INNOVATION VOUCHER REPORT

Project: Assessment of novel Resysten coating system for theinactivation of MRSA

Client: Resysten International Ltd

Knowledge Provider: Schools of Engineering and Biomedical Sciences

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Voucher Reference: IV 130266985

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Agreed work plan

1. Virtual meetings to discuss project goals and logistics of receipt of equipment/access to research labs. (Resysten to deliver coated samples to Ulster)
2. Review existing test reports on biocidal efficacy of Resysten coatings. (Resysten have supplied materials to Ulster by email)
3. Develop robust methods (based on ISO standards) to assess level of surface disinfection on a range of substrates treated with Resysten protective coatings.
4. Undertake a series of laboratory-based experiments to assess inactivation of methicillin-resistant Staphylococcus aureus (MRSA) under indoor light conditions.
5. Assess the level of MRSA inactivation as a function of exposure time. A series of time points will be investigated, Time zero (control), 10 min, 30 min, 60 min and 240 min with calculation of log inactivation as a function of exposure time.
6. Undertake replicate experiments with a full suite of microbiology and process control experiments.
7. Project output: A written technical report on the above laboratory experimentation will be submitted to the client.

(Indicative time: 8 days testing, 0.5 day for meetings with client, 0.5 day report writing.)

Summary of actions against work plan

1. Preliminary discussions took place via email with treated and untreated glass samples delivered to the labs in Coleraine on 4th August 2021.
2. Data from previous lab-based assessment and environmental monitoring of installed / Resysten systems was reviewed to ensure the work within the IV would add value to the company's portfolio of results.
3. The primary ISO method identified as relevant to the project was BS ISO 27447:2019 (Test method for antibacterial activity of semiconducting photocatalytic materials). The method was modified, as described in the technical report, to ensure assessment of the coatings under realistic illumination conditions (i.e. standard room lighting).
4. A technical report relating to the experiments undertaken with MRSA is presented below.
5. Data on the inactivation of MRSA by the coatings is presented in the technical report – exposure times were updated in-keeping with the relevant ISO method.
6. Replicate and control experiments were undertaken as described in the ISO standards.
7. A detailed report has been produced for the client. This was followed by an online meeting to discuss the findings and identify next steps.

(Actual time: 9 days lab, 2 days doc/data review, 0.5 day client meetings, 1 day report writing.)

Technical Report

1. Resysten Technology

The Resysten product is a protective surface coating system which utilises light and a catalyst (the surface coating) to initiate anti-microbial and anti-soiling effects (https://www.whatisresysten.com/). Samples of coated glass surfaces (2.5 cm^2) were prepared by the client and delivered to Ulster, along with a series of untreated glass samples - to enable direct assessment of the effect of the photocatalytic coating. All samples were subjected to a regular clean/wash stage, as described in the methods below, but were not modified in anyway prior to assessment.

2. Test Method

The primary standard method identified as relevant to the project was BS ISO 27447:2019 (Test method for antibacterial activity of semiconducting photocatalytic materials). This method was developed specifically to quantify the antibacterial activity of photocatalytic ceramics and other materials produced by either the coating or the mixing of a photocatalyst. ISO 27447 is universally accepted as the gold standard method for photocatalytic products and conducted in laboratories across the globe to permit a level of compliance, confidence in the use of photocatalytic surfaces and product benchmarking.

The test method stipulates irradiation of samples with UVA lamps, however, to create conditions which are more realistic to the environments which the client wishes to use the system, standard laboratory lights were used as the irradiation source (with lighting levels similar to that expected in standard retail, leisure, entertainment facilities etc). The efficacy of the coating to inactivate bacteria in the absence of light (i.e. under dark conditions) could also be quantified using the methods. Following discussion with the client, the organism chosen for the testing was methicillin resistant *Staphylococcus aureus* (MRSA) – a Gram positive bacterium that is resistant to several widely used antibiotics and known as a 'hospital superbug'. This is in-keeping with the ISO standard with stipulates testing with *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and permits use of other bacteria.

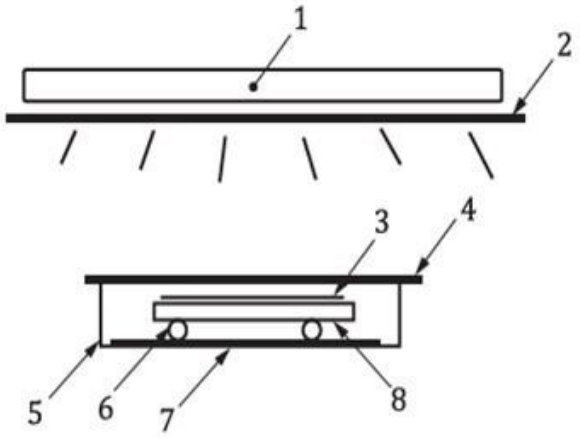
Full details of the methods for preparation and enumeration of bacteria, the diluent, interfering substance and the neutralizer can be found within BS ISO 27447:2019. In brief:

- *Staphylococcus aureus* (MRSA) ATCC43300 was grown freshly from stock in appropriate media and suspended in 1/500 nutrient broth to obtain a concentration of approx. 1 $x10^6$ CFU/mL.

- Test surfaces were washed in 5% (V/V) Decon®, washed under running water, rinsed in distilled water and sterilized by place in a bath containing 70 % (V/V) iso-propanol for 15 min. Test surfaces were removed and dried by evaporation under laminar air flow.
- The treatment environment was prepared laying moisture control filter paper into the bottom of a sterilized Petri dish, 3.5 mL of sterilized water was added to the filter paper.
- Three plastic spacers were added onto the filter paper, and subsequently a test piece was placed on top of each spacer. Coated test pieces were placed with the photocatalytic surface facing up. Non coated glass samples were prepared in the same fashion.
- Each sample was inoculated with 0.2 ml of test bacterial suspension.
- A transparent sterile polypropylene film (2 cm^2) was placed on top of the inoculum, evenly dispersing to bacterial suspension across the sample surface. Care was taken to ensure no bacterial suspension leaked beyond the film edge.
- A moisture conservation glass (Pyrex, 1 mm thick) was placed on the top of each Petri dish – see Figure 1 which shows a schematic of the test equipment from the ISO method, with a photograph of the assembly used in this work.
- Samples were subsequently left under light and/or dark (placed in a sealed light proof box) conditions for a period of 8 hours (standard ISO conditions) or 4 hours (ISO permitted 'real conditions' time period). The environmental conditions were not specifically controlled, simply standard laboratory conditions of approx. 20^{o}C with a relative humidity of between 40 and 60%. The distance between the room light and the samples was 1.7 m.
- Following treatment, each sample was transferred to a separate container containing 10 mL of neutralizer (SCDLP). Samples were vortexed for at least 1 min to release test organisms into the liquid, followed by appropriate 10-fold dilution with 0.1 mL enumerated on agar plates following 18-24 h incubation at 37 $^{\circ}$C.

Experiments were undertaken in triplicate, with a full range of control experiments as described within the ISO standard; including coated and uncoated glass samples in the dark, and coated and uncoated glass exposed to natural room light.

Bacterial counts are reported as the number of colony forming units per mL (CFU/mL) with analysis conducted via the approach described within BS ISO 27447:2019.



1	light source	5	petri dish
2	punched metal	6	glass tube or glass rod
3	cover film	7	paper filter
4	moisture preservation glass	8	test piece

A

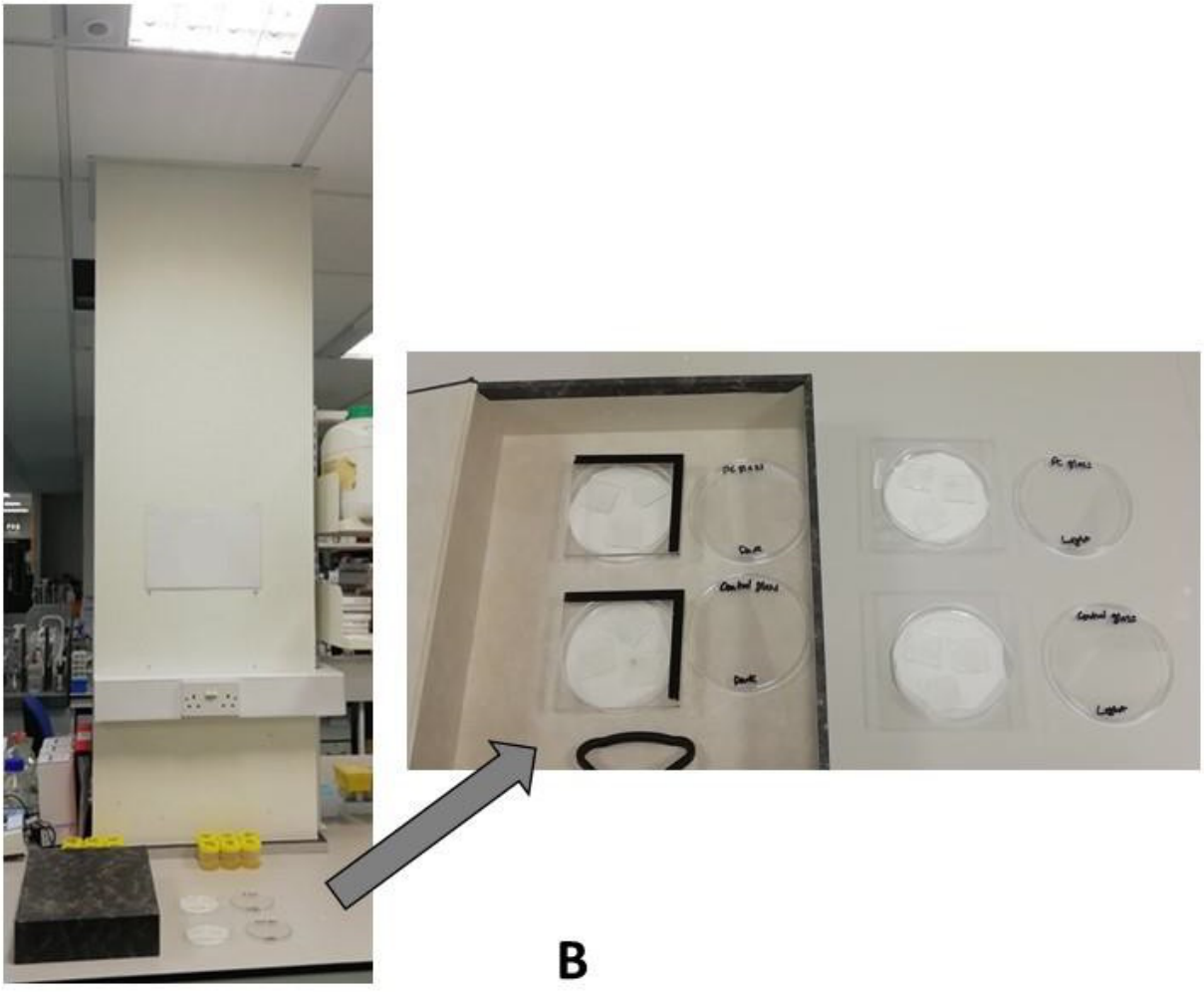


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Figure 1 A: Schematic of test assembly described within BS ISO 27447:2019; B: Photograph of the test assembly used for analysis of Resysten samples.

3. Results and Calculations

Microbial counts from all samples following 8 and 4 hours exposure are reported in Appendix 1 and Appendix 2, respectively, and summarised in Tables 1 and 2.

Table 1: Summary data relating to 8 hours treatment

Sample	Log count (CFU/mL)	Log kill vs Untreated glass dark
Untreated Glass T0	5.56	-
Untreated Glass DARK T8	5.58	0.00
Resysten DARK T8	4.00	1.58
Resysten Glass LIGHT T8	3.58	2.00
Untreated Glass LIGHT T8	5.59	-0.01

The experiment was confirmed to be valid given the data fulfils the four quality and replicate measurements stated within BS ISO 27447.

Photocatalyst antibacterial activity value (RL) was calculated to determine the log-reduction of MRSA following 8 hours light exposure of the coated glass vs uncoated glass exposed to light (see Appendix 1 for the calculation), **RL = 2.0 equating to a 2-log kill (or 99% reduction).**

A second calculation determines ΔR, the photocatalyst antibacterial activity value resulting from irradiation, i.e. a comparison of the light exposed coated glass to the coated glass maintained in the dark (see Appendix 1 for the calculation). **ΔR = 0.4** resulting from significant inactivation in dark conditions (i.e. the coating is capable of MRSA inactivation without exposure to light).

Based on the data in Table 1, it can be observed that the effect of light alone (i.e. untreated glass in the light compared to untreated glass in the dark), resulted in no significant inactivation.

Table 2: Summary data relating to 4 hours treatment

Sample	Log count (CFU/mL)	Log kill vs Untreated glass dark
Untreated Glass T0	6.52	-
Untreated Glass DARK T4	6.14	0.00
Resysten DARK T4	4.40	1.74
Resysten Glass LIGHT T4	3.78	2.36
Untreated Glass LIGHT T4	6.24	-0.10

The experiment was considered valid given the data fulfils the quality and replicate measurements stated within BS ISO 27447 – but the bacterial loading was increased by 1-log.

Photocatalyst antibacterial activity value (RL) was calculated to determine the log-reduction of MRSA following 4 hours light exposure of the coated glass vs uncoated glass exposed to light (see Appendix 2 for the calculation), **RL = 2.4 equating to a 2.4-log kill (or 99.6% reduction).**

A second calculation determines ΔR, the photocatalyst antibacterial activity value resulting from irradiation, i.e. a comparison of the light exposed coated glass to the coated glass maintained in the dark (see Appendix 1 for the calculation). **ΔR = 0.7** resulting from significant inactivation in dark conditions (i.e. the coating is capable of MRSA inactivation without exposure to light).

Based on the data in Table 2, it can again be observed by that the effect of light alone was negligible (i.e. untreated glass in the light compared to untreated glass in the dark).

4. Comments and Conclusion

Assessment of the Resysten treated surfaces demonstrates significant MRSA inactivation with 2.4 log (99.6%) reduction observed following 4 hours exposure to visible light under standard conditions room (no laboratory-based UV source was used in these experiments). Control experiments on untreated surfaces confirmed the pathogen to be stable during the length of the experiments with no loss of viability.

Significant inactivation was observed during dark exposure, which is assumed to result from inclusion of a biocidal compound in addition to the base photocatalytic semiconductor.

It is often the case that photocatalytic inactivation of bacteria proceeds via an exponential decay mechanism (i.e. a curved relationship which demonstrates lower reduction with increased time), therefore the reduction rates with extended light exposure (8 hr vs 4 hr) would agree with data commonly presented in scientific literature, where significantly greater inactivation is not observed at long exposure times. Conversely, this demonstrates that the coating quickly inactivates a high level of pathogens within 4 hours (the minimum exposure time specified within BS ISO 27447).

Based upon our academic experience in the area (>20 years working with photocatalytic materials for disinfection applications) a coating demonstrating >2-log inactivation of a high loading of a clinically relevant pathogen, assessed by the ISO standard method following exposure to basic laboratory lighting, is considered to be highly biocidal. The Resysten system is perhaps one of the most active biocidal photocatalytic coatings that we have worked with to date.

Appendix 1

8 hour exposure

N = P × V

N = number of cells on the sample

P = plate count

V = volume SCDLP

	Number of CFUs per plate (0.1 ml plated)								
	Dilutions								
	Neat	-1	-2	-3	-4	CFUs per ml (P)	CFU/ml in SCDLP (N)	Log 10 CFUs per ml	Ave CFU/mL
Untreated Glass T0	-	-	32	3	0	3.20E+04	3.20E+05	5.51	
Untreated Glass T0	-	-	36	5	0	3.60E+04	3.60E+05	5.56	
Untreated Glass T0	-	-	41	6	0	4.10E+04	4.10E+05	5.61	3.63E+05
Untreated Glass DARK T8	-	-	40	8	0	4.00E+04	4.00E+05	5.60	
Untreated Glass DARK T8	-	-	33	2	0	3.30E+04	3.30E+05	5.52	
Untreated Glass DARK T8	-	-	41	4	0	4.10E+04	4.10E+05	5.61	3.80E+05
Resysten DARK T8	97	9	1	-	-	9.70E+02	9.70E+03	3.99	
Resysten DARK T8	116	21	3	-	-	1.16E+03	1.16E+04	4.06	
Resysten DARK T8	89	5	0	-	-	8.90E+02	8.90E+03	3.95	1.01E+04
Resysten Glass LIGHT T8	41	6	0	-	-	4.10E+02	4.10E+03	3.61	
Resysten Glass LIGHT T8	37	1	0	-	-	3.70E+02	3.70E+03	3.57	
Resysten Glass LIGHT T8	36	8	0	-	-	3.60E+02	3.60E+03	3.56	3.80E+03
Untreated Glass LIGHT T8	-	-	38	2	0	3.80E+04	3.80E+05	5.58	
Untreated Glass LIGHT T8	-	-	42	3	0	4.20E+04	4.20E+05	5.62	
Untreated Glass LIGHT T8	-	-	37	5	0	3.70E+04	3.70E+05	5.57	3.90E+05

RL = Log (BL / CL)

RL = the photocatalyst antibacterial activity value for the film cover method

BL = average number of viable bacteria of non-treated specimens, after irradiation

CL = average number of viable bacteria of photocatalytic treated specimens, after irradiation

RL = 2.0 99% reduction

∆ R = Log (BL / CL) - Log (BD / CD)

∆R = the photocatalyst antibacterial activity value with UV irradiation

BD = average number of viable bacteria of non-treated specimens, after dark treatment

CD = average number of viable bacteria of treated specimens, after dark treatment

∆ R = 0.4 (calculation uses values to 1 dp)

Appendix 2

4 hour exposure

N = P × V

N = number of cells on the sample

P = plate count

V = volume SCDLP

	Number of CFUs per plate (0.1 ml plated) Dilutions								
	Neat	-1	-2	-3	-4	CFUs per ml (P)	CFU/ml in SCDLP (N)	Log 10 CFUs per ml	Ave CFU/mL
Untreated Glass T0	-	-	297	30	4	3.00E+05	3.00E+06	6.48	
Untreated Glass T0	-	-	295	38	2	3.80E+05	3.80E+06	6.58	
Untreated Glass T0	-	-	289	31	0	3.10E+05	3.10E+06	6.49	3.30E+06
Untreated Glass DARK T4	-	-	108	24	2	1.08E+05	1.08E+06	6.03	
Untreated Glass DARK T4	-	-	152	21	2	1.52E+05	1.52E+06	6.18	
Untreated Glass DARK T4	-	-	161	23	1	1.61E+05	1.61E+06	6.21	1.40E+06
Resysten DARK T4	251	15	2	-	-	2.51E+03	2.51E+04	4.40	
Resysten DARK T4	232	17	4	-	-	2.32E+03	2.32E+04	4.37	
Resysten DARK T4	268	29	2	-	-	2.68E+03	2.68E+04	4.43	2.50E+04
Resysten Glass LIGHT T4	71	2	0	-	-	7.10E+02	7.10E+03	3.85	
Resysten Glass LIGHT T4	93	6	0	-	-	9.30E+02	9.30E+03	3.97	
Resysten Glass LIGHT T4	33	1	0	-	-	3.30E+02	3.30E+03	3.52	6.57E+03
Untreated Glass LIGHT T4	-	-	184	19	2	1.84E+05	1.84E+06	6.26	
Untreated Glass LIGHT T4	-	-	178	16	4	1.78E+05	1.78E+06	6.25	
Untreated Glass LIGHT T4	-	-	165	15	4	1.65E+05	1.65E+06	6.22	1.76E+06

RL = Log (BL / CL)

RL = the photocatalyst antibacterial activity value for the film cover method

BL = average number of viable bacteria of non-treated specimens, after irradiation

CL = average number of viable bacteria of photocatalytic treated specimens, after irradiation

RL = 2.4 99.6% reduction

∆ R = Log (BL / CL) - Log (BD / CD)

∆R = the photocatalyst antibacterial activity value with UV irradiation

BD = average number of viable bacteria of non-treated specimens, after dark treatment

CD = average number of viable bacteria of treated specimens, after dark treatment

∆ R = 0.7 (calculation uses values to 1 dp)