterococcus: a model of adaptability to its environment. Clin Microbiol Rev 32:e00058-18

[2] Edward J. Bottone. Bacillus cereus*, a Volatile Human Pathogen. Clinical Microbiology Reviews Apr 2010, 23 (2) 382-398*

[3] Anna Jeffery-Smith, Surabhi K. Taori, Silke Schelenz, Katie Jeffery, Elizabeth M. Johnson, Andrew Borman, Candida auris Incident Management Team, Rohini Manuel, Colin S. Brown. Candida auris: a Review of the Literature. Clinical Microbiology Reviews Nov 2017, 31 (1) e00029-17

[a] All micro-organisms were isolated from clinical samples submitted to a NEN-EN-ISO 15189:2012 certified clinical microbiology laboratory. Identification was performed using standard Maldi-tof determination testing with ATCC strains as reference micro-organisms.

- Tryptone Soy Agar + 5% Sheep blood (TSA-SB) agar (Thermo Fisher Scientific)
- Sabouraud Dextrose (Sab) Agar (Thermo Fisher Scientific)
- Middlebrook 7H10 Agar (BD)
- E-swabs with amies medium (Copan)
- 3 wells glass carrier:



- 35°C incubator
- Sterile NaCL suspension
- Bovine serum albumine (BSA) 0,3 g/L plus sheep erythrocytes 3 ml/l

METHODS

Reference test method

This study was performed as required by NEN-EN-14885:2018, using techniques in accordance with the ASTM E2111-12(2018) standard (Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemicals). Virucidal activity of UV Smart D25 was not tested. However, UVC potency against viruses can reliably derived from tests with (myco)bacteria, yeasts and fungi using two indicators for UV susceptibility: the average UV rate constants (a marker for microbial decay curves) and D90 values (indicating the UV dose for 90% inactivation) for bacteria, fungi and viruses on surfaces. Relevant adjustments were made to the ASTM E2111-12(2018) procedure in order to allow disinfection with D25.

All experiments, including examination and interpretation of agar plates and colony counts, were performed by a trained technician with over 10 years of experience in clinical bacteriology. Test were performed in a clinical microbiology laboratory certified according to the NEN-EN-ISO 15189:2012.

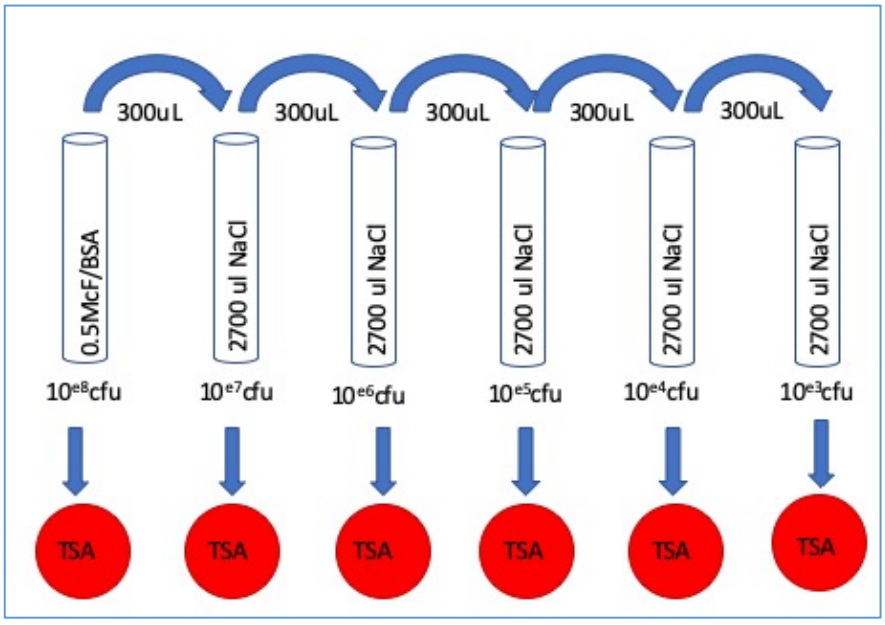
Preparing inocula of micro-organisms

Micro-organisms were grown overnight on TSA-SB agar (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecium, Bacillus cereus*) or Sabouraud agar (*Candida albicans, Candida auris, Aspergillus niger*) or 7H10 agar (Mycobacterium avium and Mycobacterium terrae) to receive fresh cultures. These fresh cultures were used to make 0.5 McFarland suspensions (corresponding to 1-2 x 10^8 CFU/mL) for the quantitation standard curves. McFarland suspensions ranging between 0.5 – 3.0 (for gram negative, gram positive and mycobacteria) and 3.0 – 4.0 (for yeasts and fungi) were prepared for inoculation of the glass carriers. All McFarland suspensions were mixed with BSA (0,3 mg/mL BSA).

Quantitation standard curve

For quantitation purposes, McFarland standard curves were created for gram positive bacteria (*S. aureus* and *B. cereus),* gram negative bacteria (*E. coli*), yeasts *(C. albicans),* fungi *(A. Niger)* and mycobacteria *(M. avium).* This was done by making 10-fold dilution series of the 0.5 McFarland/BSA suspensions (see figure 1). The McFarland dilutions were inoculated on TSA-SB agar (*S. aureus, E. coli* and *B. cereus*), Sabouraud dextrose agar (*C. albicans* and *A. niger*) or Middlebrook 7H10 Agar (*M. avium*).

Figure 1: McFarland standard curves from 1-2 x 10^8 CFU/mL to 1-2 x 10^3 CFU/mL were created using 10-fold dilutions series from a 0.5 McFarland suspension.

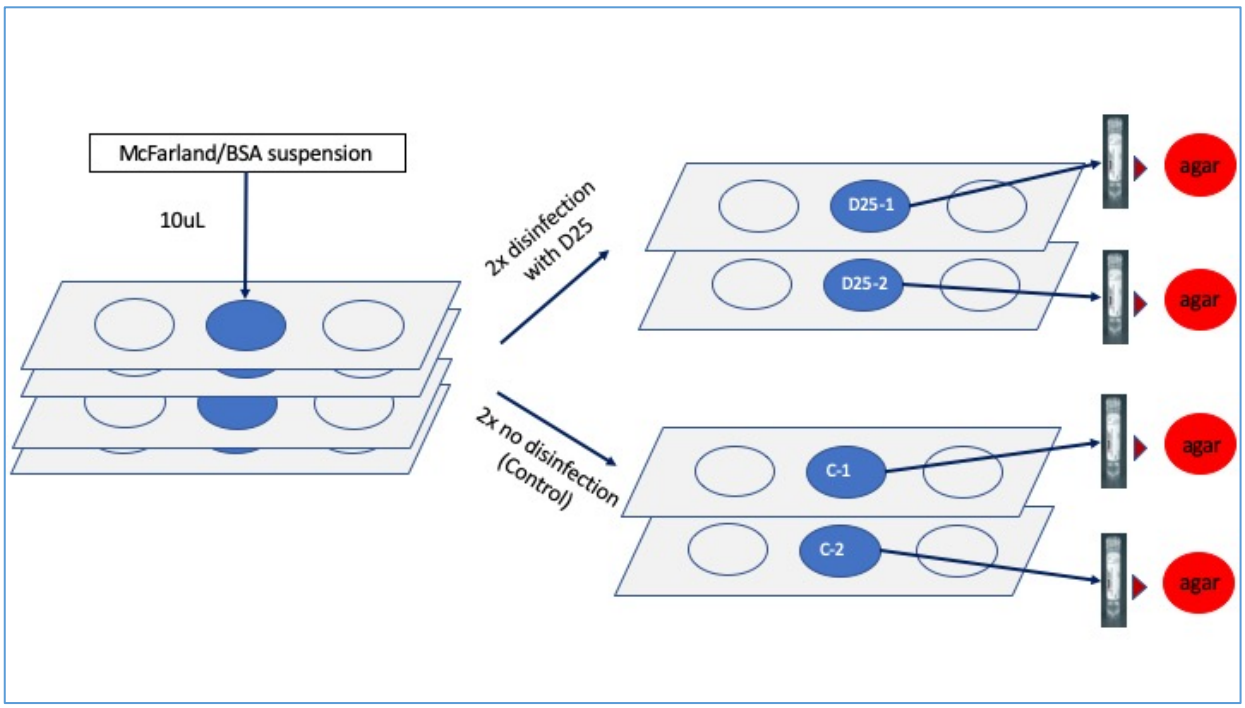


Inoculation of the glass carriers
The 3-wells glass carriers were inoculated as follows (figure 2):

a) Use four glass carriers per micro-organism (two for D25 disinfection and two non-disinfected controls).
b) Inoculate 10 *u*L of the homogenized McFarland/BSA suspensions in the middle well and allow the inoculum to become visibly dry at room temperature.

Exposure of inoculated glass carriers to radiation with D25
For each micro-organism, two inoculated glass carriers were disinfected in the D25 according to the manufacturer's instructions. The other two inoculated glass carriers were places outside the D25 during the disinfection procedure.
Following disinfection, the inoculated wells from all four glass carriers were thoroughly swabbed using a sterile moist e-swab. The e-swabs were subsequently resuspended vigorously in the copan tube containing amies medium, and vortexed before inoculating 10 ul of the amies suspension on TSA agars (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecium, Bacillus cereus*) or Sabouraud agar (*Candida albicans, Candida auris, Aspergillus niger*) or 7H10 agar (Mycobacterium avium and Mycobacterium terrae) (Figure 2).

Figuur 2: Test design



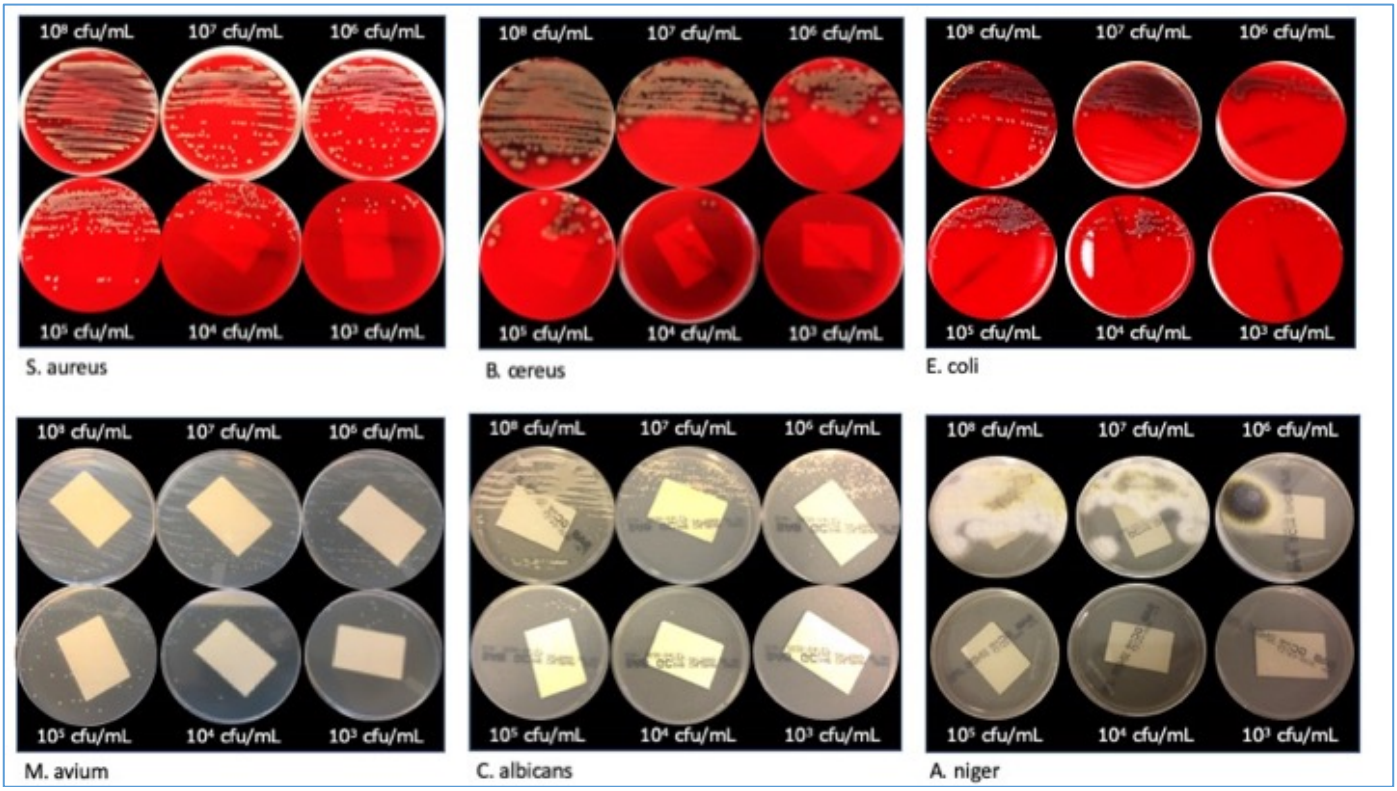
Agar plates were examined after 24 and 48 hours (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecium, Bacillus cereus*, *Candida albicans, Candida auris),* after 7 days (*Aspergillus niger*) or after 2 and 3 weeks (Mycobacterium avium and Mycobacterium terrae). Colony counts were compared between the disinfected and control glass carriers and quantitation was determined using the McFarland standard curves.

RESULTS

McFarland standard curves

McFarland standard curves ranging from 1-2 x 10^8 CFU/mL to 1-2 x 10^3 CFU/mL were created for gram positive cocci (*Staphylococcus aureus),* gram positive rods *(Bacillus cereus*), gram negative rods (Escherichia coli), yeasts (*Candida albicans),* fungi *(Aspergillus niger*) and mycobacteria (Mycobacterium avium) The standard curves demonstrates a gradually declining colony count from ~10^8 CFU/mL to ~10^3 CFU/mL (Figure 3.)

Figuur 3: McFarland standard curves



Efficacy of disinfection with D25

Inoculated micro-organisms were recovered from disinfected and non-disinfected glass carriers. The colony counts, estimated using the McFarland standard curves (Figure 3), were determined for the disinfected and non-disinfected glass carriers. The results are summarized in table 1 and corresponding pictures of the agar plates are depicted in figure 4.

For S. aureus, E. faecium, B. cereus, E. coli and P. aeruginosa, an estimated 10^7 cfu/mL were recovered from both non-disinfected glass-carriers. After disinfection, no colonies were recovered from both glass-carriers for S. aureus, E. faecium, E. coli and P. aeruginosa, and only 1 colony was recovered from a single glass-carrier for B. cereus. Similar results were obtained for both mycobacteria species, indicating a reduction of at least 10^6 cfu/mL after disinfection for all bacteria species tested.
For C. albicans and C. auris, at least 10^8 cfu/mL were recovered in duplo when no disinfection was performed. After disinfection no colonies were recovered, indicating a reduction of at least

10^7cfu/mL. Aspergillus niger was recovered in an estimated quantity of 10^6 cfu/mL from both non-disinfected glass-carriers. After disinfection, not a single cfu of A. niger could be recovered.
Virus neutralizing activity of D25 was not tested in these experiments. However, virucidal activity of UVC was deduced by comparing the well-studied average UV rate constants (a marker for microbial decay curves) and D90 values (indicating the UV dose for 90% inactivation) on surfaces, for viruses and bacteria, fungi and yeasts[4]. On surfaces, viruses are less susceptible to UVC compared to bacteria but substantially more sensitive to UVC compared to fungal cells and yeast (rate constant of 0.14 m^2/J for bacteria, 0.007 m^2/J for fungal cells/yeast and 0.03 m^2/J for viruses. D90 of 16 J/m^2 for bacteria, 229 J/m^2 for fungal cells/yeast and 72 J/m^2 for viruses[4]). In this study, it was shown that disinfection with D25 effectively reduced concentrations of Aspergillus niger as well as the two candida species, for more than Log[6] CFU. Therefore, it is evident that UVC disinfection with D25 will show excellent virus neutralizing activity.

[4] *Germicidal Irradiation Handbook; UVGI for Air and Surface Disinfection, Chapter 4, table 4.1*

CONCLUSIONS

Table 2 presents a summary of the NEN-EN-14885: 2018 requirements and the test results. In conclusion, this study demonstrates that bactericidal, fungicidal, yeasticidal as well as mycobactericidal claims for the D25 were met (table 2). Virucidal activity was deduced from results with fungi and yeast species.

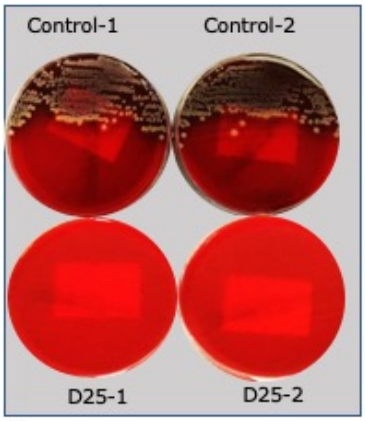
Table 1. Overview of quantitative growth of micro-organisms recovered from disinfected (D25-1 and D25-2) and non-disinfected (control-1 and control-2) glass carriers.

Micro-organism	Test specs	Semi-quantitative growth[1]	Log reduction
S. aureus	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
E. faecium	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
B. cereus	Disinfection with D25 (D25-1)	1 colonies: < 10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
P. aeruginosa	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
E. coli	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
C. auris	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^7 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^8 cfu/mL	
	No disinfection Controle-2	10^8 cfu/mL	
C. albicans	Disinfection with D25 (D25-1	0 colonies: < 10^1 cfu/mL	> 10^7 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^8 cfu/mL	
	No disinfection Controle-2	10^8 cfu/mL	
A niger	Disinfection with D25 (D25-1)	0 colonies: < 10^1 cfu/mL	> 10^5 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^6 cfu/mL	
	No disinfection Controle-2	10^6 cfu/mL	
M.avium	Disinfection with D25 (D25-1)	0 colonies: <10^1 cfu/mL	> 10^6 cfu / mL
	Disinfection with D25 (D25-2)	0 colonies: <10^1 cfu/mL	
	No disinfection Controle-1	10^7 cfu/mL	
	No disinfection Controle-2	10^7 cfu/mL	
M.terrae	Disinfection with D25 (D25-1)	1 colony: <10^1 cfu/mL	> 10^6 cfu / mL

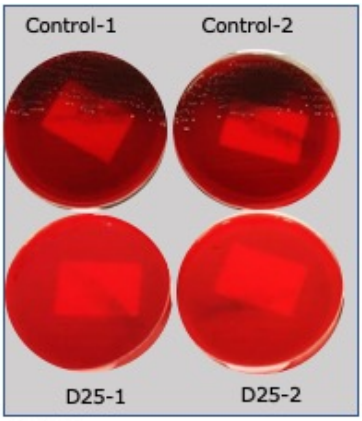
	Disinfection with D25 (D25-2)	3 colonies: <10^1 cfu/mL
	No disinfection Controle-1	10^7 cfu/mL
	No disinfection Controle-2	10^7 cfu/mL

[1]Quantitative growth was estimated using the McFarland standard curves.

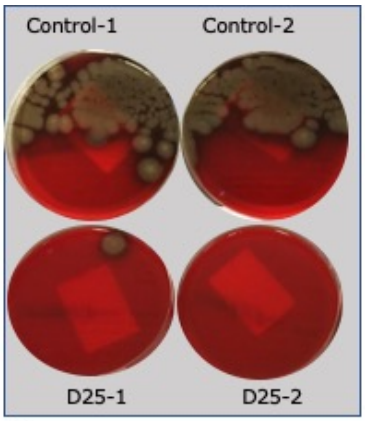
Figure 4: Quantitative growth of micro-organisms, recovered from disinfected (D25-1 and D25-2) and non-disinfected (control-1 and control-2) glass carriers. All experiments were performed in duplo. Quantitative growth was estimated using the McFarland standard curves.



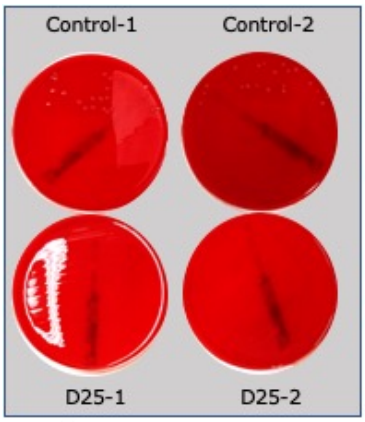
S. aureus



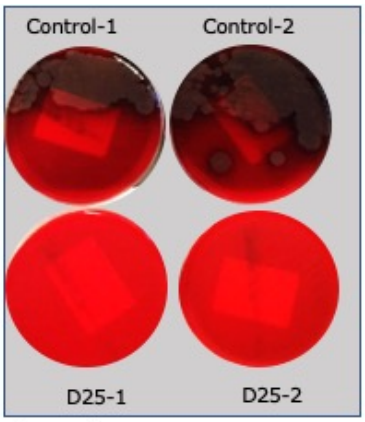
E. fecium



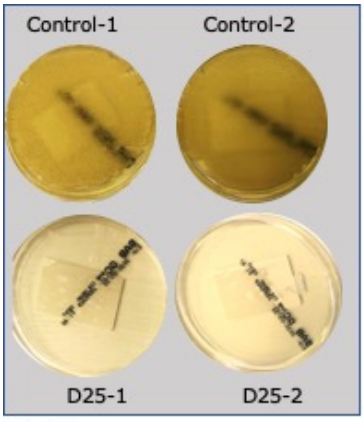
B. cereus



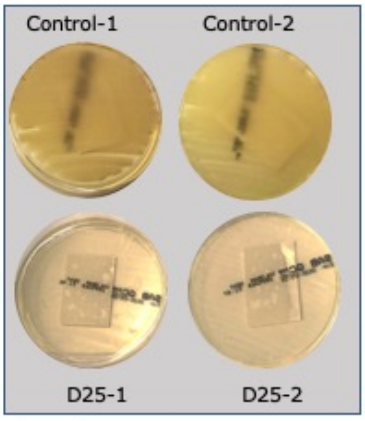
E. coli



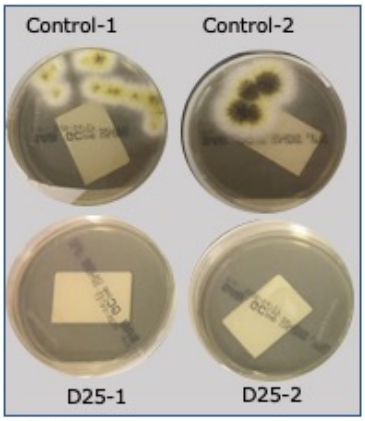
P. aeruginosa



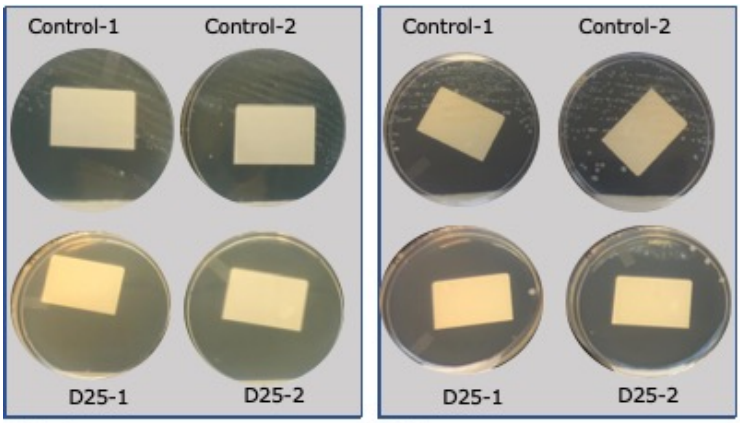
C. albicans



C. auris



A. niger



M. avium M. terrae

Table 2: Summary of NEN-EN-14885:2018 requirements and test results

Micro-organism	Claim	NEN-EN-14885:2018 requirements according to Field of application				Reduction achieved in test (cfu/mL	Compliance to NEN-EN-14885:2018
		Surface		Instrument			
		Standard (Phase, Step)	Logarythmic-reduction	Standard (Phase, Step)	Logarythmic-reduction		
Staphylococcus aureus (ATCC 6538)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0	$>10^6$	PASSED
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0		
Pseudomonas aeruginosa (ATCC 15442)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0	$>10^6$	PASSED
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0		
Enterococcus hirae (ATCC 10541)	Bactericidal	EN 13727 (2,1)	≥ 5,0	EN 13727 (2,1)	≥ 5,0	$>10^6$	PASSED
		EN 13697 (2,2) [1]	≥ 4,0	EN 14561 (2,2)	≥ 5,0		
Bacillus cereus (ATCC 12826)	Sporicidal	EN 13704 (2,1) [3]	≥ 3,0	EN 13704 (2,1)	≥ 3,0	$>10^6$	PASSED
Candida albicans (ATCC 10231)	Yeasticical	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0	$>10^7$	PASSED
		EN 13697 (2,2) [2]	≥ 3,0	EN 14562 (2,2)	≥ 4,0		
	Fungicidal	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0		
		EN 13697 (2,2) [2]	≥ 3,0	EN 14562 (2,2)	≥ 4,0		
Aspergillus brasiliensis (ATCC 16404)	Fungicidal	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0		
		EN 13697 (2,2)	≥ 3,0	EN 14562 (2,2)	≥ 4,0	$>10^5$	PASSED
Candida auris (DSM 21092)	Yeasticidal	EN 13624 (2,1)	≥ 4,0	EN 13624 (2,1)	≥ 4,0	$>10^7$	PASSED
Mycobacterium avium (ATCC 15769)	Mycobactericidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1)	≥ 4,0	$>10^7$	PASSED
				EN 14563 (2,2)	≥ 4,0		
Mycobacterium terrae (ATCC 15755)	Mycobactericidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1)	≥ 4,0	$>10^7$	PASSED
				EN 14563 (2,2)	≥ 4,0		
	Tuberculocidal	EN 14348 (2,1)	≥ 4,0	EN 14348 (2,1)	≥ 4,0		
				EN 14563 (2,2)	≥ 4,0		
Escherichia coli ATCC 10536	Bactericidal	EN 13697a (2,1)	≥ 4,0	EN 13697a (2,1)	≥ 4,0	$>10^7$	PASSED
Poliovirus type 1, LSc-2ab	Virucidal	EN 14476 (2,1)	≥ 4,0	EN 14476 (2,1)	≥ 4,0	Not tested	PASSED according to rationale as described in the text on page 8
Adenovirus type 5, strain Adenoid 75 (ATCC VR-5)	Virucidal	EN 14476 (2,1)	≥ 4,0	EN 14476 (2,1)	≥ 4,0	Not tested	PASSED according to rationale as described in the text on page 8
Murine Norovirus, strain S99 Berlin	Virucidal	EN 14476 (2,1)	≥ 4,0	EN 14476 (2,1)	≥ 4,0	Not tested	PASSED according to rationale as described in the text on page 8