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Research and development of Toucan and Biostream ECAS technology platforms

[Technical report]

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EXECUTIVE SUMMARY

WORK PACKAGE (WP1) – Assessing the antimicrobial efficacy of the 'Toucan' unit

- The Toucan unit produced consistent ECAS in terms of the physicochemical parameters, with an elevated ORP and free chlorine concentration (WP1.1).
- The 'single activation' ECAS exhibited a broad spectrum of activity, and has significant bactericidal activity against both Gram positive and Gram negative organisms (see table E1; WP1.2).
- The 'double activation' ECAS exhibited significant sporicidal activity (see table E2; WP1.3)
- Using a bespoke antimicrobial kinetic assay the 'single activation' ECAS was shown to be fast acting, eliciting a significant response within 10 seconds and completely reducing metabolic activity within 90 seconds, with no observed re-growth (WP1.4).

WORK PACKAGE 2 (WP2) – Assessing the carpet disinfection capability of a Biostream ECAS integrated Oztek cleaning system

- Under laboratory conditions, the Biostream ECAS integrated Oztek cleaning system had a significant carpet disinfection capability as measured by ATP swab tests and reduction in the number of viable recoverable bacteria and fungi (contact plating) from the surface of commercial and care home carpet samples (WP2.1).
- Under real world conditions, the Biostream ECAS integrated Host Oztek cleaning system had a significant carpet disinfection capability as measured by ATP swab tests and reduction in the number of viable recoverable bacteria and fungi (contact plating) from the surface of care home carpet tested in situ (WP2.2).
- Under real world conditions, the Biostream ECAS integrated Sebo Oztek cleaning system had a significant carpet disinfection capability as measured by ATP swab tests and reduction in the number of viable recoverable fungi only (contact plating) from the surface of care home carpet tested in situ (WP2.2).

Table E1 Significant log reduction values (log_{10} cfu), percentage (%) reduction and number of viable cells remaining after treatment (log_{10} cfu), when testing the antimicrobial efficacy of 'single activation' ECAS generated using the Toucan unit against a range of bacterial species. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± standard deviation [SD]).

Bacterial species	Log reduction values (log_{10} cfu)	Percentage (%) reduction	Viable cells remaining after treatment (log_{10} cfu)
Escherichia coli	5.803	99.9997	1.293
Salmonella enterica **serovar Typhimurium**	4.940	99.9979	2.284
Staphylococcus aureus	5.878	99.9998	1.572
Methicillin Resistant ***Staphylococcus aureus*** **(MRSA)**	6.017	99.9998	1.235
Listeria monocytogenes	6.703	99.9993	0.994

Table E2 Significant log reduction values (log_{10} cfu), percentage (%) reduction and number of viable cells remaining after treatment (log_{10} cfu), when testing the antimicrobial efficacy of 'double activation' ECAS against bacterial endospores generated using the Toucan unit against a range of bacterial species. All experiments were performed according to ***BS EN 13704:2002 Chemical Disinfectants Sporicidal Activity Testing*** (clean conditions; n=3 ± SD).

Bacterial endospore producing species	Log reduction values (log_{10} cfu)	Percentage (%) reduction	Viable cells remaining after treatment (log_{10} cfu)
Bacillus subtilis	5.650	100.00	0

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Summary of ECAS technology

Electrochemically activated solutions (ECAS) are generated from low mineral salt solutions (the electrolyte) within specially designed electrochemical cells. In the presence of a direct current, modification of ionic structures at the surface of titanium electrodes coated with porous layers of a metal oxide catalyst, results in solutions exhibiting elevated chemical reactivity[Ref1]. This releases reactive oxidants, mainly chlorine and oxygen species, into the bulk fluid, producing a solution with an elevated redox (oxidation-reduction potential; ORP). The resulting pH of a given ECAS then dictates which form of chorine is most prevalent (see figure 1[Ref2]). These solutions have been proven to have significant antimicrobial activity against bacteria, fungi and viruses[Ref3], driven by the mixed oxidants present (including hypochlorous acid). In addition, ECAS have shown significant antimicrobial efficacy against bacterial spores[Ref4] which are considered one of the most resistant microbial structures to treatment with antiseptics or disinfectants[Ref5]. Due to the safety and non-toxicity of ECAS at in-use concentrations[Ref4], they have been exploited for many applications including; potable water disinfection[Ref6], food safety[Ref7] and within medical healthcare settings[Ref4].

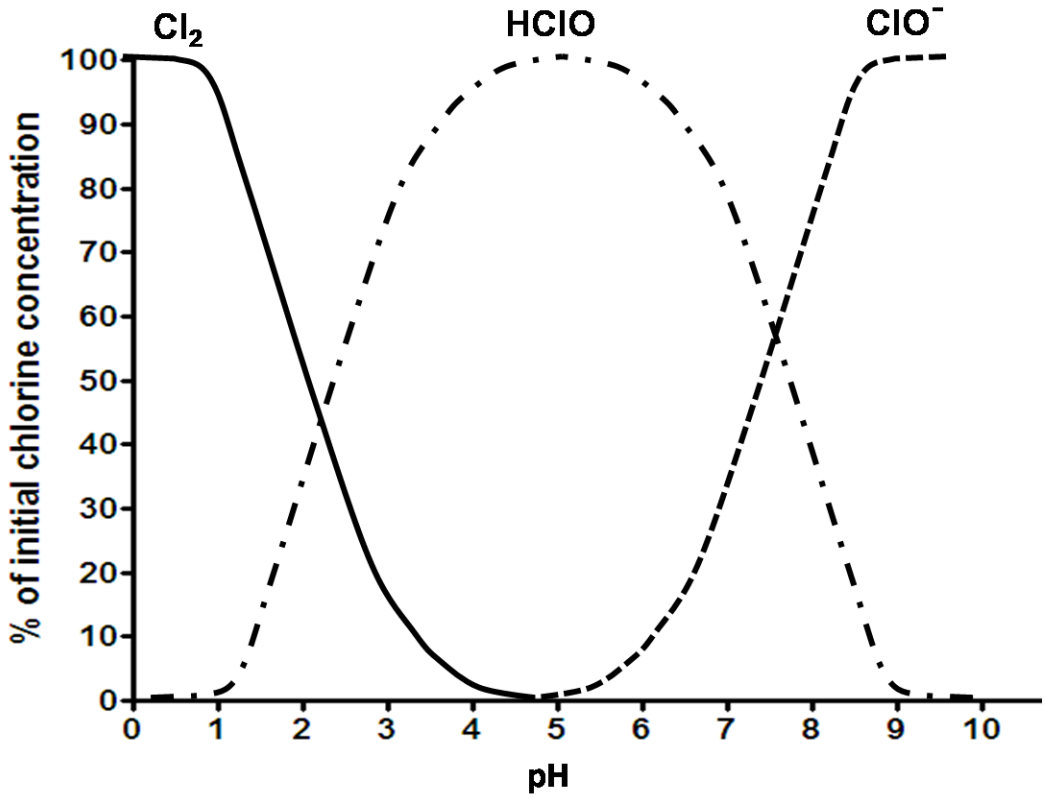

Figure 1. Theoretical pH profile of the concentration of free chlorine compounds (Cl_2, HClO, and ClO^-) when Cl_2 is dissolved in a saline aqueous medium[Ref2].

WORK PACKAGE (WP1) – Assessing the antimicrobial efficacy of the 'Toucan' unit

The physicochemical and antimicrobial properties of Electrochemically Activated Solutions (ECAS) generated using the Toucan unit were assessed according to industrial requirements.

WP1.1 - Physicochemical parameters of ECAS generated by the Toucan unit.

The physicochemical properties of ECAS generated by the Toucan unit were assessed to determine the pH, free activated chlorine (FAC; mg/L) and Oxidation Reduction Potential (ORP; mV). The Toucan unit was operated with 450 mL of tap water, 0.5 g salt (NaCl) and activated with a 5 Amp power supply (see figure 1.1). The results of physiochemical testing are shown in table 1.1. Standard operation of the Toucan unit requires a 'single activation' of 450 mL of tap water with 0.5 g of dissolved salt for 5 minutes. This resulted in a solution that had a slightly alkaline pH (~8.5), an elevated ORP of +767 mV indicating an oxidative solution had been produced, with a free chlorine concentration of 135.1 mg/L (table 1.1). To investigate the effects of 'double activation', 450 mL of tap water with 0.5 g of dissolved salt was activated for a total of 10 minutes. This resulted in a solution with a significantly higher pH (8.993; $p<0.001$), ORP (779.7 mV; $p<0.05$) and free chlorine concentration (210.0 mg/L; $P<0.001$), compared to the single activation solution (unpaired t-test; $P<0.05$ regarded as significant).

Table 1.1 Physicochemical properties of ECAS generated by a Toucan unit (n=9 ± standard deviation [SD]).

ECAS	Volume (mL ± SD)	Salt (g ± SD)	Power (A)	pH (± SD)	ORP (mV ± SD)	Free chlorine (mg/L ± SD)
Single activation	450 mL	0.5 g	5A	8.524 ± 0.129	767.0 ± 7.9	135.1 ± 3.5
Double activation	450 mL	0.5 g	5A	8.993 ± 0.052	779.7 ± 3.3	210.0 ± 5.8

WP1.2 - Bactericidal efficacy of ECAS generated using the Toucan unit.

The 'single activation' ECAS generated using the Toucan unit was assessed for antimicrobial efficacy using a standard suspension test in line with the methodology of EN testing protocol: ***BS EN 1276:2009 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1)***. Briefly, test organisms were used to prepare a standard suspension, mixed with bovine serum albumin (0.3g/L) and subjected to treatment with 'single activation' ECAS generated using the Toucan unit for 5 minutes. An aliquot of this test suspension was then neutralised and plated onto Tryptone Soya Agar to determine the number of viable survivors. Standard tests were also performed to validate the selected experimental conditions, verify the absence of toxicity of the neutralizer and validate the dilution neutralization method. The test organisms used within this assay are shown in table 1.2; these were chosen according to industrial priorities.

The results from the antimicrobial efficacy testing of 'single activation' ECAS generated using the Toucan unit are shown figures 1.2 – 1.5. The test solution elicited a significant antimicrobial effect ($P<0.001$) against all bacterial species tested (figures 1.2 – 1.5). The requirements of ***BS EN 1276:2009*** dictate that "The product shall demonstrate at least a 5 decimal log (lg) reduction". Therefore, 'single activation' ECAS generated using the Toucan unit possesses **bactericidal activity according to the condition of the test** (5 minutes at 20°C when undiluted, under clean conditions [0.3g/L bovine albumin]) for reference strains *E. coli* (figure 1.2), *S. aureus* (figure 1.4), MRSA (figure 1.5) and *L. monocytogenes* (figure 1.5).

Table 1.2 Microbial species used to test the antimicrobial efficacy of 'single activation' ECAS generated using the Toucan unit performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing.***

Bacterial Species	Strain	Brief description
Escherichia coli	ATCC 10536	Common food and environmental pathogen
***Salmonella enterica* serovar Typhimurium**	ATCC 14028	Common foodborne pathogen
Staphylococcus aureus	ATCC 6538	Common commensal and hospital acquired pathogen
Methicillin Resistant *Staphylococcus aureus* (MRSA)	Clinical strain (Llewlyn)	Antibiotic resistant commensal and hospital acquired pathogen
Listeria monocytogenes	Scott A	Common foodborne pathogen

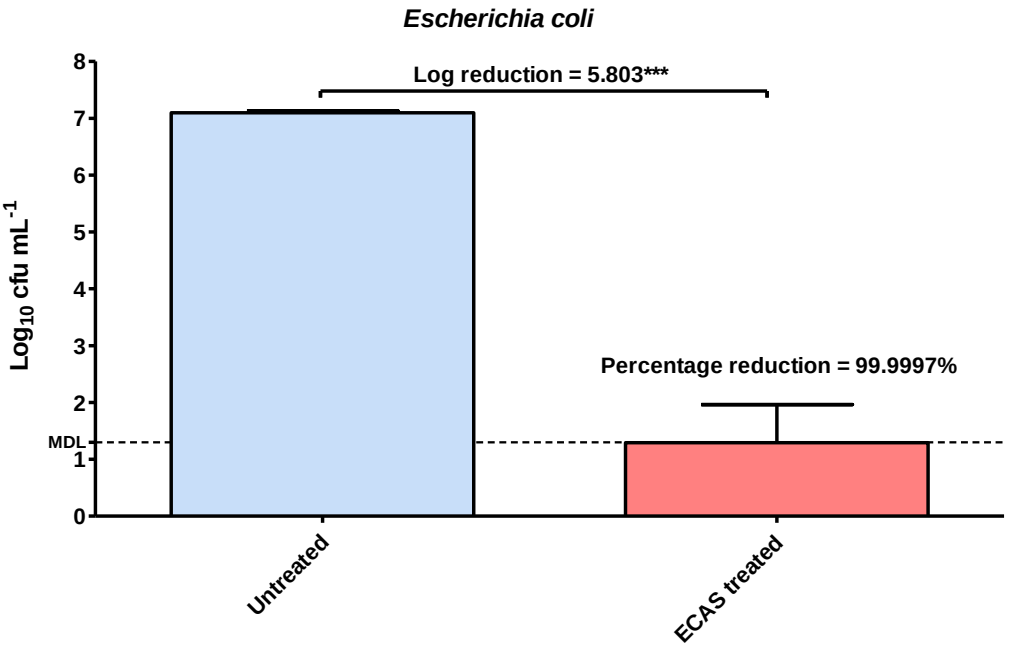

Figure 1.2 Viable *Escherichia coli* (log_{10} cfu mL^{-1}) recovered after no treatment (untreated) or after a 5 minute treatment with 'single activation' ECAS generated using the Toucan unit. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

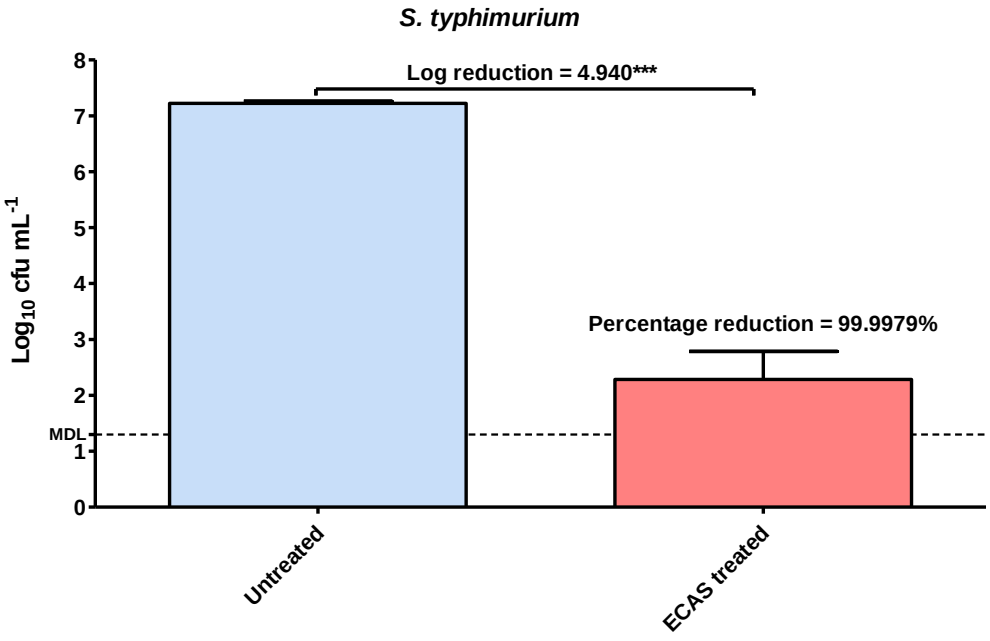

Figure 1.3 Viable *Salmonella enterica* serovar Typhimurium (log_{10} cfu mL^{-1}) recovered after no treatment (untreated) or after a 5 minute treatment with 'single activation' ECAS generated using the Toucan unit. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

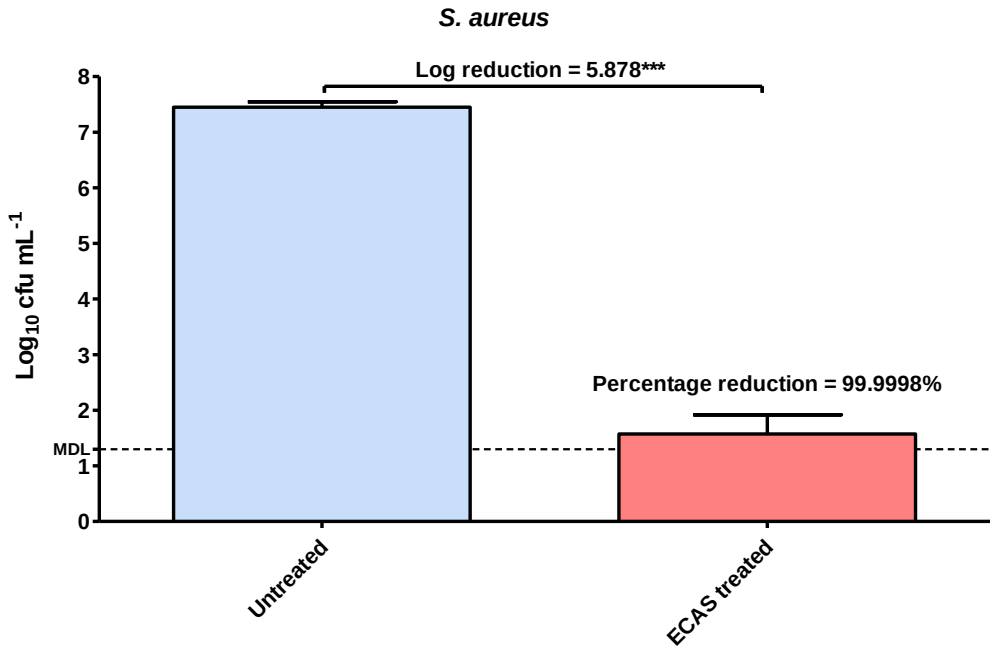

Figure 1.4 Viable *Staphylococcus aureus* (log_{10} cfu mL^{-1}) recovered after no treatment (untreated) or after a 5 minute treatment with 'single activation' ECAS generated using the Toucan unit. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

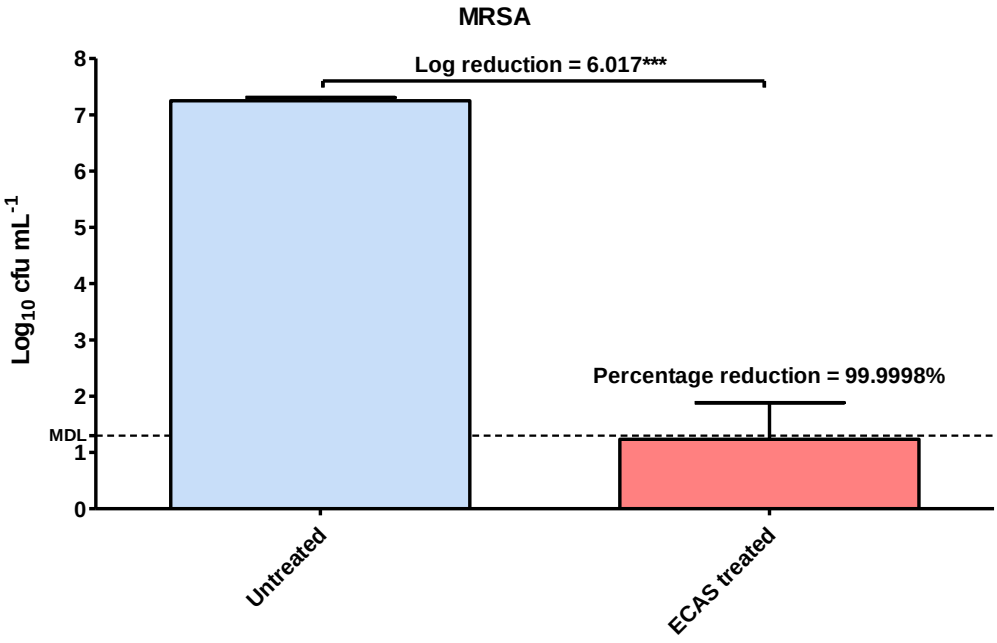

Figure 1.5 Viable Methicillin Resistant *Staphylococcus aureus* (MRSA; log_{10} cfu mL^{-1}) recovered after no treatment (untreated) or after a 5 minute treatment with 'single activation' ECAS generated using the Toucan unit. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

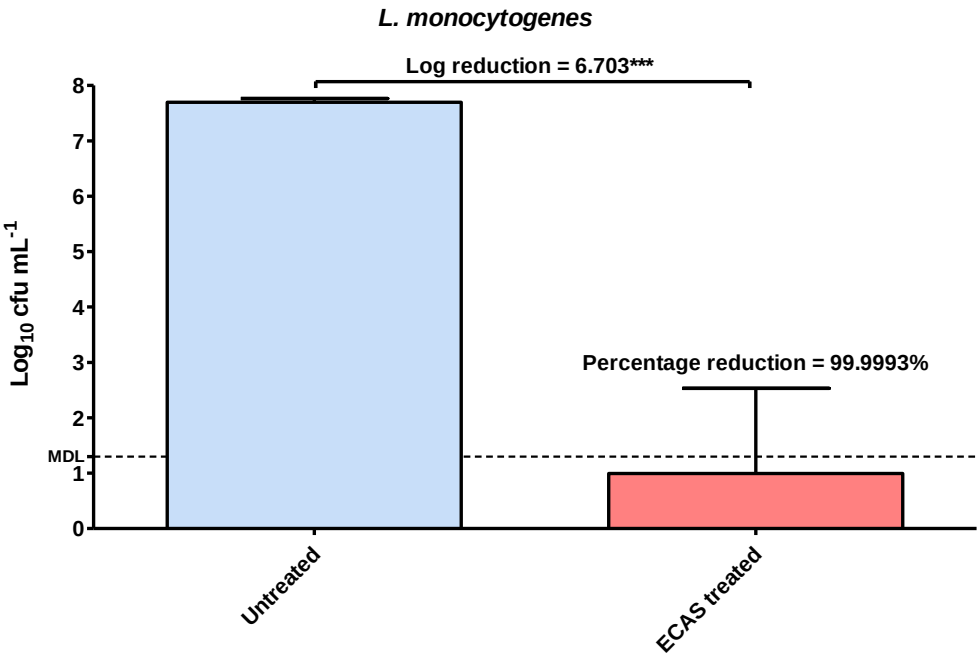

Figure 1.6 Viable *Listeria monocytogenes* (log_{10} cfu mL^{-1}) recovered after no treatment (untreated) or after a 5 minute treatment with 'single activation' ECAS generated using the Toucan unit. All experiments were performed according to ***EN 1276 Chemical Disinfectants Bactericidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

WP1.3 - Sporicidal efficacy of ECAS generated using the Toucan unit.

The 'single activation' and 'double activation' ECAS generated using the Toucan unit was assessed for antimicrobial efficacy using a standard suspension test in line with the methodology of EN testing protocol: ***BS EN 13704:2002 Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1).*** Briefly, a standard spore suspension of *Bacillus subtilis* (ATCC 6633) was mixed with bovine serum albumin (0.3g/L) and subjected to treatment with 'single activation' or 'double activation' ECAS generated using the Toucan unit for 60 minutes. An aliquot of this test suspension was then neutralised and plated onto Glucose Yeast Extract Agar (GYA) to determine the number of viable survivors. Standard tests were also performed to validate the dilution neutralization method.

The results from the antimicrobial efficacy testing of 'single activation' and 'double activation' ECAS generated using the Toucan unit are shown in figure 1.7. The 'single activation' ECAS elicited no sporicidal activity. In contrast, the 'double activation' ECAS elicited a significant 5.60 $\log_{10}$ cfu reduction in spores of *B. subtilis* after 60 minutes. The requirements of ***BS EN 13704:2002*** dictate that "The product… shall demonstrate at least a 10^3 reduction in viable counts". Therefore, 'double activation' ECAS generated using the Toucan unit possesses **sporicidal activity according to the condition of the test** (60 minutes at 20°C when undiluted, under clean conditions [0.3g/L bovine albumin]) for spores of reference strain *B. subtilis*.

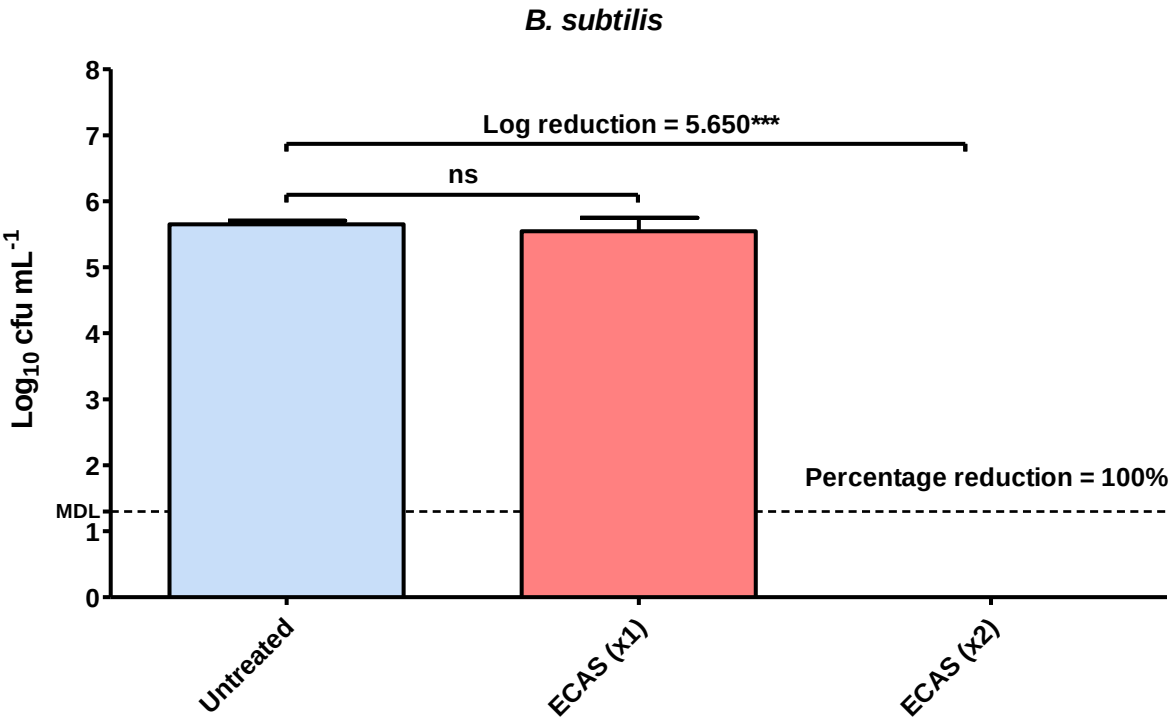

Figure 1.7 Viable spores of *Bacillus subtilis* (log_{10} cfu mL^{-1}) recovered after no treatment (untreated), a 60 minute treatment with 'single activation' ECAS (ECAS x1) or 'double activation' treatment (ECAS x2) generated using the Toucan unit. All experiments were performed according ***BS EN 13704:2002 Chemical Disinfectants Sporicidal Activity Testing*** (clean conditions; n=3 ± SD). Hatched line shows the minimum detection limit (MDL) for the assay.

WP1.4 – Real time antimicrobial kinetics of ECAS generated by the Toucan unit.

A bioluminescent reporter strain capable of continually producing light when metabolically active was used to assess the antimicrobial kinetics of **'**single activation' ECAS generated using the Toucan unit. This required the modification of a multi-well assay plate to incorporate 6 injection ports (syringe needles), to enable direct injection of test solutions. The experimental set-up can be seen in figure 1.8. A 1 mL standard suspension (1×10^8 cfu mL^{-1}) of bioluminescent *E. coli* (expressing the *lux* genes) within diluent (Tryptone 0.1%, NaCl 0.85%) was pipetted into 9 wells to be used for light control (n=3), water treatment (n=3) and ECAS treatment (n=3), an additional 3 wells were used as a dark control (see figure 1.8). The plate was then positioned below a low light photon counting camera (Andor iXon EMCCD) within a bespoke low light imaging suite. The light produced by the bacteria was continuously monitored (consecutive 10 second exposures) for ~2 minutes, before remotely injecting in 1 mL of water into each of the 'water treatment' wells, and 1 mL of 'single activation' ECAS generated using the Toucan unit into each of the 'ECAS treatment' wells (figure 1.8). The light produced by the bacteria was continuously monitored (consecutive 10 second exposures) for a further 1 hour to enable the real time antimicrobial kinetics to be determined. After 1 hour, 1 mL of Tryptone Soya Broth was added into each of the test wells and the light produced by the bacteria continuously monitored (consecutive 10 second exposures) for a further 24 hours to enable detection of microbial regrowth. All images were analysed post-capture using ImageJ (Java-based image processing program developed at the National Institutes of Health), and results reported as mean Relative Light Units (RLU) per well.

The results from the antimicrobial kinetics of **'**single activation' ECAS generated using the Toucan unit are shown in figure 1.9. For the water treated wells, a small 'spike' in mean RLU per well immediately after treatment is observed, due to oxygenation of the bioluminescent bacteria (as would be expected). Over the next 1 hour a steady decrease in mean RLU per well after water treatment is observed, likely to be the result of nutrient (and therefore metabolic) exhaustion. For the ECAS treated wells, a rapid and significant reduction in mean RLU per well is observed, and is indistinguishable from the dark control (background light levels) after 90 seconds, indicative of complete metabolic inhibition. The results from the regrowth experiment are shown in figure 1.10. For the water treated wells, a steady increase in mean RLU per well is observed over the first 18 hours, indicative of active microbial growth and metabolism, before nutrient (and therefore metabolic) exhaustion

occurs again at 24 hours. For the ECAS treated wells, there is no increase in mean RLU per well observed after addition of nutrient. The mean RLU per well is not significantly different from dark control levels, and therefore it can be concluded that the reduction in light level observed after ECAS treatment is a true reflection of the fast acting antimicrobial kinetics of ‘single activation’ ECAS generated using the Toucan unit.

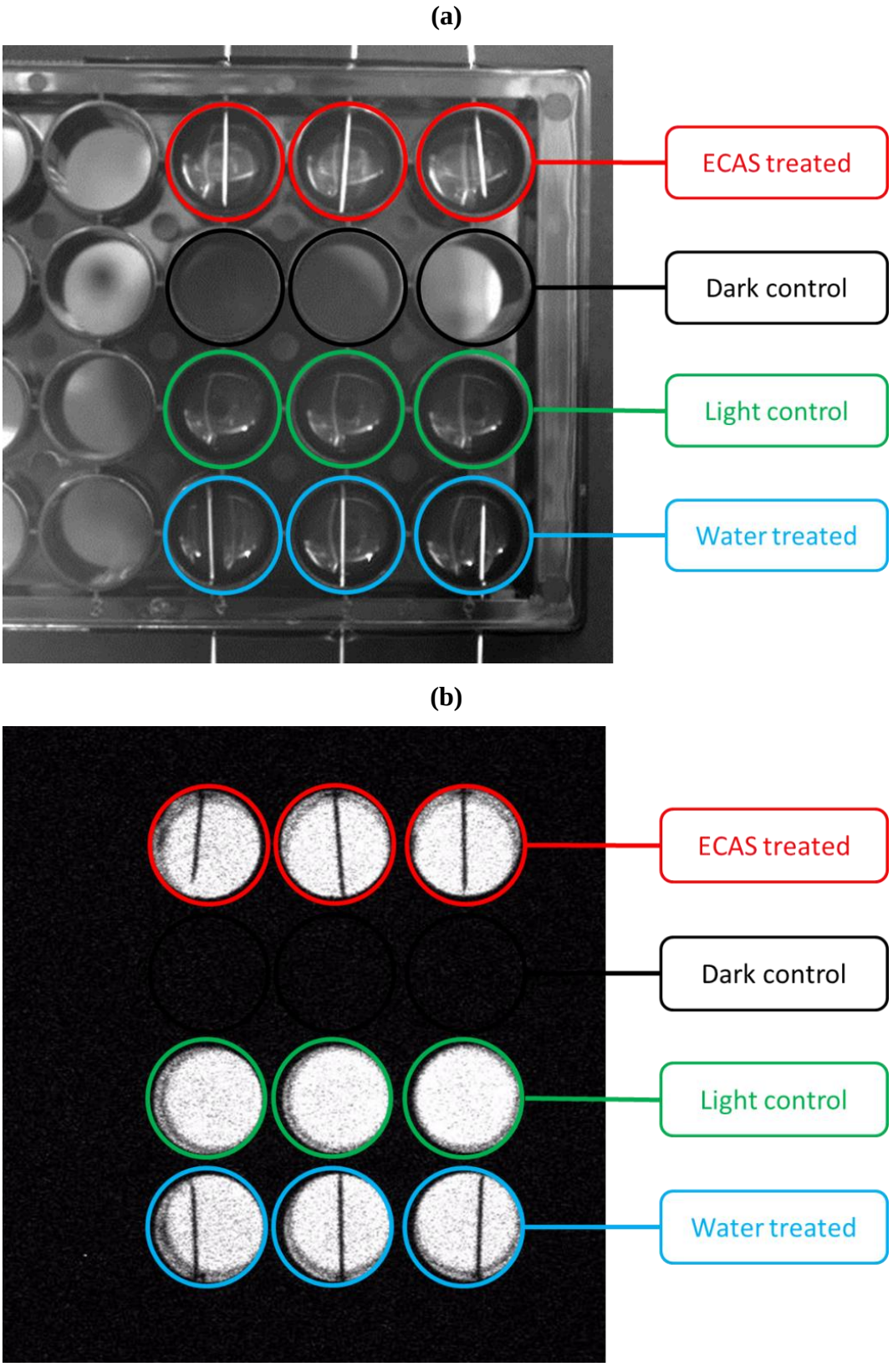

Figure 1.8 (a) Light and **(b)** dark image of bioluminescent *Escherichia coli* within a multi-well assay plate at the beginning of an assay used to assess the effects of ECAS generated by the Toucan unit *in-situ* and in real time.

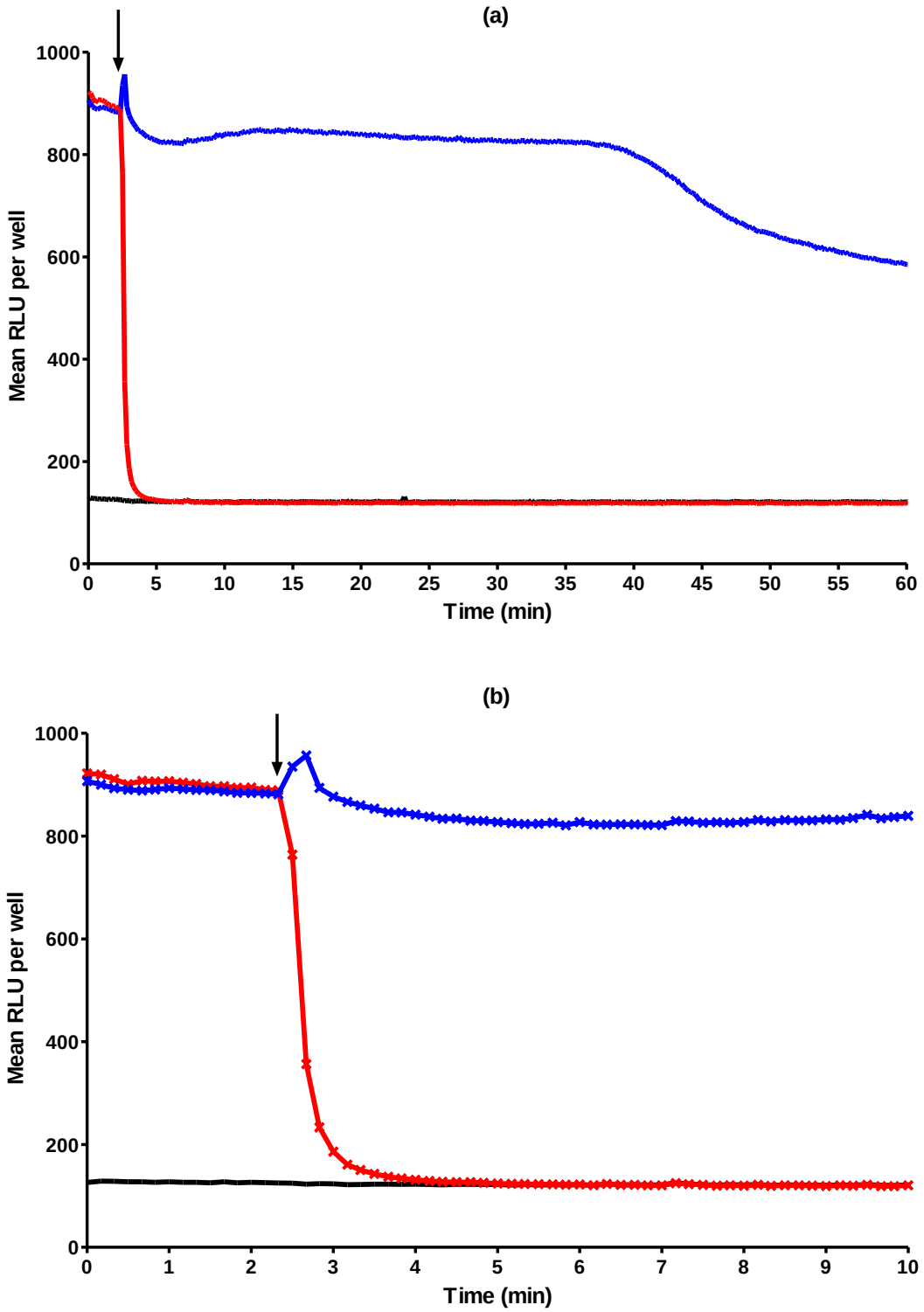

Figure 1.9 Mean light output measured in Relative Light Units (RLU) over **(a)** 1 hour and **(b)** 10 minutes, from a bioluminescent *Escherichia coli* treated with water (**blue line**) or ECAS generated by the Toucan unit (**red line**) within a multi-well plate assay (n=3). Black line shows the dark control background light levels and therefore the minimum detection limit for the assay. Arrow signifies when treatment occurred.

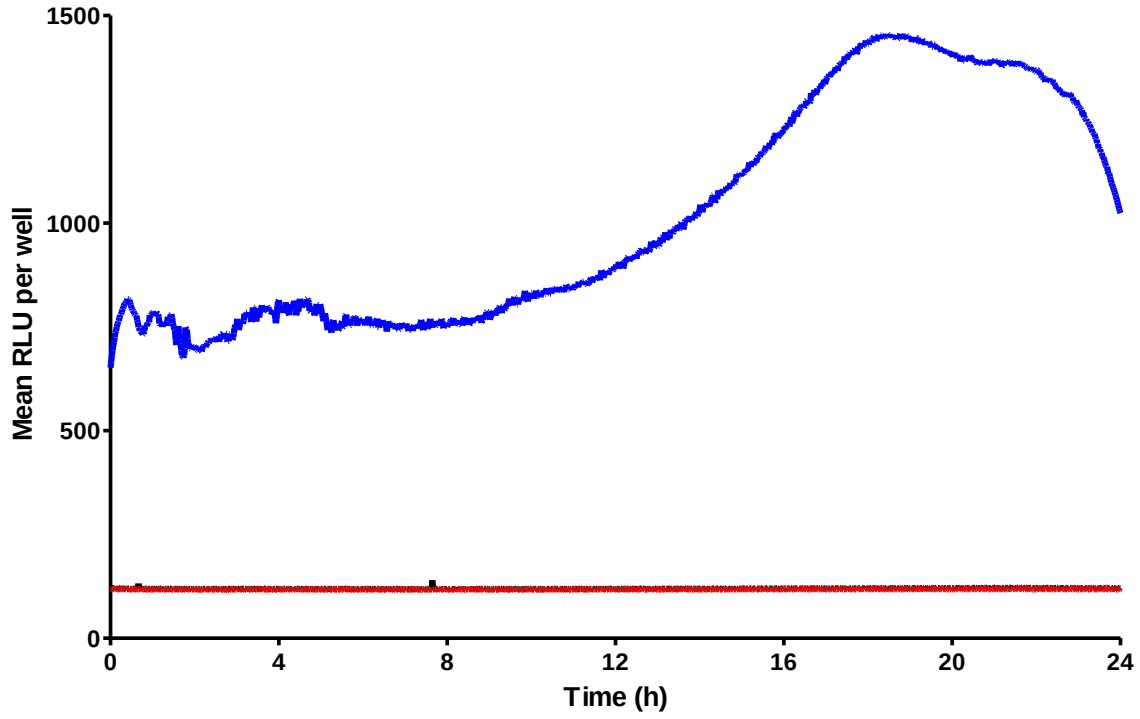

Figure 1.10 Mean light output measured in Relative Light Units (RLU) over 24 hours from a bioluminescent *Escherichia coli* after addition of nutrients (at time 0) 1 h after treatment with water (**blue line**) or ECAS generated by the Centrego Toucan unit (**red line**) within a multi-well plate assay (n=3). Black line shows the dark control background light levels and therefore the minimum detection limit for the assay.

WORK PACKAGE 2 (WP2) – Assessing the carpet disinfection capability of a Biostream ECAS integrated Oztek cleaning system

Electrochemically Activated Solutions (ECAS) at 1000 ppm were generating using a Biostream™ 500 anolyte generator using 2.0 g/L of salt (termed Biostream ECAS). These solutions were then used in conjunction with Oztek (UK) Ltd. carpet cleaning equipment.

WP2.1 - Laboratory trial of the carpet disinfection capability of a Biostream ECAS integrated Oztek cleaning system

A Biostream ECAS integrated Oztek cleaning system was assessed for carpet disinfection capability using a laboratory based assay. Two dirty carpet sample types (commercial and care home) were provided by Oztek for experimentation. Carpet tile samples (1000 x 1000 mm) were assessed for microbial loading pre and post treatment using:

1. ***Adenosine Triphosphate (ATP) swab testing;*** detection of ATP was used as an indicator of surface cleanliness. Carpet sample squares (40 x 40 mm; n=5) were swabbed pre and post treatment using an UltraSnap™ ATP surface test (Hygiena International, UK).
2. ***Contact plating;*** the level of viable recoverable bacteria or fungi on the surface of the carpet samples (40 x 40 mm; n=5) was assessed pre and post treatment using Plate Count Agar contact plates or Sabouraud Dextrose Agar contact plates, respectively.

All data was analysed using GraphPad Prism for windows using an unpaired t-test ($P<0.05$ regarded as significant).

Commercial carpet results

The results from the ATP swab test of commercial carpet pre and post cleaning with the Biostream ECAS integrated Oztek cleaning system are shown in figure 2.1. The Biostream ECAS integrated Oztek cleaning system resulted in a significant reduction in the RLU per swab pre and post treatment ($P<0.001$). However, it should be noted that all RLU per swab levels obtained were below the cautionary or fail levels for the assay. The results from the contact plate testing of commercial carpet pre and post cleaning with the Biostream ECAS integrated Oztek cleaning system are shown in figure 2.2. The Biostream ECAS integrated Oztek cleaning system resulted in a significant reduction ($P<0.001$) in the number of viable recoverable bacteria (figure 2.1a) and viable recoverable fungi (figure 2.2b).

Care home carpet results

The results from the ATP swab test of care home carpet pre and post cleaning with the Biostream ECAS integrated Oztek cleaning system are shown in figure 2.3. The Biostream ECAS integrated Oztek cleaning system resulted in a significant reduction in the RLU per swab pre and post treatment ($P<0.001$). However, it should be noted that all RLU per swab levels obtained were below the cautionary or fail levels for the assay. The results from the contact plate testing of care home carpet pre and post cleaning with the Biostream ECAS integrated Oztek cleaning system are shown in figure 2.4. The Biostream ECAS integrated Oztek cleaning system resulted in a significant reduction ($P<0.001$) in the number of viable recoverable bacteria (figure 2.4a) and viable recoverable fungi (figure 2.4b).

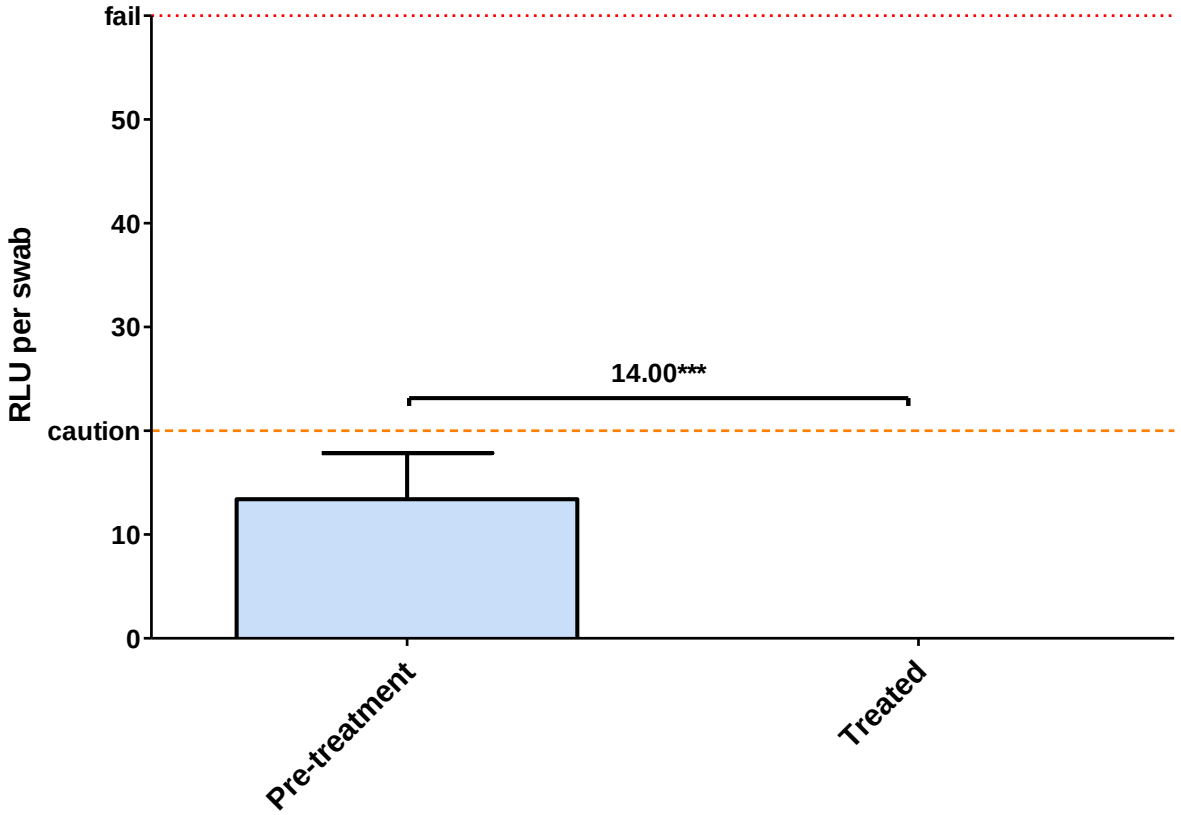

Figure 2.1 Relative Light Units (RLU) per ATP Surface Test swab from 'commercial environment' carpet samples pre-and post- treatment with an ECAS integrated Oztek cleaning system (n=5 ± SD).

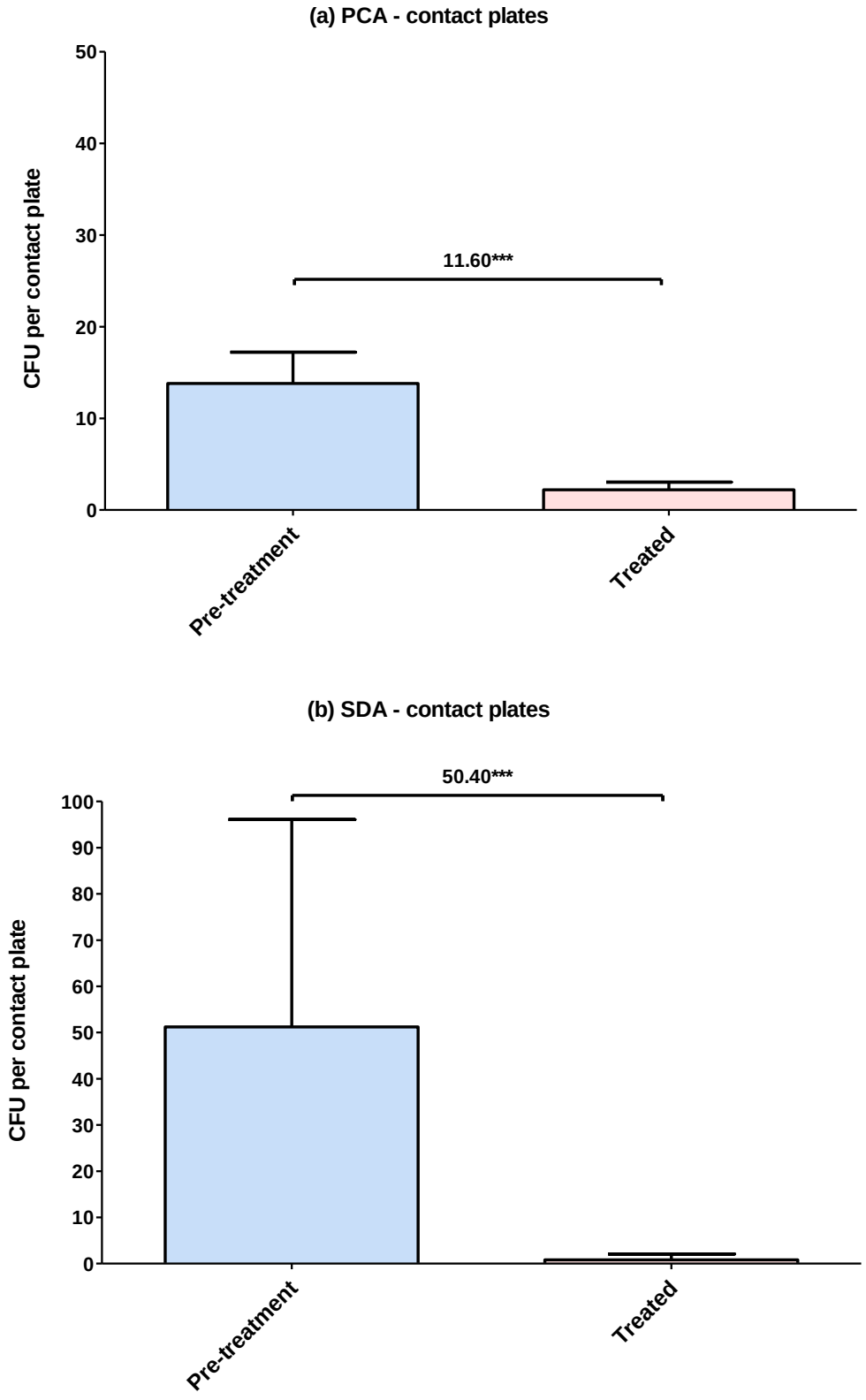

Figure 2.2 Colony Forming Units (CFU) of **(a)** bacteria recovered on Plate Count Agar (PCA) contact plates and **(b)** yeast/fungi recovered on Sabouraud Dextrose Agar (SDA) contact plates from 'commercial environment' carpet samples pre-and post- treatment with an ECAS integrated Oztek cleaning system (n=5 ± SD).

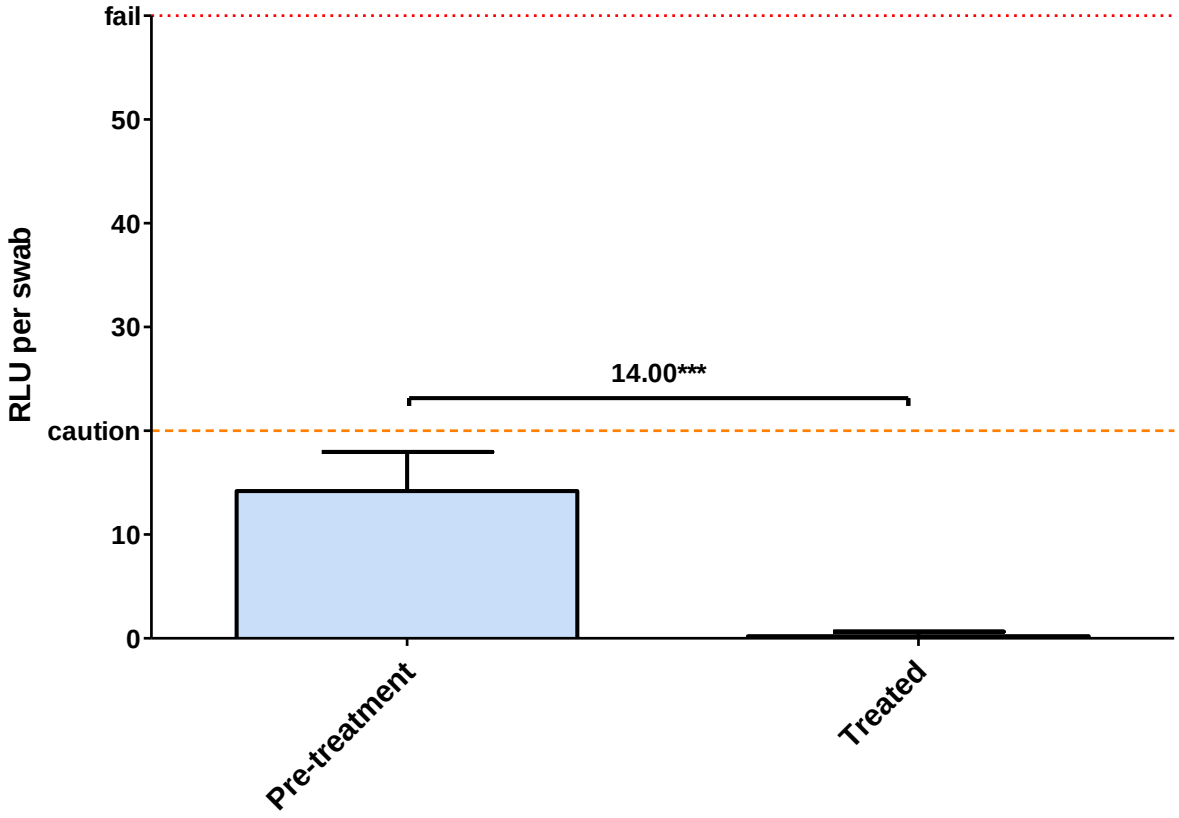

Figure 2.3 Relative Light Units (RLU) per ATP Surface Test swab from 'care home' carpet samples pre-and post- treatment with an ECAS integrated Oztek cleaning system (n=5 ± SD).

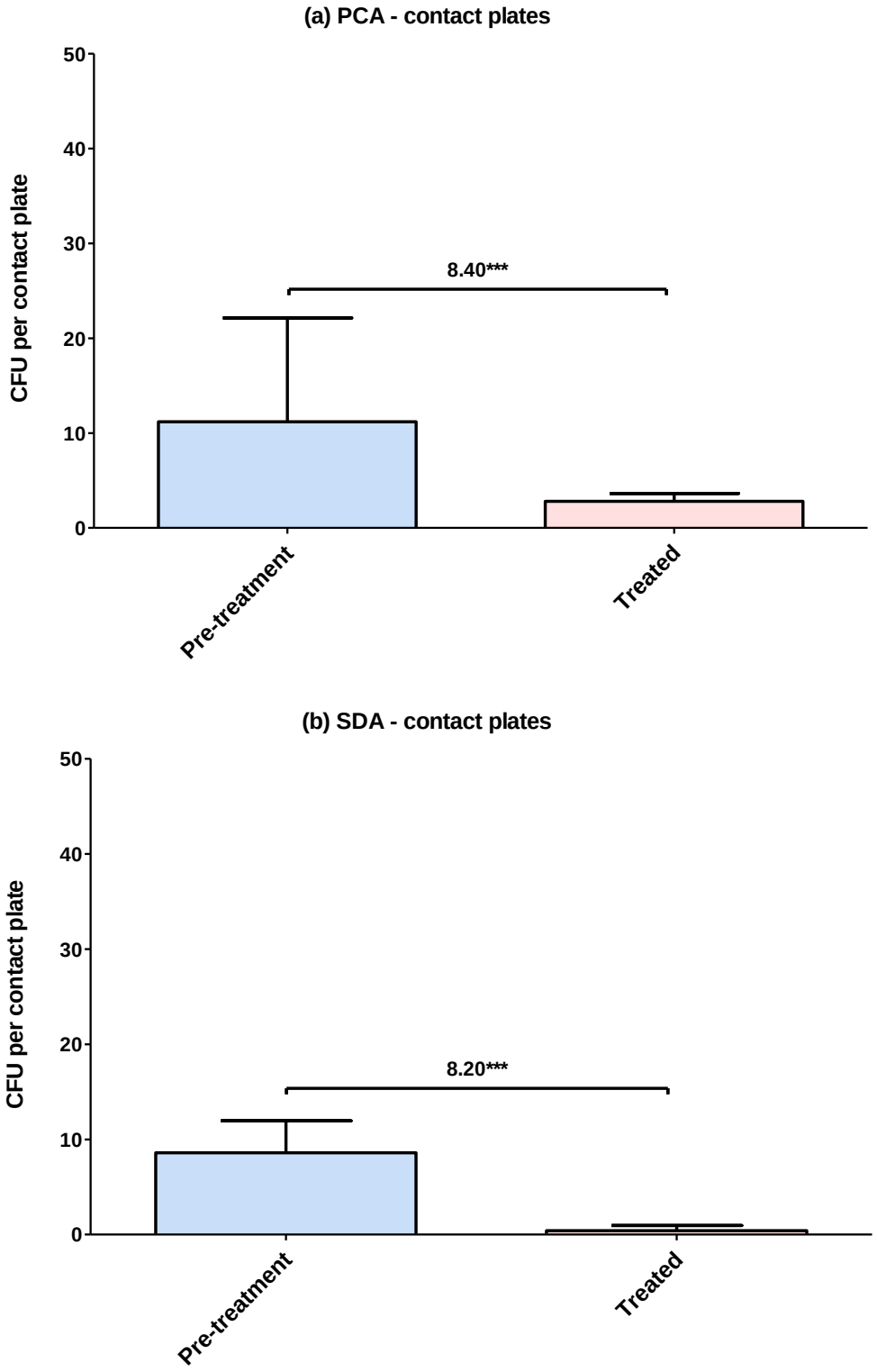

Figure 2.4 Colony Forming Units (CFU) of **(a)** bacteria recovered on Plate Count Agar (PCA) contact plates and **(b)** yeast/fungi recovered on Sabouraud Dextrose Agar (SDA) contact plates from 'care home' carpet samples pre-and post- treatment with an ECAS integrated Oztek cleaning system (n=5 ± SD).

WP2.2 – Real world trial of the carpet disinfection capability of a Biostream ECAS integrated Oztek cleaning system within a care home setting

A Biostream ECAS integrated Oztek cleaning system was assessed for carpet disinfection capability within a Bristol care home. Two 0.5 x 1 m areas of an in-use care home unit were delineated and assigned to either a ***Biostream ECAS integrated Host Oztek cleaning system*** treatment group or ***Biostream ECAS integrated Sebo Oztek cleaning system*** treatment group. The carpet areas were assessed for cleanliness and microbial loading pre and post treatment using:

1. ***Adenosine Triphosphate (ATP) swab testing;*** detection of ATP was used as an indicator of surface cleanliness. Carpet sample squares (40 x 40 mm; n=5) were swabbed pre and post treatment using an UltraSnap^{TM} ATP surface test (Hygiena International, UK).
2. ***Contact plating;*** the level of viable recoverable bacteria or fungi on the surface of the carpet samples (40 x 40 mm; n=5) was assessed pre and post treatment using Plate Count Agar contact plates or Sabouraud Dextrose Agar contact plates, respectively.

All data was analysed using GraphPad Prism for windows using an unpaired t-test ($P<0.05$ regarded as significant).

Biostream ECAS integrated Host Oztek cleaning system

The results from the ATP swab test of commercial carpet pre and post cleaning with the Biostream ECAS integrated Host Oztek cleaning system are shown in figure 2.7. The Biostream ECAS integrated Host Oztek cleaning system resulted in a significant reduction in the RLU per swab pre and post treatment ($P<0.001$). However, it should be noted that all RLU per swab levels obtained were below the cautionary or fail levels for the assay. The results from the contact plate testing of commercial carpet pre and post cleaning with the Biostream ECAS integrated Host Oztek cleaning system are shown in figure 2.8. The Biostream ECAS integrated Host Oztek cleaning system resulted in a significant reduction ($P<0.001$) in the number of viable recoverable bacteria (figure 2.8a) and viable recoverable fungi (figure 2.8b).

Biostream ECAS integrated Sebo Oztek cleaning system

The results from the ATP swab test of commercial carpet pre and post cleaning with the Biostream ECAS integrated Sebo Oztek cleaning system are shown in figure 2.9. The Biostream ECAS integrated Sebo Oztek cleaning system resulted in a significant reduction in the RLU per swab pre and post treatment ($P<0.001$). However, it should be noted that all RLU per swab levels obtained were below the cautionary or fail levels for the assay. The results from the contact plate testing of commercial carpet pre and post cleaning with the Biostream ECAS integrated Sebo Oztek cleaning system are shown in figure 2.10. The Biostream ECAS integrated Sebo Oztek cleaning system resulted in a significant reduction ($P<0.001$) in the number viable recoverable fungi (figure 2.10b), but no significant reduction in the number of viable recoverable bacteria (figure 2.10a).

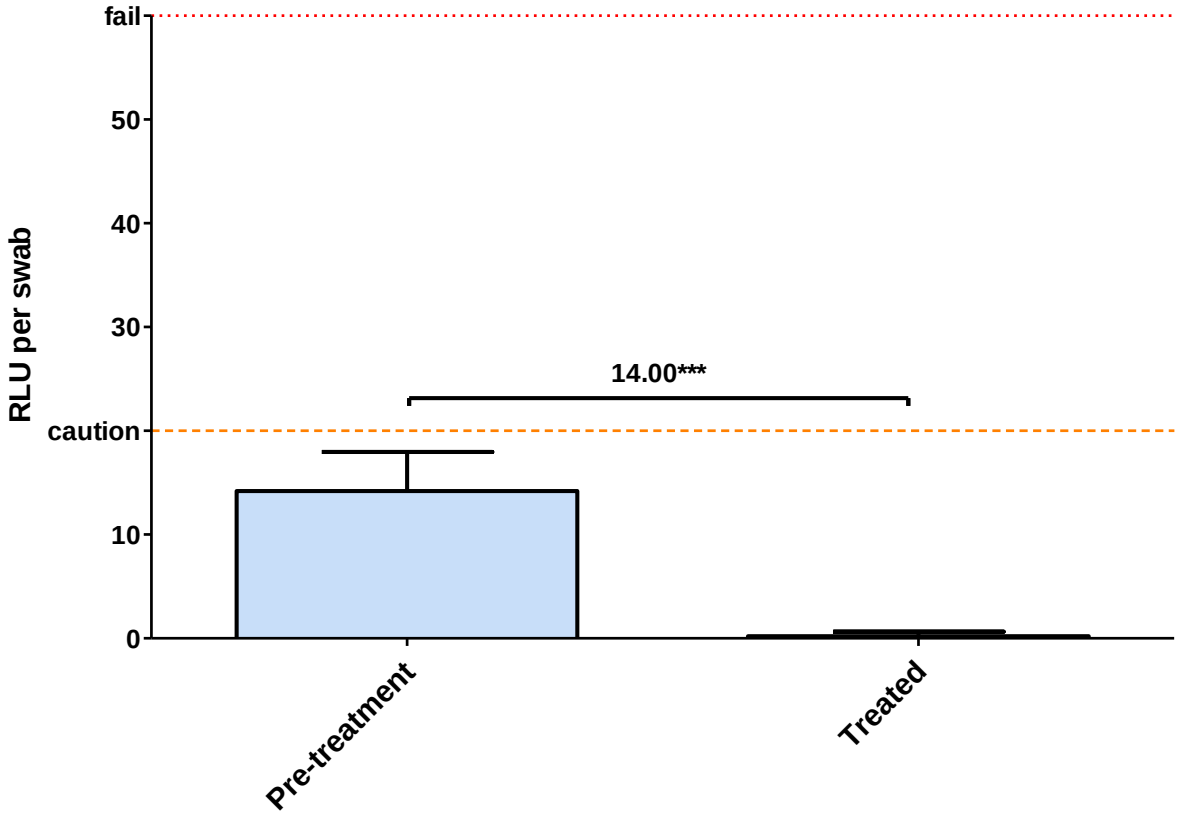

Figure 2.7 Relative Light Units (RLU) per ATP Surface Test swab from 'care home' carpet tested in situ pre-and post- treatment with an ECAS integrated Host Oztek cleaning system (n=5 ± SD).

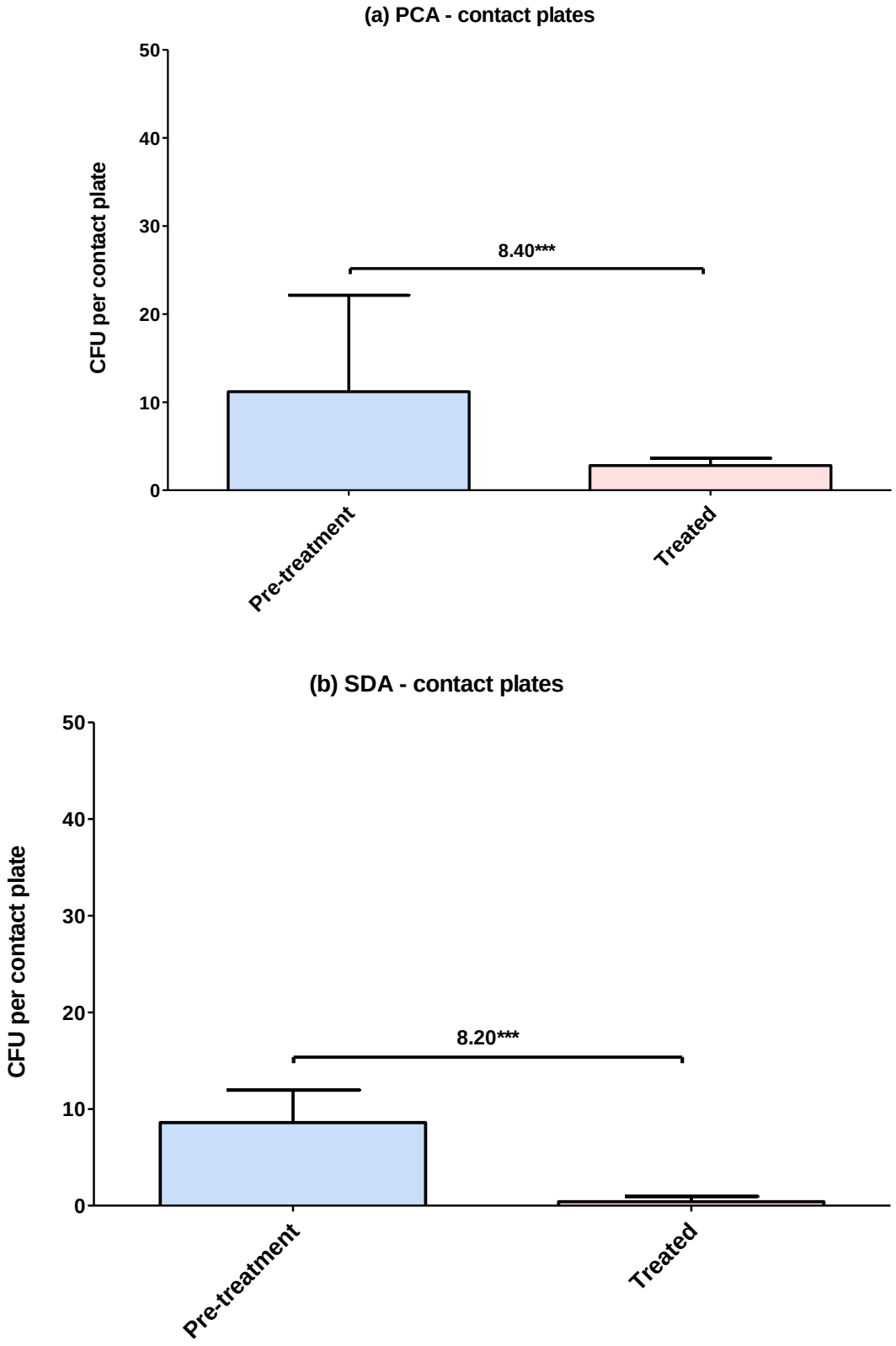

Figure 2.8 Colony Forming Units (CFU) of **(a)** bacteria recovered on Plate Count Agar (PCA) contact plates and **(b)** yeast/fungi recovered on Sabouraud Dextrose Agar (SDA) contact plates from 'care home' carpet tested in situ pre-and post- treatment with an ECAS integrated Host Oztek cleaning system (n=5 ± SD).

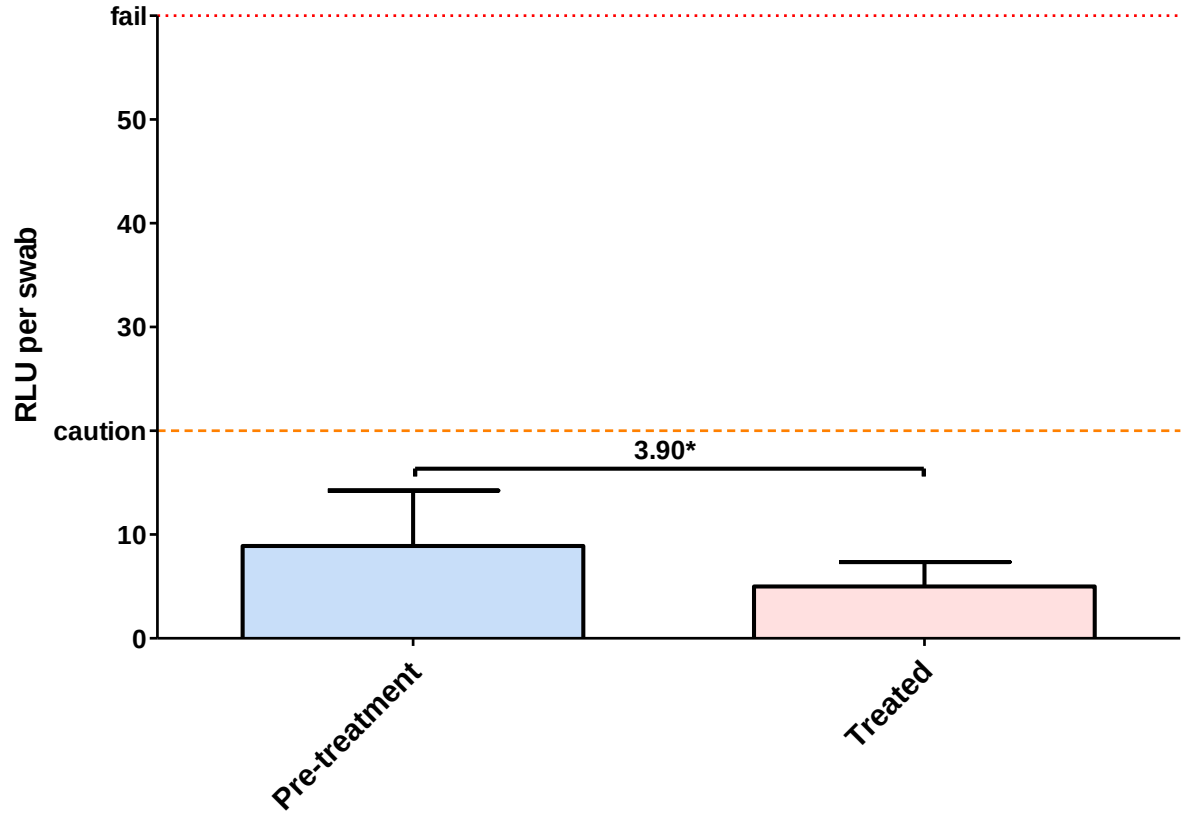

Figure 2.9 Relative Light Units (RLU) per ATP Surface Test swab from 'care home' carpet tested in situ pre-and post- treatment with an ECAS integrated Sebo Oztek cleaning system (n=5 ± SD).

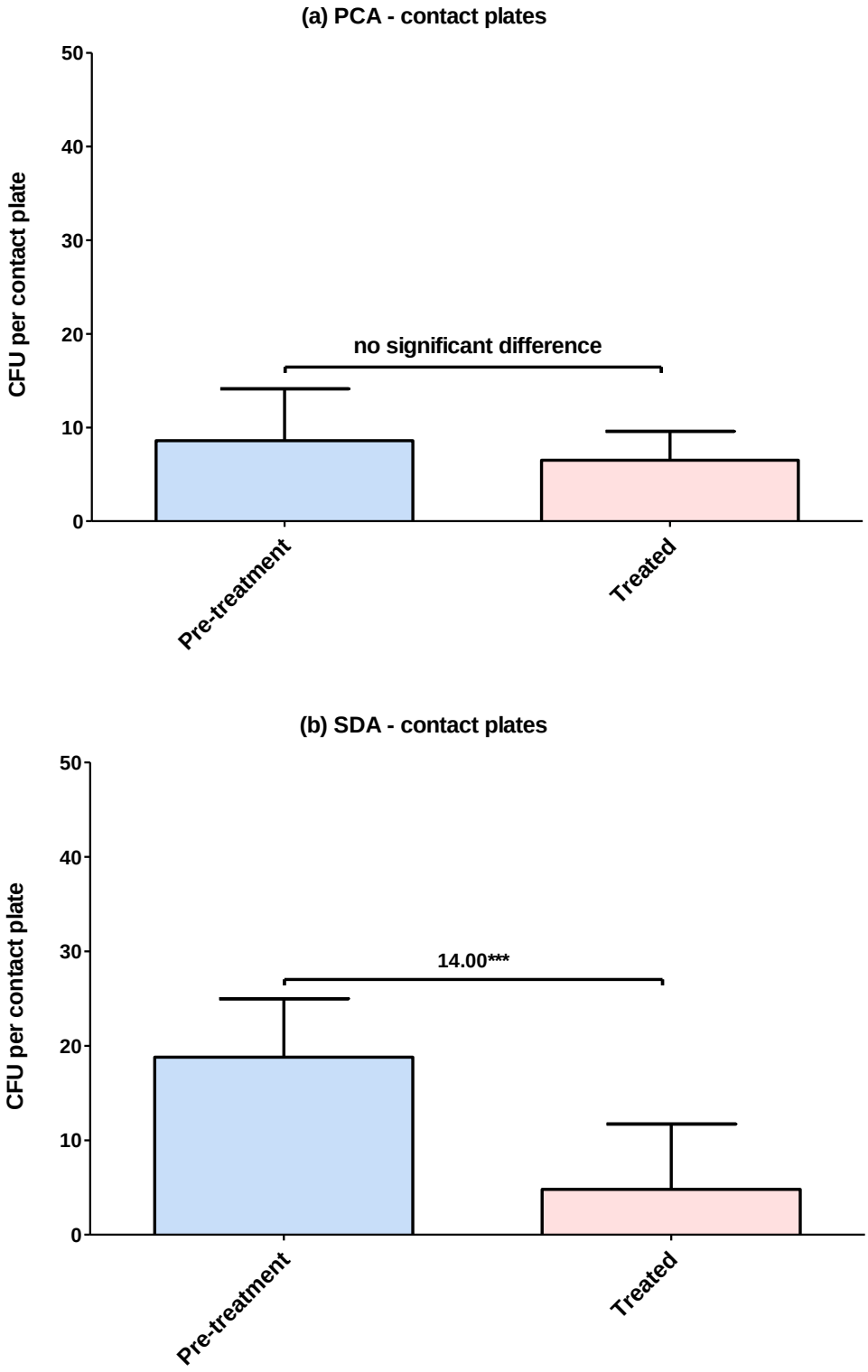

Figure 2.10 Colony Forming Units (CFU) of **(a)** bacteria recovered on Plate Count Agar (PCA) contact plates and **(b)** yeast/fungi recovered on Sabouraud Dextrose Agar (SDA) contact plates from 'care home' carpet tested in situ pre-and post- treatment with an ECAS integrated Sebo Oztek cleaning system (n=5 ± SD).

SUMMARY DISCUSSION & FUTURE WORK

The toucan unit has been shown to produce consistent ECAS in terms of physicochemical parameters, with an elevated ORP and free chlorine concentration. The 'single activation' ECAS exhibited a broad spectrum of activity against both Gram positive (*S. aureus*, MRSA and *L. monocytogenes*) and Gram negative (*E. coli* and *S.* Typhimurium) microorganisms. The 'single activation' ECAS did not exhibit sporicidal activity; however, by performing a 'double activation' the ECAS produced by the toucan unit was able to exhibit significant sporicidal activity, due to the elevated free chlorine concentration (some of which will be in the form of hypochlorous acid). Bacterial endospores are one of the most resistant microbial structures to disinfectants and antiseptics[Ref4], therefore good level of activity against this target is likely to indicate good levels of anti-viral and anti-fungal activity, given the non-specific antimicrobial mode of action ECAS.

A bespoke antimicrobial kinetic assay was used to assess the real time antimicrobial activity of 'single activation' ECAS. Under the conditions of this test, ECAS was shown to be fast acting against a high microbial load, eliciting a significant response within 10 seconds and completely reducing metabolic activity within 90 seconds, with no observed regrowth. This is in line with previous research at UWE, which has shown that ECAS are fast acting, and in the absence of organic loading outperform ethanol, virkon and bleach in terms of the speed of kill[Ref8].

It is important to understand the in-use application of ECAS, to ensure that the ECAS delivered is fit for purpose and capable of achieving significant levels of microbial control relevant to the setting in which it is applied. This decision making process should be evidence based and supported by experimental research. This study has demonstrated that under laboratory

conditions ECAS is capable of eliciting significant antimicrobial activity, but further research on microbial communities more relevant to in situ conditions (e.g. biofilms) will be required to truly understand these processes.

A clear example of this was the collaborative design and implementation of the use of ECAS generated by the Biostream™ 500 anolyte generator and Oztek cleaning system for carpet disinfection. Within both the laboratory and in a real world setting (care home unit) this approach was found to significantly reduce surface microbial loading.

Overall, this study has shown ECAS generated by the Toucan unit to have significant antimicrobial activity against bacteria and spores, and has the potential to be exploited for a range of applications. One such application is carpet disinfection, and a Biostream integrated Oztek cleaning system has been shown capable of significantly reducing the surface microbial loading of commercial and care home carpets.

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