

## Hakuzo Environment Cloth Double Block Bactericidal Effect Experiment Results

### Bactericidal Effect Experiment

#### I . Testing Liquid

Hakuzo Environment Wipes Double Block solution

#### II . Experiment

##### 1) Experimeted bacteria

*Acinetobacter calcoaceticus*, *Bordetella bronchiseptica*, *Chlamydia psittaci*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecalis*, multi drug resistance *Enterococcus faecalis*, multi drug resistance *Enterococcus faecalis*, Vancomycin-Resistant *Enterococcus*, *Escherichia coli*, *Fusobacterium necrophorum*, Multi drug resistance *Klebsiella pneumoniae*, *Legionella pneumophilia*, *Listeria monocytogenes*, *Pasteurella multocida*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, Multi drug resistanc *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Serratia marcescens*, *Shigella flexnen* , *Shigella sonnei* , *Staphylococcus aureus* , Methicillin-resistant *Staphylococcus aureus* , Vancomycin-intermediate *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptcoccus pyogenes*,

##### 2) Culture Medium

Nutrient broth

Fluid thioglycolate medium USPXX

Lethen broth (Add 0.5% of lecithin 0.07% and Tween 80 in Nutrient broth)

Tryptic soy agar

##### 3) Tools Used

Test tube :25mm × 150mm glass tube

Carrier: Stainless steel penicillin cup

Outer diameter 8mm, Inner diameter 6mm, Length10mm

Constant temperature reservoir : Set at 20°C

##### 4) Experiment method

Experiment was done based on AOAC Use-dilution method.

Contact time : 10 mins

Organic pollutant: Horse serum 5%

Water hardness: 400ppm(CaCO<sub>3</sub> conversion )

- A 48 hour culture of an individual species of bacteria is dried onto a number of small, cylindrical, stainless steel test surfaces called penicylinders.
- If the test includes "organic soil load," then the culture is amended with some percentage of organic matter (animal serum), before application to the test surfaces.
- Each dry, contaminated test surface is transferred, individually, to a test tube filled with 10 mL of disinfectant using a wire hook, where they incubate for a specified contact time, typically

near ambient temperature (~20-25 C). The contact time used in the test will be the contact time EPA allows on the product label.

- After the contact time has elapsed, the treated test surfaces are transferred, individually at intervals, to test tubes containing a liquid growth medium that has been amended with chemical agents to immediately neutralize the action of the disinfectant.
- After transfer from the disinfectant, the treated test surfaces are incubated in the neutralizing growth medium for 48 hours.
- After incubation, the number of tubes showing growth of the target microorganism is recorded.

### III. Results

Fungus Scientific name	ATCC#	Repeat count	Results
<i>Acinetobacter calcoaceticus</i>	23055	10 times × 2 sets	All negative
<i>Bordetella bronchiseptica</i>	31427	10 times × 2 sets	
<i>Chlamydia psittaci</i>	VR-854	10 times × 2 sets	
<i>Enterobacter aerogenes</i>	13048	10 times × 2 sets	
<i>Enterobacter cloacae</i>	23355	10 times × 2 sets	
<i>Enterococcus faecalis</i>	19433	10 times × 2 sets	
multi drug resistance <i>Enterococcus faecalis</i>	19433	10 times × 2 sets	
<i>Vancomycin-Resistant Enterococcus</i>	51299	10 times × 2 sets	
<i>Escherichia coli</i>	11229	10 times × 2 sets	
<i>Escherichia coli</i>	Clinical isolate	10 times × 2 sets	
<i>Fusobacterium necrophorum</i>	27852	10 times × 2 sets	
Multi drug resistance <i>Klebsiella pneumoniae</i>	13883	10 times × 2 sets	
<i>Legionella pneumophila</i>	33153	10 times × 2 sets	
<i>Listeria monocytogenes</i>	15313	10 times × 2 sets	
<i>Pasteurella multocida</i>	7707	10 times × 2 sets	
<i>Proteus mirabilis</i>	25933	10 times × 2 sets	
<i>Proteus vulgaris</i>	13315	10 times × 2 sets	
<i>Pseudomonas aeruginosa</i>	15442	60 times × 3 sets	
Multi drug resistanc <i>Pseudomonas aeruginosa</i>	Clinical isolate	10 times × 2 sets	
<i>Salmonella choleraesuis</i> ( <i>Salmonella enterica</i> serovar Choleraesuis)	10708	60 times × 3 sets	
<i>Salmonella enteritidis</i>	13076	10 times × 2 sets	
<i>Salmonella typhi</i> ( <i>Salmonella enterica</i> serovar Typhi)	6539	10 times × 2 sets	

<i>Salmonella typhimurium</i>	14028	10 times × 2 sets	All negative
<i>Serratia marcescens</i>	8100	10 times × 2 sets	
<i>Shigella flexnen</i>	12022	10 times × 2 sets	
<i>Shigella sonnei</i>	9290	10 times × 2 sets	
<i>Staphylococcus aureus</i>	6538	60 times × 3 sets	
<i>Methicillin-resistant Staphylococcus aureus</i>	33592	10 times × 2 sets	
<i>Methicillin-resistant Staphylococcus aureus</i>	NRS384 USA300	10 times × 2 sets	
<i>Vancomycin-intermediate Staphylococcus aureus</i>	CDC HIP-5836	10 times × 2 sets	
<i>Staphylococcus epidermidis</i>	Clinical isolate	10 times × 2 sets	
<i>Streptococcus pyogenes</i>	19615	10 times × 2 sets	
<i>Aspergillus niger</i>	6275	10 times × 2 sets	

### Fungal Elimination Experiment

Experiment method: AOAC Fungal Elimination Experiment

Experiment conditions:

Contact time: 10 mins
Organic pollutant: Not added
Water hardness: 400ppm(CaCO <sub>3</sub> conversion )

### 《Fungal Elimination Results》

Fungus Scientific name	ATCC#	Repeat count	Results
<i>Trichophyton mentagrophytes</i>	9533	4times	Fungus are killed in 10 mins
<i>Candida albicans</i>	11651	4times	Fungus are killed in 10 mins