Final Report of the Study

Inactivity examination for Corona Virus DF2

(Kind of SARS induction virus) by Virus_killer

August 2004

Virus Examination Institute
appointed by National Environment Research Institute
Medical Science Research Institute of Medical College

Kangwon National University

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I. Introduction

It is very important that is not caught from several kind of microorganism diseases that can be exist in air while human runs life. Specially, several disease producing viruses can keep infectivity during a certain period among air and hereafter possibility is high to be demolished in human body. There is no effective cure in most occasion after caught virus, Can bring disease of serious illness from populations of child and senescence and immunity shortage person etc. by the second infectivity by several kind of bacteria. Also, when consider that more than 50% of direct cause of human's death depends to microorganism, it has great meaning by health science to remove virus in living environment.

There are various Virus that can demolish contagium to human or an animal existing in aerosol or floating state among air and cause sanitary danger and injury.

Hepatitis A, adeno virus, RHINOVIRUS etc. can disperse pathogen existing among air for relative long term without membrane. These virus is possible dissemination by contact or water in addition beside aerosol. Specially, RHINOVIRUS is common that cause most of a cold everyday. There are smallpox, Influenza, measles, rubella, parotitis, varicella etc. virus that can demolish big disease with problem to human transmit through air except these virus without membrane.

All these viruses have membrane that stability for survival among air is lower than virus without membrane. All RNA viruses except smallpox and varicella Virus has special quality of virus with membrane. Specially, Influenza and measles are relatively strong disease producing that

pathogen should intercept certainly by communicable disease control. Extremely happily, these viruses were developed vaccine that prevention is available by method of immunity.

it is coronavirus to be problem of health science in air. Coronavirus has membrane and relatively big virus that cause disease in human as well as an animal. Specially, it was known as virus existing in air because this virus leads to 15% or 30% of common cold. In most occasion, even if infected by coronavirus with RHINOVIRUS routinely, it has special character that gets back after slight symptoms so that was not differentially stared as contagion. But, coronavirus leads to acute respiratory disease in old man as like RHINOVIRUS, influenza virus, respiratory syncytial virus, RSV. Natural infection frequency of coronavirus is about half of RHINOVIRUS and is known as degree similar with influenza virus and RSV.

It is relativity severe acute respiratory syndrome, SARS that happened in the China in 2002 second half of the year that coronavirus became problem to human specially. This time, SARS that is designated clinically produced patient more than 8,000 people from 29 countries and lethality was about 9.6%. Because suspected case happens in domestic, the importance was increased. SARS instituted big problem by social economy enemy such as command of travel at point that lethality is high than other most virus diseases and communicates through air.

Therefore, device that can remove this virus among air is very important subject because is to improve economical advantage that gain reducing person's health and communication of contagious disease.

Therefore, in this study, Viruskiller that INB company manufactured specially for virus removal to test exclusion availability of high danger

virus in air, wished to select SARS's cause pathogen that becomes most problem recently to examination data and test virus exclusion ability. So, in this study, there used in an experiment the Coronavirus DF 2 belonging to family such as SARS induction virus that feature is identical by all virology, and described here the result and contents.

Ⅱ. The experiment device and material

1. Equipment and gratuitous dispensation of medicine necessary for research

Virus study is achieved dividing into virus injection, virus concentration, virus detection and necessary basis equipment and reagent for study were presented in <Table 1>.

Table 1) Necessary equipment and reagent in virus study

Purpose	Equipment	Reagent
virus injection	- Virus_killer 99.999%up of INB Corp - Spray gun (virus for injection), - MDS Filter (Virus collection by charge, CUNO company)	- 0.05M glycine - 2% Thiosulfate - 0.1% chlorine
virus concentration	- Refrigeration high speed centrifuge (studied virus concentration) - pH metre(sample pH control), - CO_2 incubator(Animal cell culture) - milipore filterDevice (microbial pollution prevention 0.2㎛ syringe filter)	- 1%beef extract (GIBCO co.) - 2% or 10% fetal calf serum(FCS)의 DMEM (Gibco) - Penicillin - streptomycin
virus detection	- Optical microscope (Cellular form observation caught in normal cell and virus)	
Others	- Autoclave (experiment connection reagent and equipment pasteurization) - Super low temperature refrigerator (- 80℃, virus sample storage) - Nitrogen tank (virus strain storage)	

2. An experiment material

1) Experiment virus host and titer

Virus used in this examination used Corona virus DF 2 that is loted out from ATCC(American Type Culture Collection) in inactivity examination. As size of coronavirus is 120?160㎚, it is RNA viruses with membrane. Titer of virus used in Viruskiller's inhalation of air making this by bubble imposing 106 plaque forming unit(PFU) to 50 ml's PBS.

2) Animal Cell Host

Corona virus' potency test and infectivity examination(EX) used CRFK call(Crandell feline kidney call) that cultivate at a laboratory which is loted out from KOREAN CELL LINE BANK.

Cell host was cultivated under cultivation condition of 37℃ and 5% CO2 and saturated relative humidity state and inoculated virus sample after cultivated (grow 80 ~ 90%) 2 ~ 3 days.

3) Disinfection and pasteurization of utensil and reagent

Instrument and equipment used at virus injection behaved pasteurization and disinfection process compulsorily in 121℃ during 15 minutes and 1 hour. And MDS Filter is Pasteurized in high pressure at 121℃ for 30 minutes, scissor or tweezers etc. soaks in 95% ethanol and used after sterilizing in flame before use. Glassware or metal goods and virus eruption and necessary beef extract used in

virus concentration were moisture pasteurized in 121℃ for 30 minutes.

Utensil and equipment(plastic) impossible for high pressure pasteurization, after do to deactivate bacillus and virus by soaking for 30 minutes to 0.1% Chlorine(pH 7.0), and made counteraction in 2% Thiosulfate solution(sodium thiosulfate) and then used drying after wash several times in pasteurization distilled water. Culture fluid used for cell culture filtered by 0.2㎛ pasteurization filter.

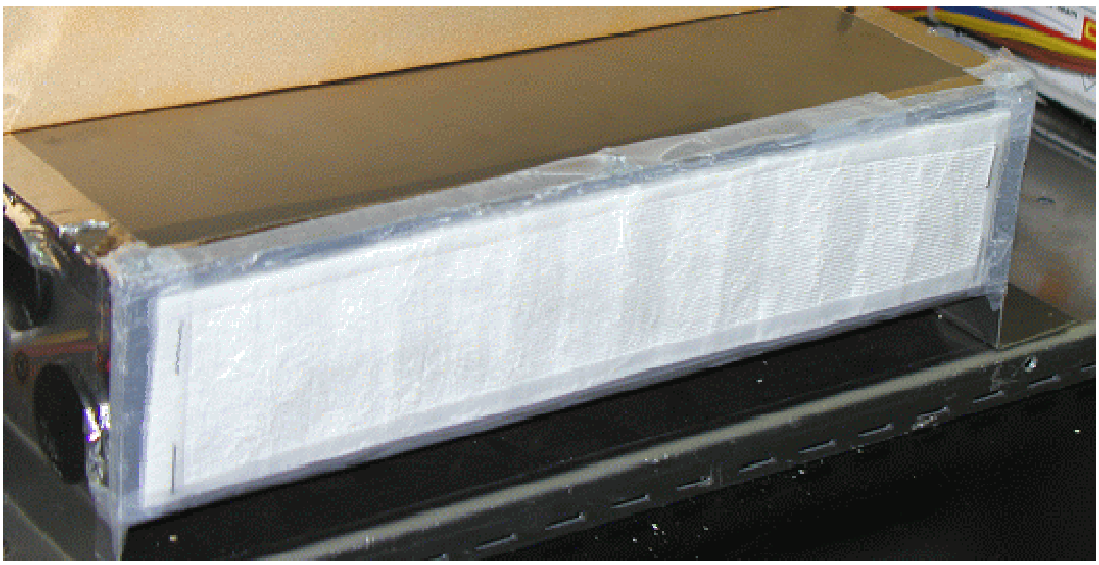
Ⅲ. Experiment method

1. Experimental condition

If inject virus to Virus_killer without any device, injected virus moves to other place by flowing of air, quantitative virus does not pass Virus_killer. Also, spouted virus had possibility to contaminate surroundings, established duct on inlet port like <Figure 1> for safe virus atomizing.

For collection experimental virus host passed through Viruskiller that Duct is placed, attached MDS filter in exhaust region as like <Figure 2>. Also, to collect Virus before passing Viruskiller, attached MDS filter on inlet port where duct is placed as like <Figure 3>.

<Figure 1> Duct establishment on inlet ergion of Virus_killer



<Figure 2> MDS filter establishment on exhaust region (for experimental group)

<Figure 3> MDS filter establishment on inlet region

(For Negative control group and positive contrast group)

2. Virus Injection and Collection

Made warming-up for 1 hour for smooth operation of Viruskiller before passing experimental virus host through Viruskiller. Operated Viruskiller after thread experimental condition of experimental group and control group suitably each to Viruskiller. Atomized correct experiment virus host and virus diluted solution(PBS) in inlet region of Virus-killer using ejector in each experimental group and control group into operating Viruskiller and kept 10 minutes so that may be attained enough adsorption(Figure 4).

Executed first negative control group in this experiment to be principle, and negative and positivity control group enforced by each 1 time about 3 experimental virus hosts.

Also, experimental group of experiment virus host achieved each 3 times. Always sterilized all experiment connection equipments and laboratory inside after 1th adsorption experiment of each experiment host finishes.

<Figure 4> Injection Experimental virus host on inlet region of Viruskiller

3. Eruption and Concentration of Virus

After finished virus injection experiment, remove MDS filter by aseptic exhaust region of Viruskiller as like <Figure 5>, After finished virus injection experiment, remove MDS filter by aseptic exhaust region of Viruskiller as like <Figure 5>, virus may be emitted in eruption solution by soaking and shaking for 10 minutes MDS filter to Beef extract (pH 9.5) 500ml <Figure 6>. Put magnetic bar without bacilli in effluent and mix carefully so that bubble may not occur. Catch Combination type pH meter's standard, and soak electrode for 1 minute in 0.1% chlorine solution and wash in the third distilled water after making neutral by decholoring solution and make pH of effluent to be proper.

Controlled firstly by pH 7.0~7.5 using 1 M HCl, Accounted for quantity of effluent to use pasteurized measuring cylinder. <Figure 7>. Then, to remove microbiological pollution factor, and filtered using pasteurized filter(0.2 ㎛) in asepsis worktable, filter is used after preprocess by beef extract of pH 7.0~7.5. <Figure 8>. Like this, recorded gotten supernatant liquid to Virus Data Sheet by finally volume(FCSV, Final Concentration Sample Volume).

4. Sample inoculation

When inoculate samples, inoculated each sample group together negative*positivity control group. Result that appears after inoculate sample, deciphered after confirm cell war effect(Cytophathic effect, CPE) seen in normal cell shape of negative control group and positive control group.

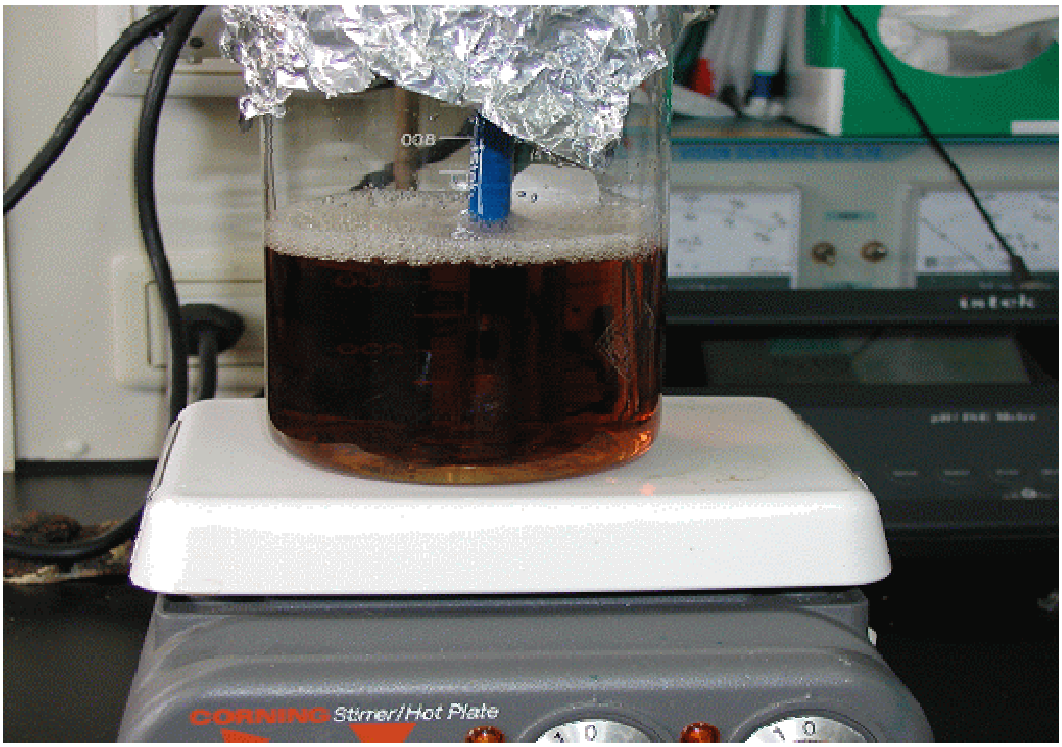
Cell that use at free medical care inoculation used that pass 3 ?s 4 day after subculture, and culturing cell passing over 6~7 days did not use considering responsiveness about virus.

First, throw away culture medium of cell culture container, after wash by DMEM culture medium that serum is not added, and concentrated sample 15ml was inoculated to total 20 flasks each 75ml/T-25flask for all T-25 flasks. Left 80~120 minutes in 37℃ for virus adsorption, shook to interval 20 minutes and avoid to be dry the cell in central of cultivation container while adsorption process, and made sample spread equally. After ends adsorption process, DMEN media contained 2% and 5% Fetal Calf Serum(FCS) are distributed in cultivation receptacle so that total volume becomes

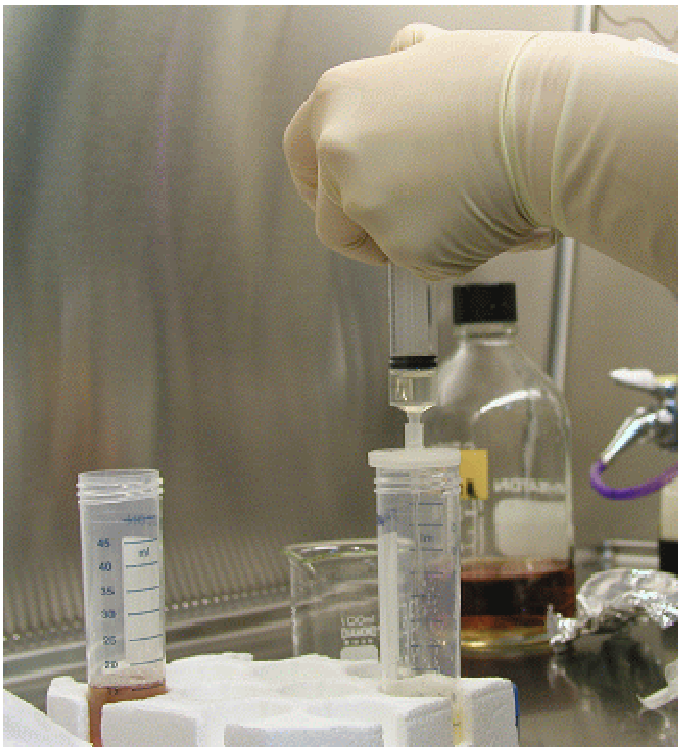
10 ㎖ in each cultivation receptacle, and cultivated in agar <Figure 9> .. Observed virus Cytopa-thic effect, CPE with microscope during total 14 days, and observed every day for 3 days after sample inoculation and observed to interval 2 days by the next. Decided by positivity that cell regeneration is seen more than 75% at observation.

<Figure 1)> Removing MDS filter from exhaust region of Viruskiller

<Figure 2)> Virus releasing from sample to effluent



<Figure 3)> Effluent pH control (pH7 ~ 7.5)



<Figure 4)> Sample pasteurization by pasteurization filter(0.2 ㎛)

<Figure 5)> Sample inoculation

Ⅳ. Experiment result

1. Infective virus of positivity and control group

Normal CRFK cell that observed to negative control group of Corona virus sample can be confirmed growing as single layer on floor of cell culture receptacle as like <Figure 10>. Contrary to this, cell that inoculated Corona virus to positivity control group can be observed secession phenomenon that decline without growing up sticking in the floor like grape as round each. <Figure 11>.

Can know that cell change from disease happened by this rounding and secession phenomenon, and affected cell can confirm easily as empty space that occurred by increase obstacle normally to virus.

2. Virus from sample that passed Viruskiller

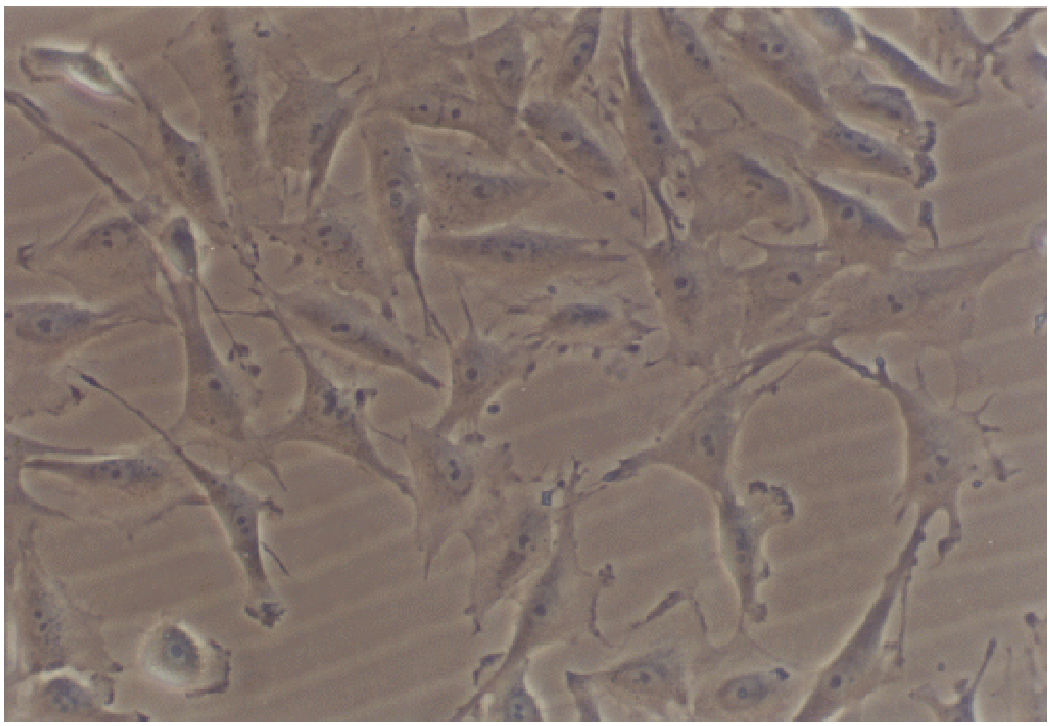
Sample of each experiment virus host(Corona virus) that passed Viruskiller, inoculated in each 20 cell culture receptacles, and observed continuously using optical microscope.

If observe CRFK cell that inoculated Corona virus experimental group, in all inoculation processes, secession phenomenon does not appear similarly with negative control group, and cell could be observed in well cultivation as single layer on floor of culture receptacle. <Figure 12~13>. It means that there is not material causes cell toxicity or virus as the result of non-existance cellular growth control or killing cell material in inoculation sample.

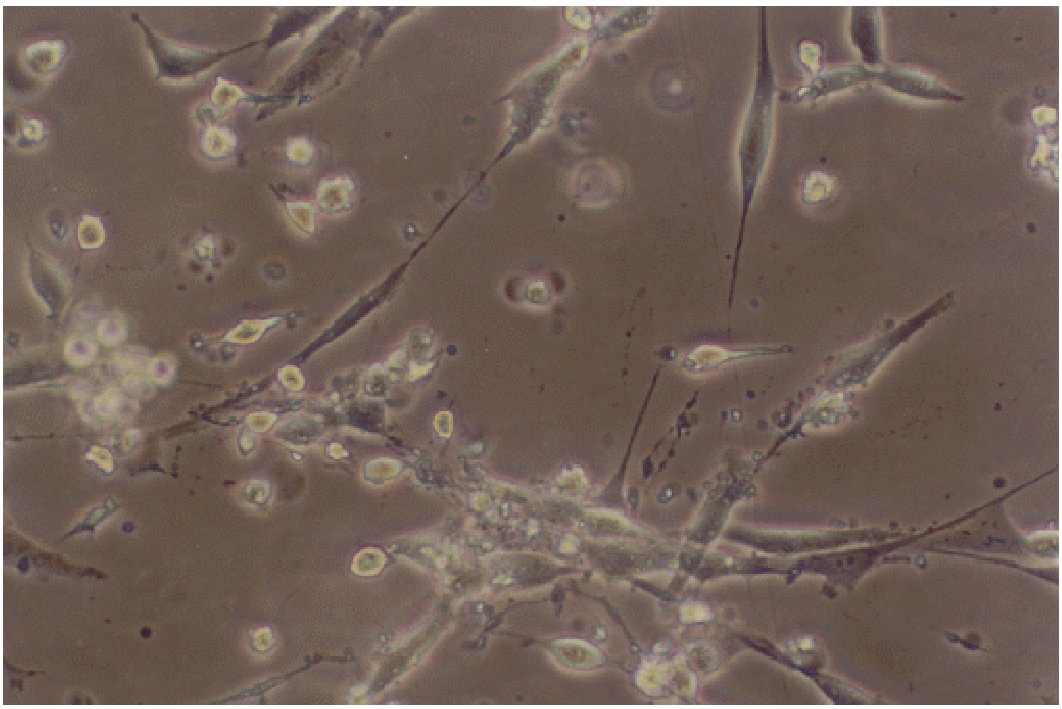
When synthesize all above results, Corona viruses are estimated that became inactive after passed Viruskiller (second cover).

Table 2) Virus study result

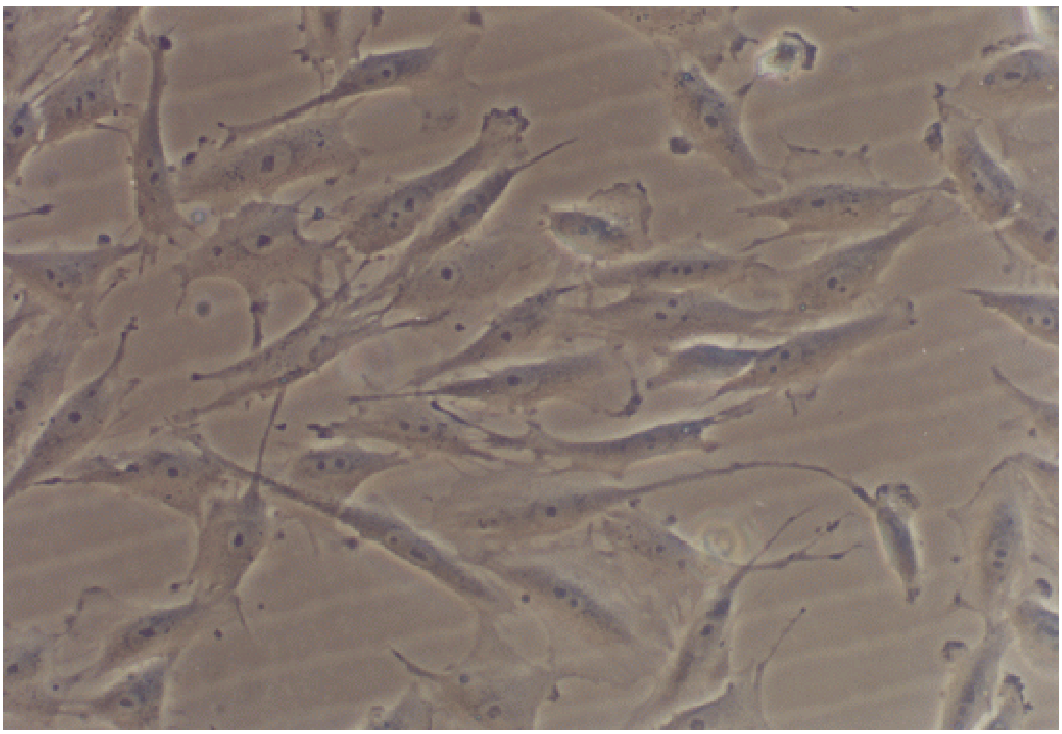
virus kind		virus injection amount	virus detection amount
Corona virus	Experimental group 1	10^6 pfu/50 ㎖	non-detection
	Experimental group 2	10^6 pfu/50 ㎖	non-detection
	Experimental group 3	10^6 pfu/50 ㎖	non-detection



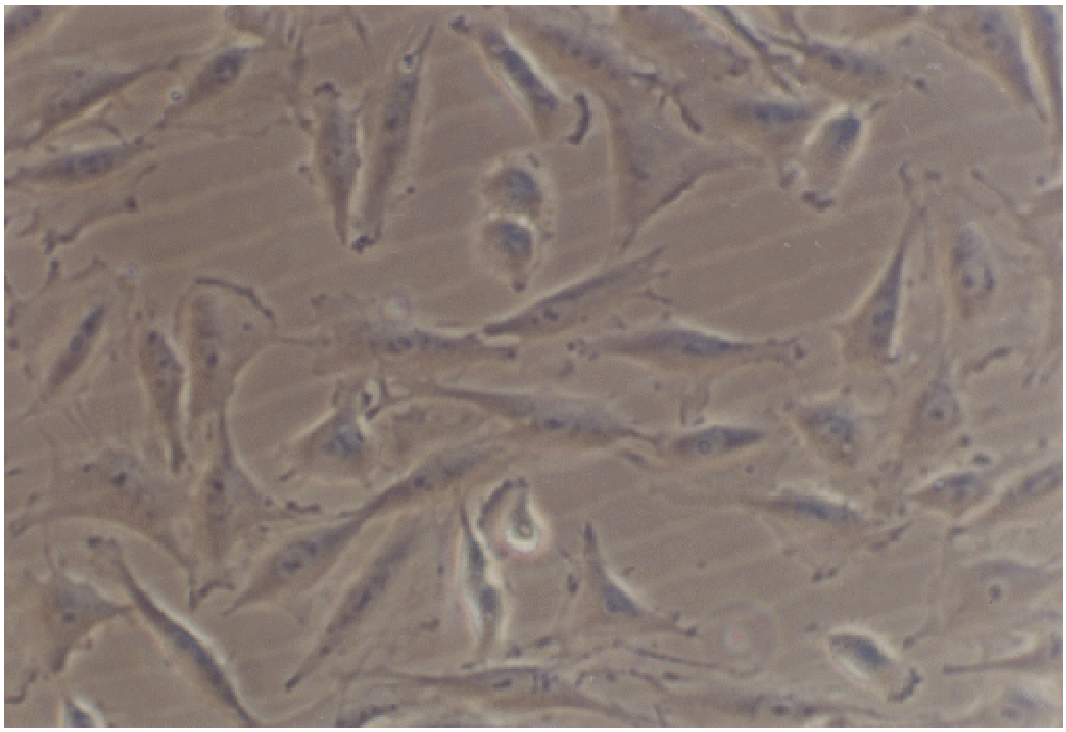
<Figure 6)> Normal CRFK cell (Negative, 200×)



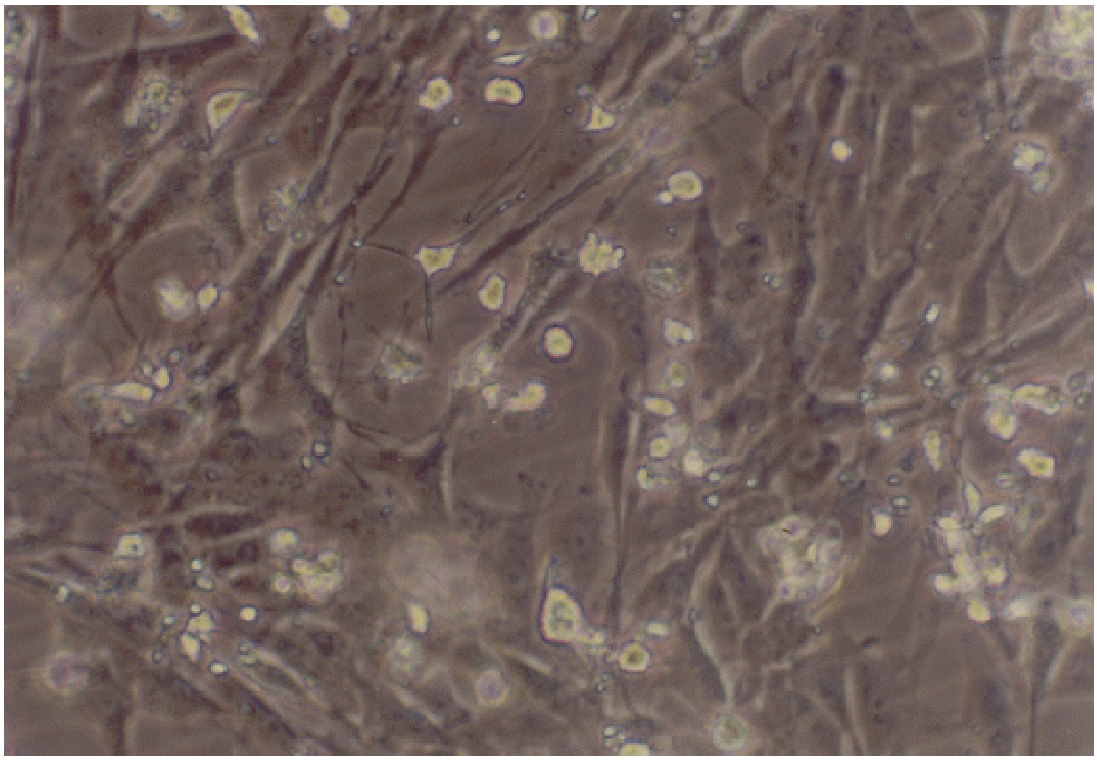
<Figure 7)> CRFK cell (Positive, 200 x) that Corona virus is inoculated



<Figure 8)> Cell that inoculate Corona virus experimental group 1(Negative, 200×)



<Figure 9)> Cell that inoculate Corona virus experimental group 2(Negative, 200×)



<Figure 10)>Cell that inoculate Corona virus experimental group 3(Negative, 200×)

Ⅴ. Conclusion

Viruskiller developed by INB Co., Ltd was tested exclusion availability of SARS induction virus that is air conduit one of most dangerous virus that is virus such as this and contagious examination uses possible Coronavirus DF 2 virus stock in examination and got following conclusion actually.

1) To achieve infectivity examination(EX) about almost impossible SARS induction virus to do infectivity examination(EX) to use actuality cell culture, could establish infectivity examination(EX) of SARS induction virus practically by an experiment that use Coronavirus DF 2 virus stock belonging to same family such as this.

2) Put million viral prticle in air inlet of Viruskiller, and tested infectivity virus among flowed exhaust air but test result was indeterminable because there was not even a infectivity virus. As this, even if many virus comes in the inhalation air of Viruskiller, can confirm that all of these became inactivity.

3) Experiment result of preceding descriptions could get conclusion that health science problem can not become entirely for basis, even if many SARS virus among air exists, when this air passes Viruskiller, can remove all infectivity SARS viruses.

4) Also, when do examination(EX) about coronavirus of singularity for basis, Viruskiller can be presumed that coronavirus with RNA virus having membrane such as the similar measles can be done inactivity.