# Removing Ability Experiment for Various Bacilluses and Fungus by VirusKiller

July, 2004.

Sungkyunkwan University
Kyunggi medicine research center

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# Ⅰ. Introduction

Various kind of microorganisms are coexisting in human's living space and these can bring human or animals various disease and some is being responsible for fatal disease. Human urbanization and a lot of migrations are heightening spread possibility of these infectivity disease more. Recently, SARS, Avian influenza etc.. new infectivity diseases are appearing continuously, and these are giving mankind and animals serious damage. Therefore, it has great meaning by health medical treatment science to get rid of these infectivity microorganism in living environment.

In this study, VirusKiller manufactured by INB Co., Ltd wished to verify removal ability of microbial that exist in air. Chose bacillus or mold that is being responsible for infectivity disease existing much in air or is used as indicator microorganism by target sample of this experiment.

Used Staphylococus aureus subsp. aureus, Streptococcus pyogenes, Streptococcus pneumoniae by Gram positivity strain, and Escherichia coli, Klebsiella pneumoniae by Gram negative strain, and Aspergillus niger, Rhizopus oryzae by fungus.

In this study, adopted clinical material in exit after inhale microorganism strain of standard number to VirusKiller and report the contents authorized the removal ability from comparison with control group.

# Ⅱ. Experiment Method

## 1. Materials for Experiments, Device and Reagent

### 1-1. Materials for Experiments

VirusKiller 99.9999%up(large size) that offered by INB for the performance test Equipment was use for this experiment..

### 1-2. Experimental Strain

Strains that used in an experiment are as following.

① Staphylococcus aureus subsp. aureus KCTC 1928

② Streptococcus pyogenes KCTC 3096

③ Streptococcus pneumoniae CP 1200

④ Escherichia coli DH 5α

⑤ Klebsiella pneumoniae KCTC 2241

⑥ Aspergillus niger KCTC 6089

⑦ Rhizopus oryzae KCTC 6062

### 1-3. Device and Reagent

#### 1-3-1. Device

- Autoclave ; LABO Autoclave (Sanyo)
- Incubator ; KMC Incubator (Vision Scientific CO., LTD)
- Clean bench ; CLEAN BENCH (Deajin Plant)
- Microscope ; Olympus CK40 (Olympus)
- Air sampler ; RCS Air sampler (Biotest HYCON)
- Colony counter ; RCS Colony-Counter set (Biotest HYCON)
- Digita camera ; Olympus CAMEDIA C-4040 ZOOM
- Super low temperature refrigerator ; -86℃ ULT Freezer (Thermo Forma)

### 1-3-2. Reagent

Culture media that used in microbial cultivation and examination are as following.

- LB Broth, Miller(Luria-Bertani) (DIFCO)
- Bacto™ Agar (BD)
- Brain Heart Infusion (DIFCO)
- Malt Extract Broth (DIFCO)
- Bacto™ Yeast Extract (BD)
- Potato Dextrose Broth (DIFCO)
- Difco™ Nutrient Broth (BD)
- Bacto™ Todd Hewitt Broth (DIFCO)
- BBL™ Lowenstein-Jensen Medium Slants (BD)

Exclusive cultivate media for RCS air sampler was used Luftkeimindikater HS (Biotest HYCON) for fungi and by botryose micrococcus for Luftkeimindikater S (Staphylokokken) (Biotest HYCON), and remainder used in examination after make suitable cultivation media in each strain using Luftkeimindikater KIT (Leer folien) (Biotest HYCON) blank strip kit.

## 2. Experimental Method

### 2-1. Experimental Conditions

VirusKiller was located in isolation hermetical space(258cm × 216cm × 216cm), and duct was established on inlet port to be situated and poured standard number of strain.

Started in examination after pasteurize by UV light more than 3 hours in isolated hermetical space before all experiments.

Instrument and equipment that used in an experiment were pasteurized in autoclave or kept under UV light after clean enough by 70% EtOH and some of them were pasteurized by flame and used.


Figure 1. Establishment VirusKiller for examination

Figure 2. Establishment for duct of VirusKiller inlet port and chamber of exhaust port and air sampler

### 2-1-1. Experimental Group

Operated VirusKiller for prewarming for 30 minutes and poured extract that mixed strain of standard number in pasteurization distilled water on inlet port through duct using receptacle for atomizing.

Established chamber in exhaust port and situated Air sampler attached proper cultivation media in near position the inside 20Cm of it, and adopted by experimental group cultivating for optimum time at optimum temperature after pick specimen for 4 minutes.

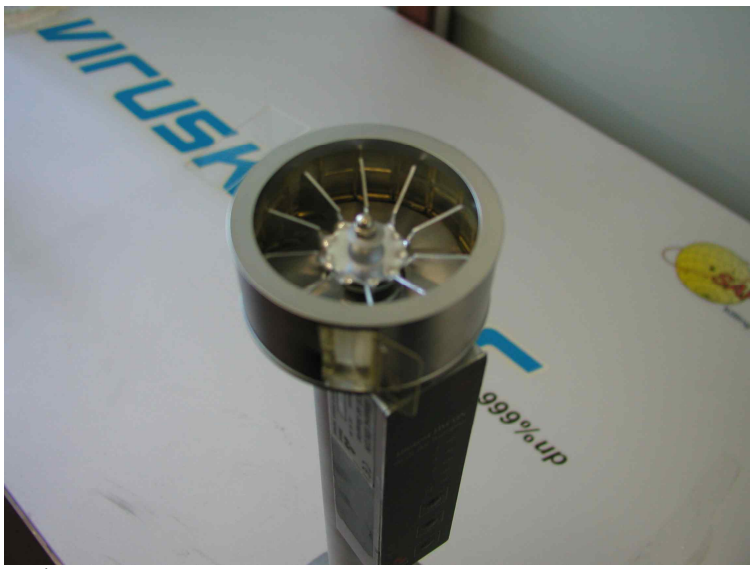



Figure 3. Attaching strip cultivation media on air sampler

### 2-1-2. Positive Experimental Group

Adopted to positivity control group through same handling such as experimental group after intercept catalyst response putting out UV lamp in VirusKiller.

### 2-1-3. Negative Experimental Group

Adopted as negative control group pouring only same quantity of pasteurized distilled water instead of distilled water contained standard number of strain handling through same process with positivity control group.

## 2-2. Method

① Inoculate strain on suitable cultivation media and cultivate from 1 day to 3 day in incubator at 37℃. In the case of fungus, cultivate for 5 days in incubator at 25℃.

② Mix calculate fungus of standard number in pasteurized distilled water after calculate fungus.

③ Remove microorganism in air putting on UV light more than 3 hours in isolated and enclosed space.

④ Operate VirusKiller for prewarming for 30 minutes.

⑤ Inject atomizing standard number of Strain on inlet through duct

⑥ Installed exclusive cultivation media at pasteurized air sampler and did sampling for 4 minutes after locating 20㎝ away from exhaust port center.

⑦ Cultivated exclusive strip cultivation media used for sampling on normal temperature and time

⑧ Calculation according to CFU (Colony Forming Units) calculation after cultivation

⑨ Calculation fungus exclusion ability of VirusKiller from comparison of positivity control group and experimental group.

$$\text{fungus exclusion ability (\%)} = \frac{\text{Calculation for Fungus removal ability of VirusKiller from comparison of positivity control group and experimental group}}{\text{CFU of Positivity control group}} \times 100$$

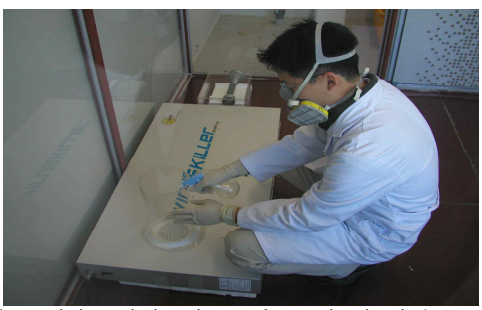


Figure 4. Atomizing injection of strain in inlet port of VirusKille

## 2-3. Cultivation Conditions

Cultivation condition and cultivation media of each strain are same as following Table.

**Table-1. Cultivation media and condition of used strain**

| Name of Strain | Cultivation Media | Cultivation Temperature |
|---|---|---|
| S. aureus subsp. aureus KCTC 1928 | Nutrient Agar | 37℃ |
| S. pyogenes KCTC 3096 | Brain Heart Infusion Agar | 37℃ |
| S. pneumoniae CP 1200 | Todd Hewitt Yeast Agar | 37℃ |
| E. coli DH 5α | LB Agar | 37℃ |
| K. pneumoniae KCTC 2241 | Nutrient Agar | 37℃ |
| A. niger KCTC 6089 | Malt Extract Agar | 25℃ |
| R. oryzae KCTC 6062 | Potato Dextrose Agar | 25℃ |

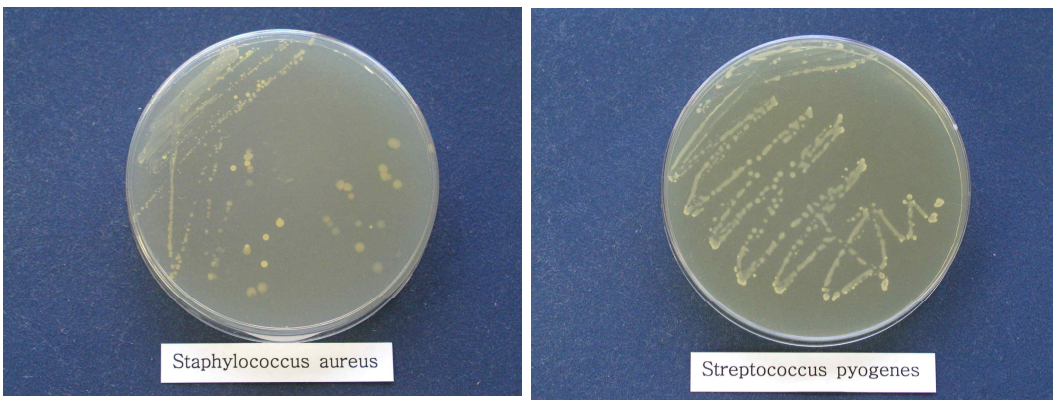



Figure 5. Cultivation S. aureus and of S. pyogens for test

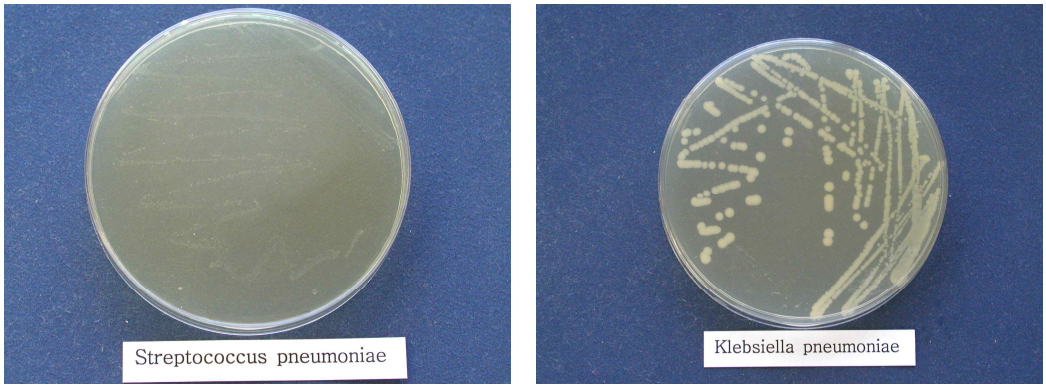



Figure 6. Cultivation of S. pneumoniae and K. pneumoniae for test

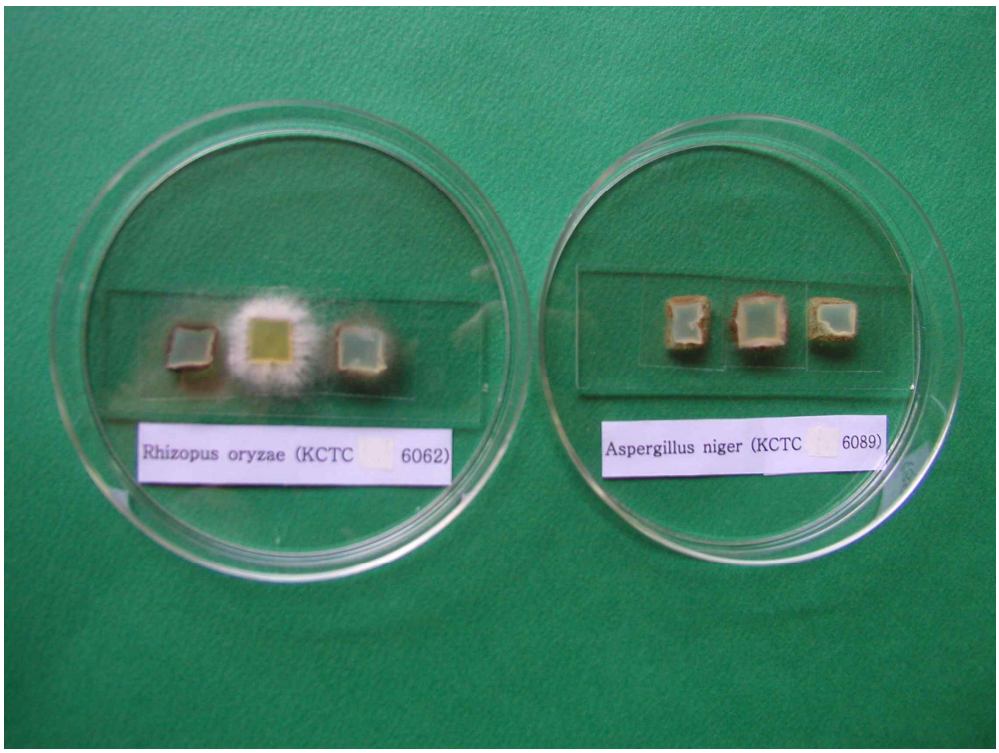



Figure 7. Cultivation of test funges

Exclusive cultivation media and cultivation condition for experimental groups and positivity, negative control group of various strain are recorded in following Table.

**Table-2. Exclusive cultivation media and cultivation condition of used strain**

| Namr of Fungus | Exclusive Cultivation Media(Agar strip) | Cultivation Condition |
|---|---|---|
| S. aureus subsp. aureus KCTC 1928 | Manntol-salt-Agar(Agar strip S) | 35℃, 48hrs |
| S. pyogenes KCTC 3096 | Brain Heart Infusion Agar | 37℃, 48hrs |
| S. pneumoniae CP 1200 | Todd Hewitt Yeast Agar | 37℃, 48hrs |
| E. coli DH 5α | MacConkey-Agar(Agar strip C) | 35℃, 48hrs |
| K. pneumoniae KCTC 2241 | Nutrient Agar | 37℃, 48hrs |
| A. niger KCTC 6089 | Rose-Bengal-Agar(Agar strip HS) | 30℃, 120hrs |
| R. oryzae KCTC 6062 | Rose-Bengal-Agar(Agar strip HS) | 30℃, 120hrs |

# Ⅲ. Experiment result

## 1. Removal ability of Gram positive fungus and Gram negative fungus by VirusKiller

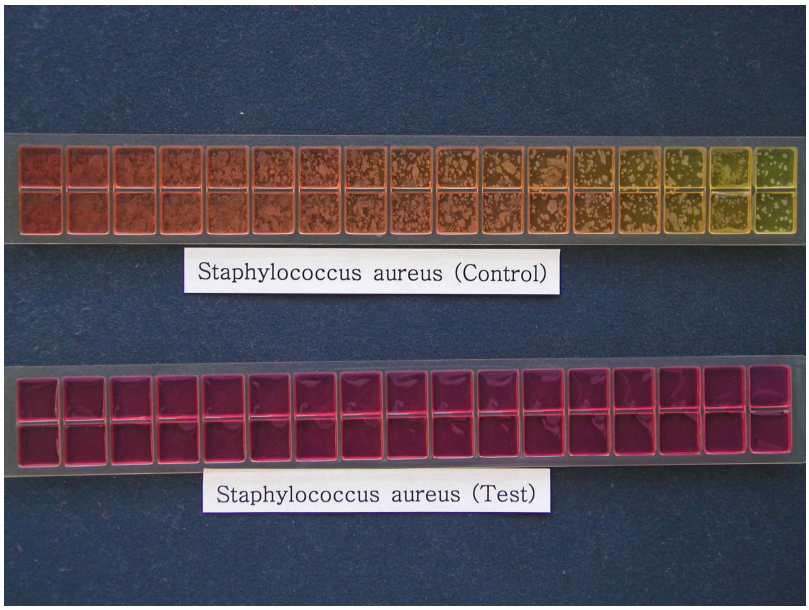
Number used in experimental group of each strain and positivity control group and calculated the number by diluting after measured OD value in 600nm by each 106 and poured on inlet port after mix each strain(106) into pasteurized distilled water 30ml.

Experimental group was tested 3 times repeatedly and positivity control group and negative control group were tested each 1 time.

Fungus was not detected in representative positivity and all experimental groups of negative fungus and negative control group.

**Table-3. result of experimental group against each strain and positivity control group, negative control group**

| Kind and Classification of Strain | | Strain dosage | Detected amount of Strain ($CFU/m^3$) |
|---|---|---|---|
| S. aureus subsp. aureus KCTC 1928 | Experimental group 1 | $10^6$/30ml | None detection |
| | Experimental group 2 | $10^6$/30ml | None detection |
| | Experimental group 3 | $10^6$/30ml | None detection |
| | Positive Control Group | $10^6$/30ml | 256 |
| | Negative Control Group | 0/30ml | None detection |
| S. pyogenes KCTC 3096 | Experimental group 1 | $10^6$/30ml | None detection |
| | Experimental group 2 | $10^6$/30ml | None detection |
| | Experimental group 3 | $10^6$/30ml | None detection |
| | Positive Control Group | $10^6$/30ml | 290 |
| | Negative Control Group | 0/30ml | None detection |
| S. pneumoniae KCTC 2241 | Experimental group 1 | $10^6$/30ml | None detection |
| | Experimental group 2 | $10^6$/30ml | None detection |
| | Experimental group 3 | $10^6$/30ml | None detection |
| | Positive Control Group | $10^6$/30ml | 312 |
| | Negative Control Group | 0/30ml | None detection |
| E. coli DH 5α | Experimental group 1 | $10^6$/30ml | None detection |
| | Experimental group 2 | $10^6$/30ml | None detection |
| | Experimental group 3 | $10^6$/30ml | None detection |
| | Positive Control Group | $10^6$/30ml | 275 |
| | Negative Control Group | 0/30ml | None detection |
| K. pneumoniae KCTC 2241 | Experimental group 1 | $10^6$/30ml | None detection |
| | Experimental group 2 | $10^6$/30ml | None detection |
| | Experimental group 3 | $10^6$/30ml | None detection |
| | Positive Control Group | $10^6$/30ml | 391 |
| | Negative Control Group | 0/30ml | None detection |




Figuer 8. positivity control group and experimental group of S. aureus

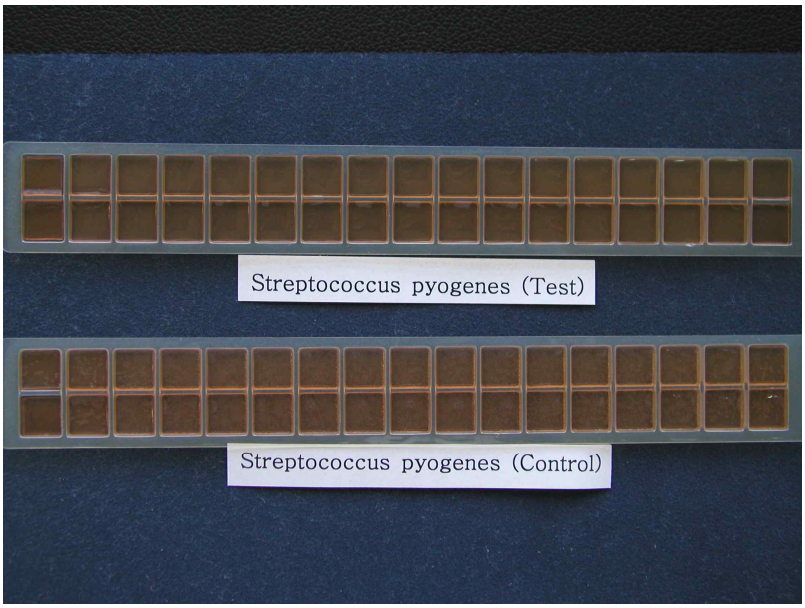



Figure 9. S. Positive control and experimental group of pyogenes

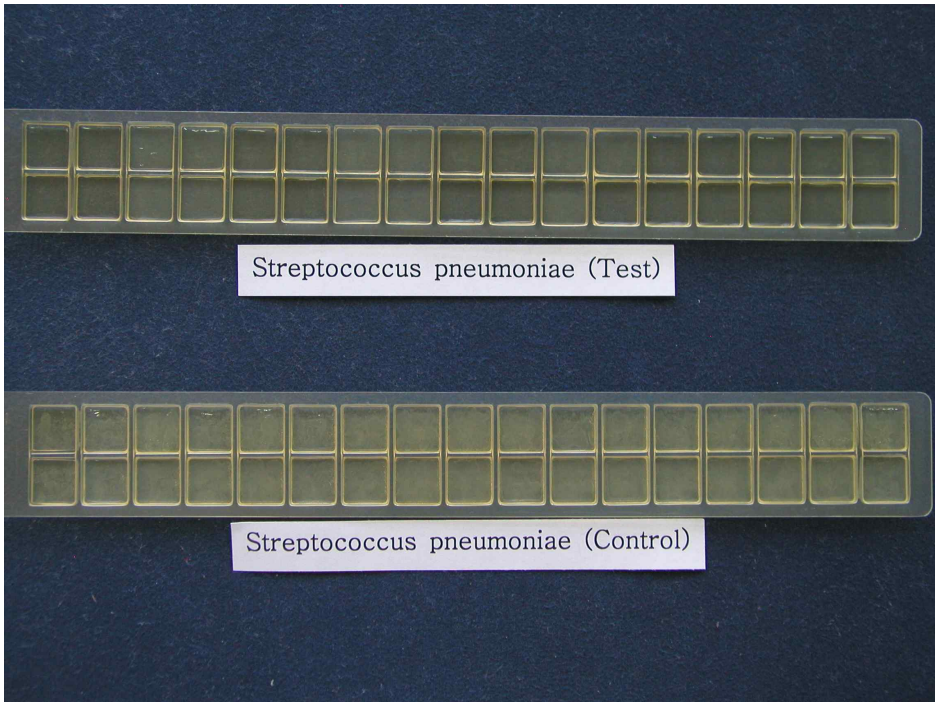



Figure 10. positivity control group and experimental group of S. pneumoniae

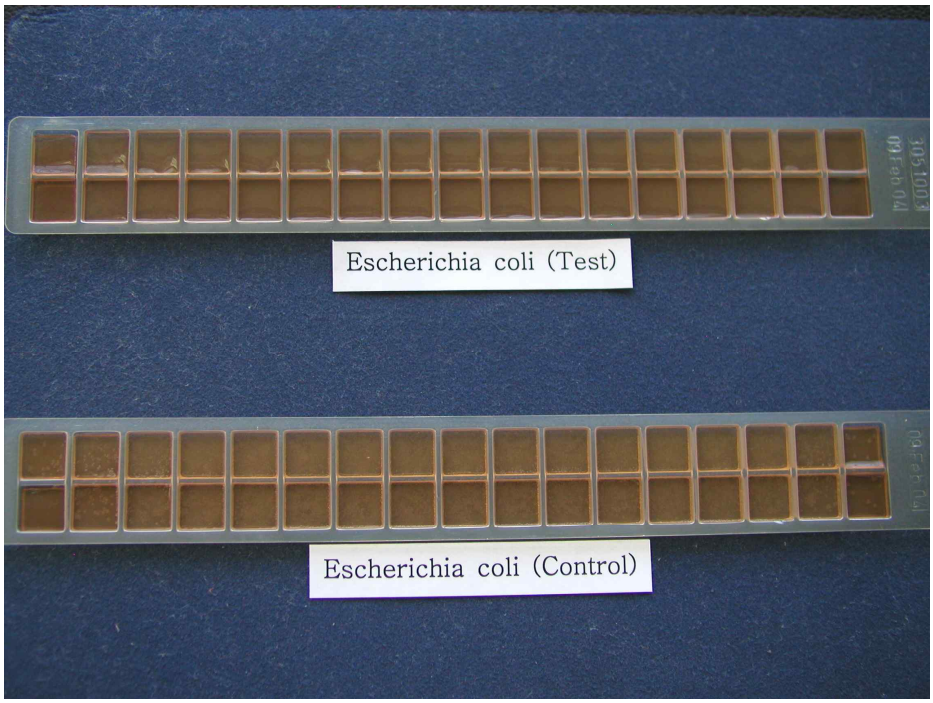



Figure 11. Positivity contrast and experimental group of E. coli

## 2. Removal ability of fungus by Viruskiller

In the case of funges, number used in experimental group and positivity control group was poured on inlet port after mix each 103 to pasteurization distilled water 30ml calculating by number of spore.

Experimental group was tested 3 times repeatedly, positive control group and negative control group was tested each 1 time.

Table-4. Result of experimental and positivity control group, negative control group against each fungus.

| Kind and Classification of Strain | | Strain Dosage | Detected amount of Strain (CFU/$m^3$) |
|---|---|---|---|
| A. niger KCTC 6089 | Experimental group 1 | $10^3$/30ml | None detection |
| | Experimental group 2 | $10^3$/30ml | None detection |
| | Experimental group 3 | $10^3$/30ml | None detection |
| | Positive Control Group | $10^3$/30ml | much |
| | Negative Control Group | 0/30ml | None detection |
| R. oryzae KCTC 6062 | Experimental group 1 | $10^3$/30ml | None detection |
| | Experimental group 2 | $10^3$/30ml | None detection |
| | Experimental group 3 | $10^3$/30ml | None detection |
| | Positive Control Group | $10^3$/30ml | much |
| | Negative Control Group | 0/30ml | None detection |

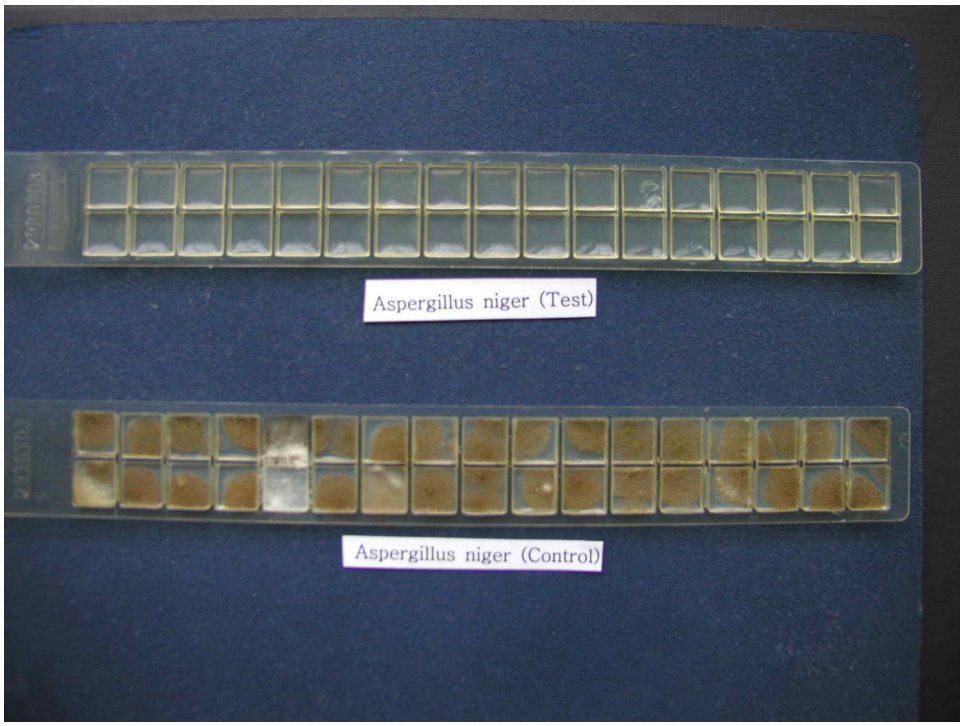



Figure 12. Experimental and Positivity Control Group of A.niger

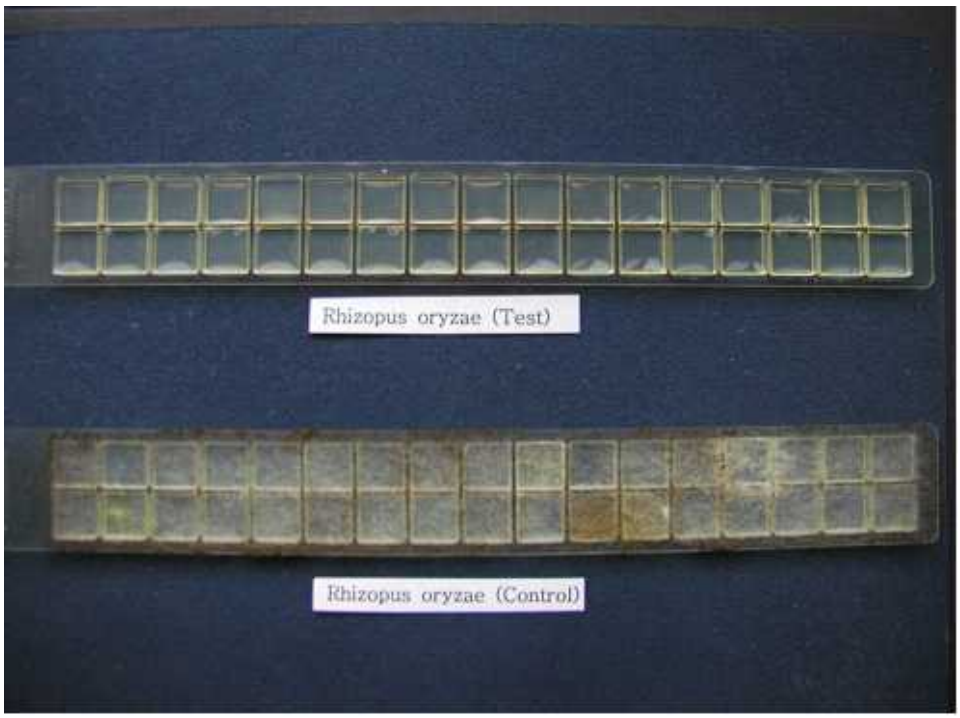



Figure 13. R. Experimental and Positivity Control Group of Oryzae

## Ⅳ. Results

In removal ability examination(EX) of various bacilluses and funges by VirusKiller of INB Co., Ltd., got following conclusion in outlet through specimen collection examination after inhale atomizing standard number of Strain.

Tested exclusion ability of VirusKiller for 3 kinds of Gram positivity fungus and 2 kinds of Gram negative fungus, 2 kinds of fungus that exist much in air and cause infectivity disease or are used by indicator strain.
Through the effect examination(EX) of VirusKiller against S. aureus causing purulent inflammation disease as Gram positive fungus and S. pyogenes the fungus causing skin purulent inflammation etc. and S. pneumonie as pneumonia germ, but there were not detected each strain in all experimental groups, therefore, it could be recognized that VirusKiller could remove these fungus perfectly.
And also colitis germs(E . Coli) and pneumobacillus(K.pneumoniae) that is Gram negative fungus were removed perfectly by VirusKiller and could recognize that Aspergillus niger and Rhizopus oryzae as fungus become inactive.

From result of above, VirusKiller of INB Co., Ltd is considered that can remove killing other similarity Gram positive fungus and negative fungus and fungus as well as strain that is used in the examination .