LENOVA®

DESIGN INSPIRED BY FUNCTION

Table of Contents

FINAL REPORT

Antimicrobial Performance of Ozone System

Mechanical and Electronic Engineering

School of Engineering, Physics and Mathematics

University of Dundee

1. Introduction

Lenova produces an ozone device for generating ozone water for a range of applications. The University of Dundee will collaborate with the company to evaluate the anti-microbial efficiency of ozone water from the device. As ozone has strong antimicrobial properties, ozoned water can be used as a surface sanitizer in food preparation, cooking and serving areas and general kitchen surfaces. Furthermore, ozone is at least 100x stronger and reacts 3100x faster than chlorine as a disinfectant. Chlorine reacts with organisms (bacteria, meat) forming highly toxic carcinogen compounds called THMs (tri-halomethanes). By comparison, ozone leaves no trace of residual products upon oxidative reaction. The use of ozone water will reduce chemicals needed by 50% and food spoilage by up to 50%. The results will help to improve their ozone generator efficiency and their products' competitiveness.

Biofilms occur in a wide variety of systems, such as in food preparation, cooking and serving areas and general kitchen surfaces. The bacteria in a biofilm are more resistant to antimicrobial agents as microbes excrete polymeric exopolysacharrides (EPS) film to protect them from the conventional antimicrobial reagents. The objective of this project is to evaluate the efficacy of ozonated water against biofilm formation originated from both gram-positive bacteria and gram-negative bacteria. Supported by Innovation Voucher Scheme Ay 2013-14, Scottish Funding Council, the anti-bacterial efficiency of Lenova' s ozone device has been evaluated.

2. Materials and methods

2.1 Types of bacteria

In this study, the antimicrobial assays of ozonated water collected from the ozone device (Lenova) were performed at the Biological and Nanomaterials Lab, University of Dundee. The antimicrobial effects of ozonated water were evaluated against both gram-positive bacteria (*Staphylococcus aureus* (F1557)) and gram-negative bacteria (*Escherichia coli WT* (F1693), *Pseudomonas aeruginosa* (ATCC 33347)), which were obtained from Institute of Infection and Immunity, Nottingham University, UK. The strains were subcultured and preserved in 15% glycerol in TSB (Tryptone Soya Broth, Oxoid®, UK) as frozen stock at −80 °C. For all microbial tests, TSA (Tryptone Soya Agar) plates were streaked out with a loop from the frozen stock and grown overnight at 37 °C. A single colony was inoculated in 10 ml TSB and grown statically overnight at 37 °C. 500 ml from this culture were further inoculated into 100 ml TSB in a conical flask and grown in a shaker-incubator at 37 °C and 250 rpm. The culture was grown to mid-exponential phase. The strains were harvested by centrifuging at 4500 rpm for 5 min at −4 °C, washed once in sterile distilled water and re-suspended in PBS (Phosphate Buffered Saline) at a 10^9 CFU/ml concentration.

2.2 Antimicrobial assays

Antimicrobial assays were performed using standard protocols. Briefly, the bacterial suspension with a 10^6 CFU/ml concentration for each type of the 3 bacteria was prepared. Six replicate standard glass slides were immersed vertically in a glass tank containing 500 ml of a bacterial suspension and were incubated on a shaker at 20 rpm for 24 hours at 37˚C. After that, each glass slide was taken out from the tank using sterile forceps and was dipped twice vertically in sterile distilled water with a custom-made automated dipper apparatus under a constant speed of 0.03 m/s in order to remove loosely attached bacteria. In order to evaluate the antimicrobial effects of ozonated water, samples were vertically immersed in a tank containing 500ml freshly collected ozonated water at 25 ˚C for 1s, 5s, 10s, 30s and 1min, respectively. In this study, the LIVE/DEAD BacLightbacterial viability kit was used for the enumeration of bacteria on the glass slides. The kit consists of two nucleic acid stains: SYTO 9, which penetrates most membranes freely, and propidium iodide, which is highly charged and normally does not permeate cells but does penetrate damaged membrane. Simultaneous application of both dyes therefore results in green fluorescence of viable cell with an intact

membrane, whereas dead cells, because of a compromised membrane, show intense red fluorescence. Bacteria on samples were then stained using the LIVE/DEAD BacLightbacterial viability kit for 15 minutes and observed under the fluorescence microscope (OLYMPUS BX 41, Japan) and counted using Image Pro Plus software (Media Cybernetics, USA).

3. Result and discussion

Fig 1 shows the effect of contact time on inactivation *E. coli*. When contact time increases to 60 second (1 min), 99.9% *E. coli* were killed or only 0.1% bacteria were alive (see Fig.2).

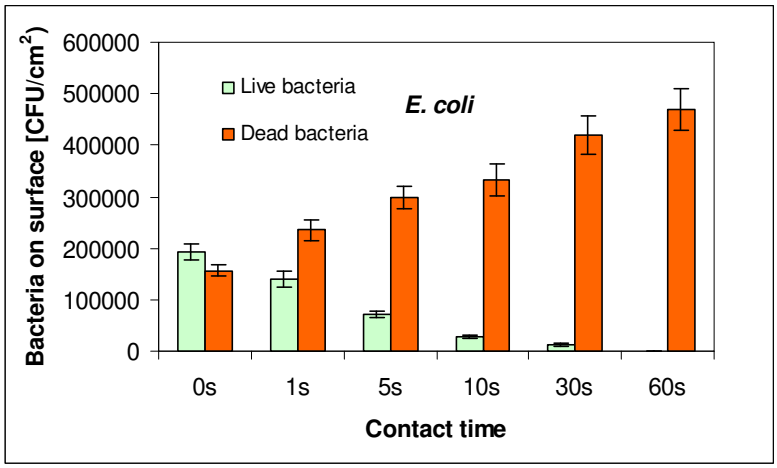


Fig 1 Effect of contact time on inactivation *E. coli*

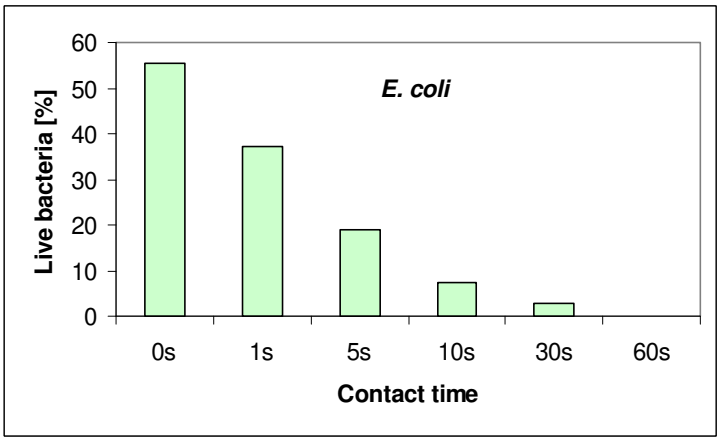


Fig 2 Effect of contact time on live *E. coli* percentage

Fig 3 shows the effect of contact time on inactivation *Staphylococcus aureus*. When contact time increases to 60 second (1 min), 99.9% *E. coli* were killed or only 0.1% bacteria were alive (see Fig.4).

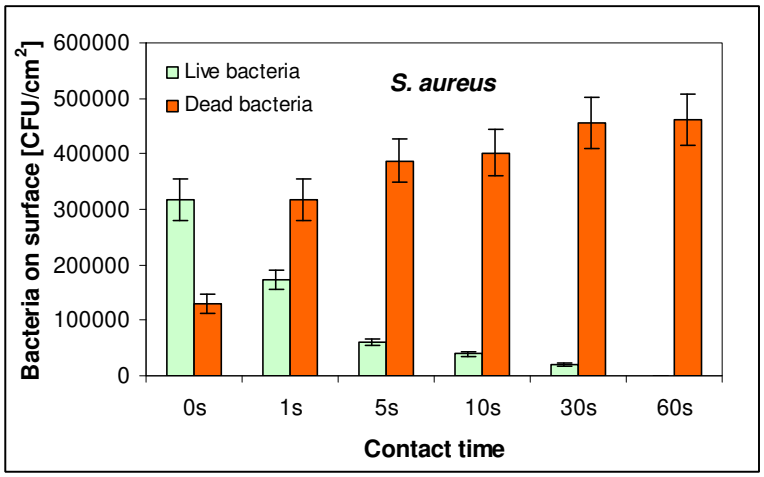


Fig 3 Effect of contact time on inactivation *S. aureus*

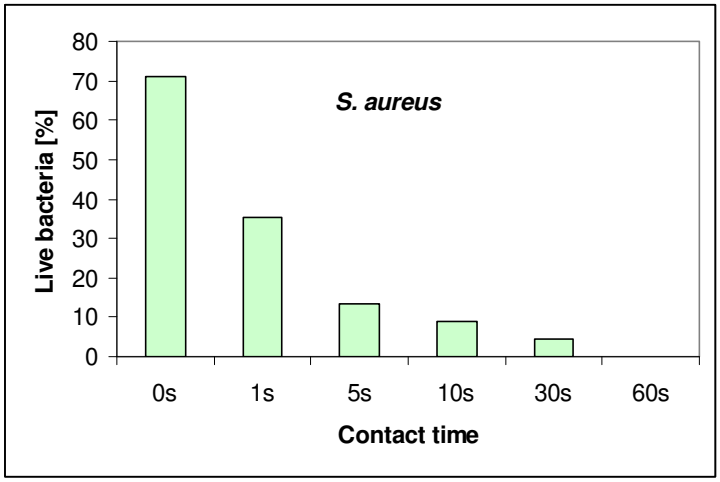


Fig 4 Effect of contact time on live *S. aureus* percentage

Fig 5 shows the effect of contact time on inactivation *Pseudomonas aeruginosa*. When contact time increases to 60 second (1 min), 99.8% *E. coli* were killed or only 0.2% bacteria were alive (see Fig.6).

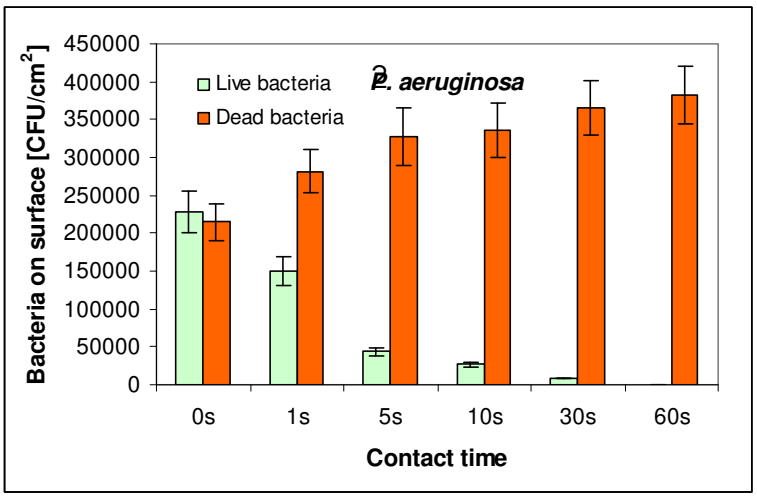


Fig 5 Effect of contact time on inactivation *P. aeruginosa*

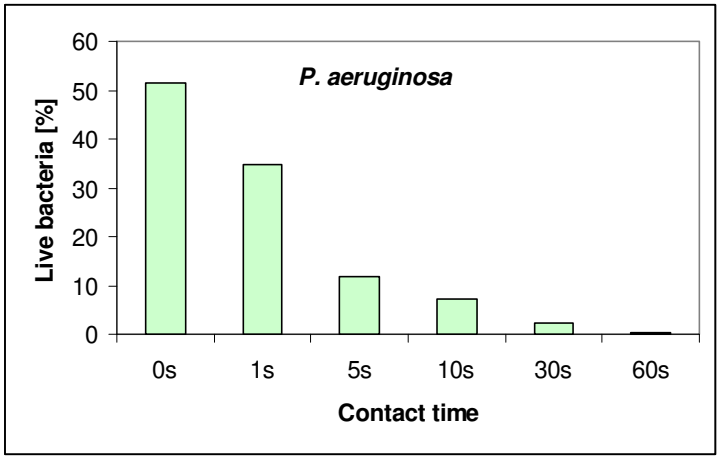
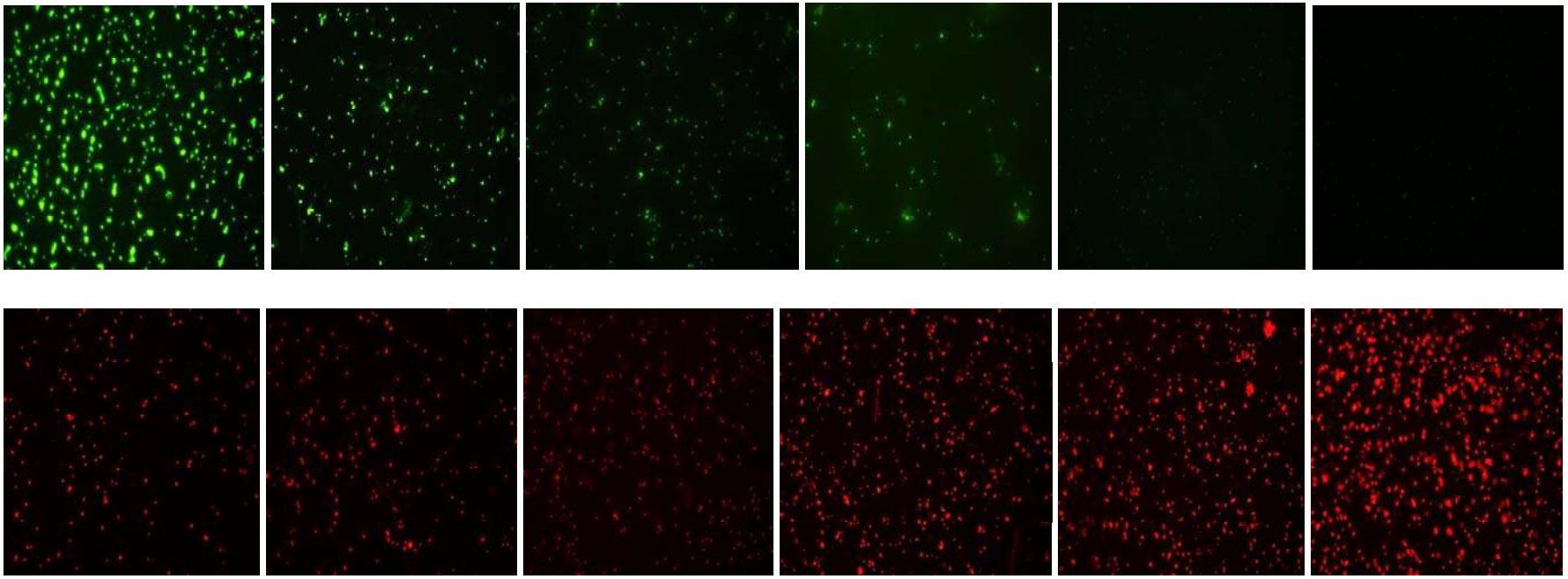


Fig 6 Effect of contact time on live *P. aeruginosa* percentage

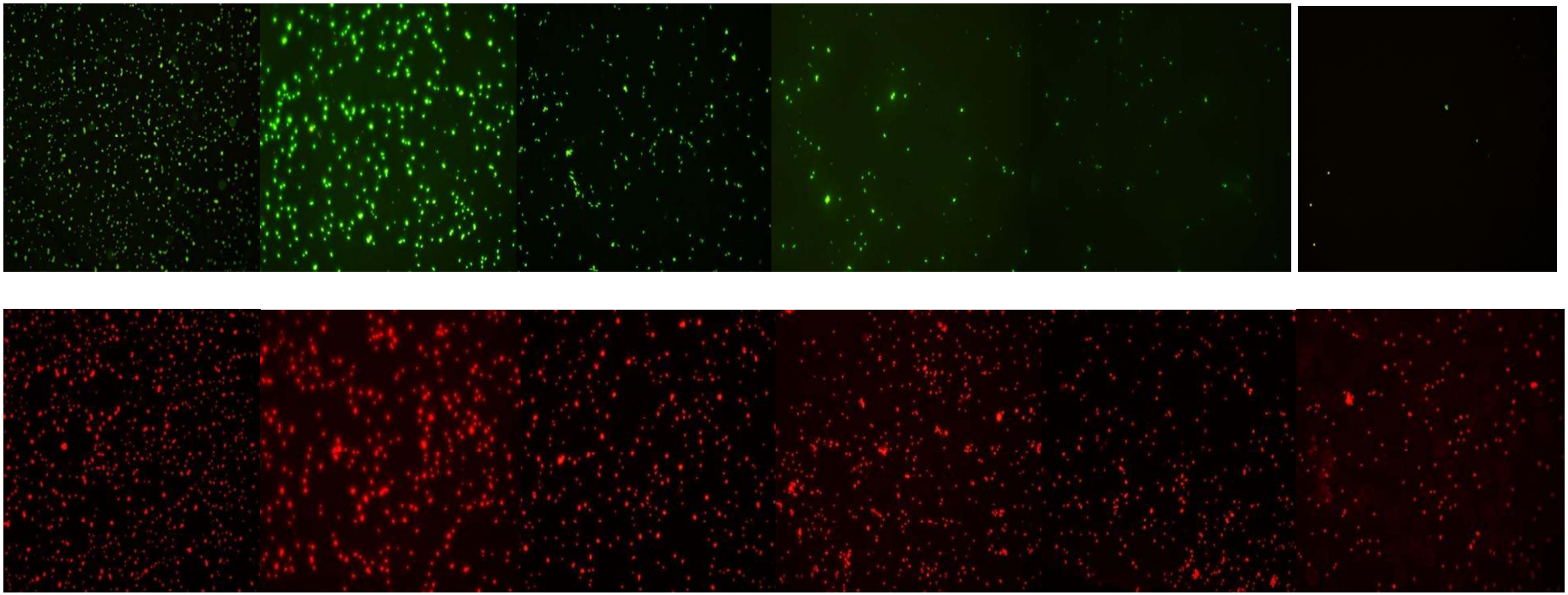
Fig. 7 shows that the typical photos of the attached live and dead *E. coli* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 7 The live and dead *E. coli* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

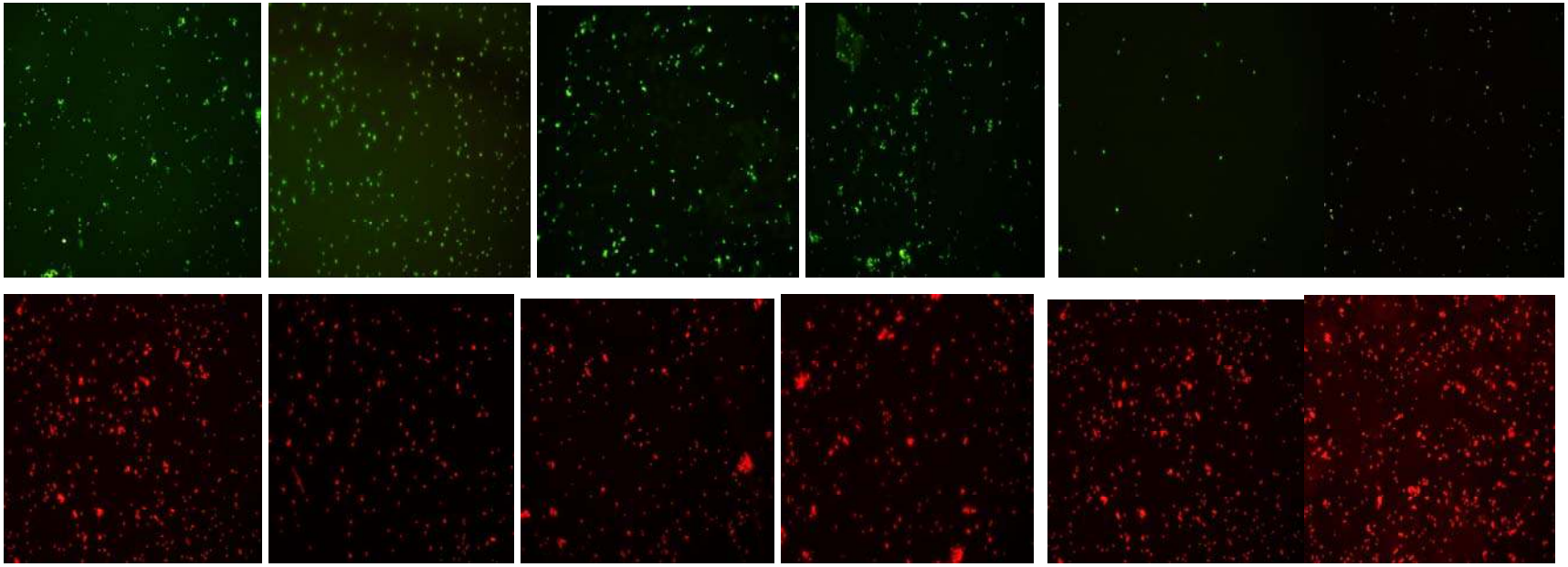
Fig. 8 shows that the typical photos of the attached live and dead *S. aureus* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 8 The live and dead *S. aureus* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

Fig. 9 shows that the typical photos of the attached live and dead *P. aeruginosa* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 9 The live and dead *P. aeruginosa* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

4. Conclusion

Lenova' ozonated water can inactivate bacteria on solid surfaces by over 99.8% within 1 min.

Summary of Ozone Testing

Independent Surface Testing for Food Safety Tayside Scientific Services, based in the UK, provides chemical and microbiological analytical testing service mainly to local authorities and public bodies as well as the private sector. They also support Local Authority Environmental Health and Trading Standards Departments to enforce the provisions of the Food Safety Act and Agriculture Act in the UK. Tayside Scientific Services has conducted surface testing using a microbiological swab to confirm the effectiveness of our ozone water, comparing against that of an approved chemical disinfectant in the UK.

For the test, some raw meat, seafood and vegetables were rubbed onto a chopping board and then left for bacteria to form. A first swap was then taken from the chopping board surface filled with bacteria. The chopping board was then cleaned with an approved chemical disinfectant before taking a second swap. The results would show satisfactory kill rate of bacteria between the first and the second swap.

The same test was repeated, this time using the ozone water from Lenova Ozone System as the cleaning agent instead of chemical disinfectant.

In both tests, the results were approved as effective.

Tayside Scientific Services, James Lindsay Place, Dundee, DD1 5JJ
Tel: 01382 307170 Fax: 01382 202085
Scientific Services Manager/ Public Analyst: Jane Couper

Test Report

Test Report No: 30150259
Issue No: 1

^ **Client Ref No:** **BUZZ/BACDET/01**
Report Date: 29 November 2013

ECOS
FAO Mr Scott Brady
9 East Haddon Road
DUNDEE
DD4 7LD

Sample Details :

^ Client's Description: MICROBIOLOGICAL SWAB
^ Site: ELLIOTS
^ Point: PREPARATION TABLE R/H/S
^ Date taken: 21/11/2013
Date received: 21/11/2013
^ Sampled by: SCOTT BRADY

Test Details :

Determination	Result	Units	Test Method
Total Viable Count @ 30C	< 1	per cm sq	PMF015
Enterobacteriaceae	< 1	per cm sq	PMF023
E. coli	< 1	per cm sq	PMF004

Opinions/Comments:

Having considered the guidance published in the International Journal of Environmental Health Research (2009 Dec;19(6):431-43), I consider the above results to be satisfactory.

Signature:

Name: Garry Ahrens BSc(Hons)
Status: Microbiologist/ Food Examiner

TEST REPORT

Received Date： Dec. 17, 2013
Report Number： PX/2013/C0092A
Report Date： Feb. 10, 2014
The number of Page： 1 OF 1

Following Test Sample is provided and confirmed by client

Client: Lenova
Product Name: OZONE ANTI-BACTERIAL FAUCET
Model/Type： OZONE SERIES
Sample Number： PXC009201~02
Test Item and Method: Performance test

1. The test solution was prepared in SGS laboratory.
2. Using tap-water processing through the product continually for 30 seconds then collecting 2000mL water.
3. Put 0.4mL test solution into 2000mL water(after Step 2) and well mixed.
4. The test solution was analyzed after step 3 and control test.

Control test:
Put 0.4mL test solution into 2000mL DI water and well mixed

Test Result：

Test Item	Unit	Before processing	After processing	Elimination ratio(%)
Coliform	CFU/mL	3.6×10^3	<5	>99.9
Escherichia coli	CFU/mL	4.7×10^3	<5	>99.9
Total Plate Count	CFU/mL	8.8×10^3	<5	>99.9
Staphylococcus aureus	CFU/mL	4.0×10^5	<5	>99.9
Pseudomonas Aeruginosa	CFU/mL	5.1×10^5	<5	>99.9
Candida albicans	CFU/mL	3.3×10^4	<5	>99.9
Legionella pneumophila	CFU/mL	8.6×10^5	<5	>99.9

Remark： 1. This report is for reference, not for advertisement or publication.
2. Sample and title of the report are provided by the client. Environment Lab is only responsible for testing and analyzing.
3. Test results are valid only for test samples.
4. This test document cannot be reproduced in any way, except in full content, without the prior approval in writing of the laboratory.
5. If there are any discrepancies between the English and Chinese report, the Chinese version shall prevail.
6. The client entrusts the product or trademark of the examination, belonging to the client all, or have already got the ownership person's authorization.
7. The report number " PX/2013/C0092A " replaces the "PX/2013/C009201~08".

Steven Chuang / Manager
SIGNED FOR AND ON BEHALF OF
SGS TAIWAN LTD.

TEST REPORT

Received Date : Dec. 17, 2013
Report Number : PX/2013/C009208-1
Report Date : Jan. 06, 2014
The number of Page : 1 OF 1

Following Test Sample is provided and confirmed by client

Product Name: OZONE ANTI-BACTERIAL FAUCET
Model/Type : OZONE SERIES
Sample Number : PXC009201~02
Performance test

Test Item and Method:

1. The test solution was prepared in SGS laboratory.
2. Using tap-water processing through the product continually for 30 seconds then collecting 2000mL water.
3. Put 0.4mL test solution into 2000mL water(after Step 2) and well mixed.
4. The test solution was analyzed after step 3 and control test.

Control test:

Put 0.4mL test solution into 2000mL DI water and well mixed

Test Result :

Test Item	Unit	Before processing	After processing	Elimination ratio(%)
Legionella pneumophila	CFU/mL	8.6×10^5	<5	>99.9

Remark : 1. This report is for reference, not for advertisement or publication.
2. Sample and title of the report are provided by the client. Environment Lab is only responsible for testing and analyzing.
3. Test results are valid only for test samples.
4. This test document cannot be reproduced in any way, except in full content, without the prior approval in writing of the laboratory.
5. If there are any discrepancies between the English and Chinese report, the Chinese version shall prevail.
6. The client entrusts the product or trademark of the examination, belonging to the client all, or have already got the ownership person's authorization.

SGS TAIWAN LTD SGS TAIWAN

Steven Chuang / Manager
SIGNED FOR AND ON BEHALF OF
SGS TAIWAN LTD.

TWA9681546

1003