



Culture Collection of Antimicrobial Resistant Microbes
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TEST REPORT

1. **Customer : Enputech Co., LTD**
2. **Testing laboratory : Culture Collection of Antimicrobial Resistant Microbes**
3. **Test Purpose : Antibacterial activity of Pure Light**
4. **Date of Request : 21. 08. 2008**
5. **Date of Report : 02. 10. 2008**

TEST REPORT

1. Purpose of experiment

Assay the death rate of four kinds of bacteria (Table 1) after irradiation of the Pure Light

2. Date of experiment

25. 8. 2008 ~ 24. 9. 2008

3. Method

3.1 Method

Microorganisms tested in this study were *Staphylococcus aureus*, *Enterococcus faecalis*, *Acinetobacter baumannii* and *Clostridium difficile*.

Each microorganism (*S. aureus*, *E. faecalis*, *A. baumannii*) was suspended in saline making appropriate turbidity. The cell density was determined by serial dilution and an aliquot containing ($10^7 \sim 10^6$ CFU) was spread onto Plate Count Agar (PCA, BBL, Sparks, MD, USA) in triplicate. UV irradiation was performed for 10 s and 20 s in 10 cm distance from the test plate. The number of colonies appeared on the plates were counted after incubation. *C. difficile* was grown on Brain heart infusion agar containing 5 % sheep blood agar, hemin, vitamin K and cysteine at 37°C for 48 h anaerobically in an anaerobic jar (BBL). Colonies were suspended to thioglycollate medium (BBL). The cell density was determined by serial dilution and spreading on BHI agar containing 5% sheep blood agar, hemin, vitamin K and cysteine in triplicate. Aliquots containing 6.0×10^5 CFU and 6.0×10^4 CFU were spread on Brain heart infusion agar containing 5% sheep blood agar, hemin, vitamin K and cysteine in triplicate. UV irradiation was performed as mentioned above. Then the plates were anaerobically incubated at 37°C for 48 h and the number of colonies were counted.

Death rate was determined as the ratio of the number of CFU after irradiation vs. total inoculated CFU.

3.2 Test Strains

Test strains are shown at Table 1.



Table 1. List of bacterial strains

No.	Name	CCARM No.	Characteristics
1	<i>S. aureus</i>	3504	MRSA
2	<i>E. faecalis</i>	5021	VRE
3	<i>C. difficile</i>	0007	Anaerobic, spore forming
4	<i>A. baumannii</i>	12001	MDR

4. Result

CFU after irradiation and death rate are shown in Table 2, Fig 1, 2, 3 and 4. At Table 2.1,

Table 2. 1 Antibacterial effect of UV irradiation

Strain	Total CFU	UV irradiation (10 sec)		UV irradiation (20 sec)	
		CFU	death rate (%)	CFU	death rate (%)
<i>S. aureus</i>	8.5×10^7	2.7×10	99.99997	3	99.999996
<i>E. faecalis</i>	6.2×10^7	5.7×10	99.99991	5.2×10	99.999916
<i>A. baumannii</i>	3.4×10^7	5.1×10	99.99985	3.5×10	99.999979
<i>C. difficile</i>	6.0×10^5	1.64×10^2	99.97267	1.22×10^2	99.979667

Table 2. 2 Antibacterial effect of UV irradiation

Strain	Total CFU	UV irradiation (10 sec)		UV irradiation (20 sec)	
		CFU	death rate (%)	CFU	death rate (%)
<i>S. aureus</i>	8.5×10^6	4	99.99995	1	99.999988
<i>E. faecalis</i>	6.2×10^6	1.7×10	99.99973	5	99.999919
<i>A. baumannii</i>	3.4×10^6	1×10	99.99997	1×10	99.999971
<i>C. difficile</i>	6.0×10^4	3.5×10	99.94167	2.1×10	99.965000

Fig 1. Effect of UV irradiation on *S. aureus*

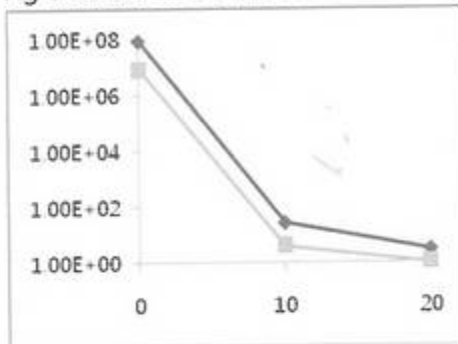


Fig 2. Effect of UV irradiation on *E. faecalis*

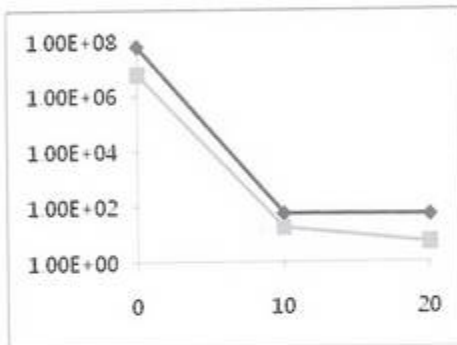


Fig 3. Effect of UV irradiation on *A. baumannii*

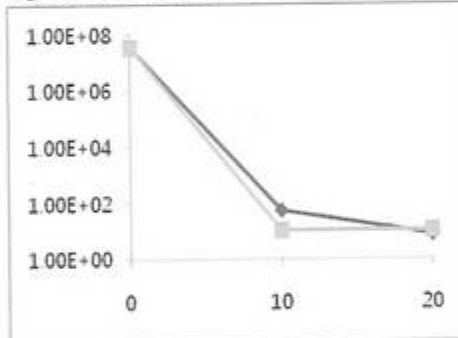
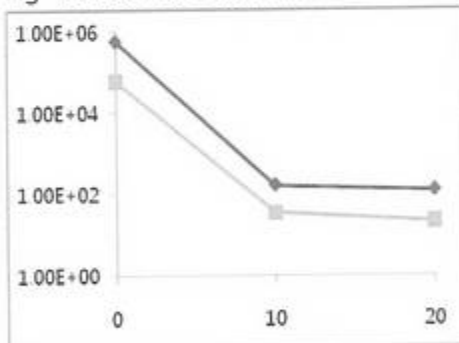


Fig 4. Effect of UV irradiation on *C. difficile*



4. Discussion

Pure light showed more than 99.9% antibacterial activity on 4 test strains. Especially its antibacterial activity on *S. aureus*, *E. faecalis* and *A. baumannii* was >99.999%, while antibacterial effect on anaerobic, spore forming bacteria-*C. difficile* was >99.9%.

Results showed UV irradiation of the Pure Light was very effective on aerobic, spore non-forming bacteria than spore-forming, anaerobic bacteria.

5. Reference

Alfred E. Brown. 2008. Benson's Microbiological Applications: Laboratory Manual in General Microbiology 10th ed. The Mcgraw-Hill companies.

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