

# Performance Verification Report of Virus Killer VK-401 on Respiratory Pathogen and SARS-CoV2 Nuclei Acid

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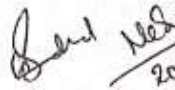
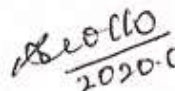
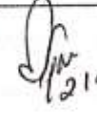
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Performance Verification Report of Virus Killer Instrument – VK - 401

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## 1.0 INTRODUCTION

Human beings are constantly adapting to the changes in their environment. Some of these changes are due to the threats that are encountered. One such threat is that of infections from micro-organisms in air. There are various theories and hypothesis of Virus particles circulating in air either as aerosols or droplets causing infection. There are studies that several diseases are caused by viruses and bacteria as they are still circulating for certain period in air.

When people with certain infections cough, sneeze or talk, airborne diseases can spread. This happens as they spew nasal and throat secretions into the air. Some viruses or bacteria take flight and hang in the air or land on other people or surfaces.

Airborne pathogenic organisms can take up residence inside you when you breathe them. You can also pick up germs when you touch a surface that harbours them, and then touch your own eyes, nose, or mouth. A certain section of population can become seriously ill due to lower immunity. Because these diseases travel in the air, they are hard to control.

Therefore, there is a great importance in health science to remove such pathogens from living environment. There are various viruses that exist in aerosols or floating state that can cause grave danger and illness. Hepatitis A, Adeno virus, Rhinovirus etc. are pathogens that can exist in air for relative long term without membrane. Viruses like smallpox, Influenza, measles, rubella, parotitis, varicella etc. can also transmit through air. Vaccines has been developed for many of these viruses that help us develop immunity.

However, it is the Coronavirus that has been a big problem in health science in recent years. The have caused large scale pandemics with SARS CoV1 in 2003, MERS-CoV in 2012 and SARS CoV2 in 2019 (Covid19). Currently, there are no vaccines or anti-viral drugs to prevent or treat human corona virus infections.

Therefore, a device that can remove pathogens from air, including Corona viruses, is of great importance as it can improve the living conditions of a large section of the population. It can also help in starting the economic activity by reducing the chances of infections in workplaces and places of commerce.

VirusKiller device by Radic8 is tested in this study for its ability to remove SARS CoV2 (Covid19) along with other respiratory viruses and bacteria.

## 2.0 TEST EQUIPMENT USED

### A) VirusKiller Device

Principle of Virus Killer VK 401:

VirusKiller is an air purifier and sterilizer. The purification function works using a combination of filters – a pre-filter, a carbon filter and a HEPA filter. This filters out most of the common air pollutants like dust, VOCs, fungus and some bacteria. The device then uses Photocatalytic Oxidation (PCO) using a combination of nano filter tubes that are coated with titanium dioxide (TiO<sub>2</sub>) and UVC lights. Hydroxyl radicals (-OH) are created when UVC lights fall on the TiO<sub>2</sub> nano filter tubes. These hydroxyl radicals then react with the RNA of the viruses to oxidize (kill) them. The nano filter tubes are filled with activated carbon in order to catch harmful gas and odour, creating time for the -OH radicals to decompose them.

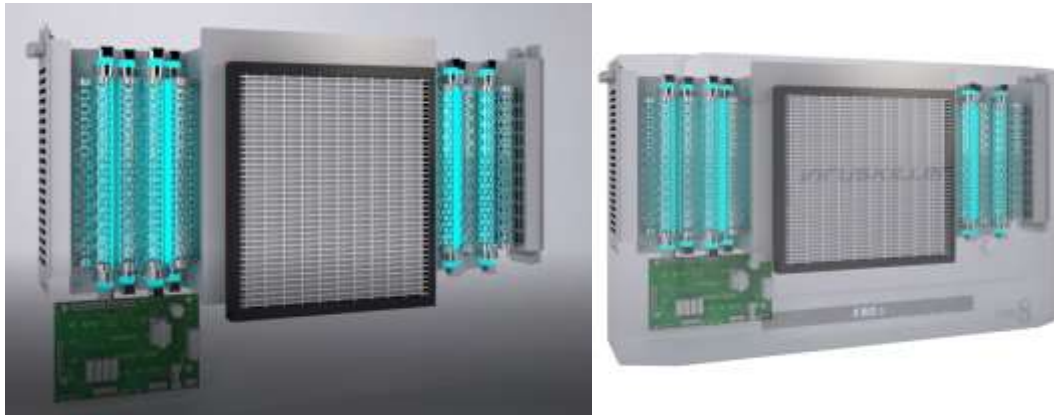


FIGURE 1 – Cross Sectional Picture of Virus Killer VK-401



FIGURE 2 – Working Principle of Virus Killer VK-401



FIGURE 3 - Virus Killer Instrument- Front with LED Light On.

B) Biosafety Level 2 Cabinet

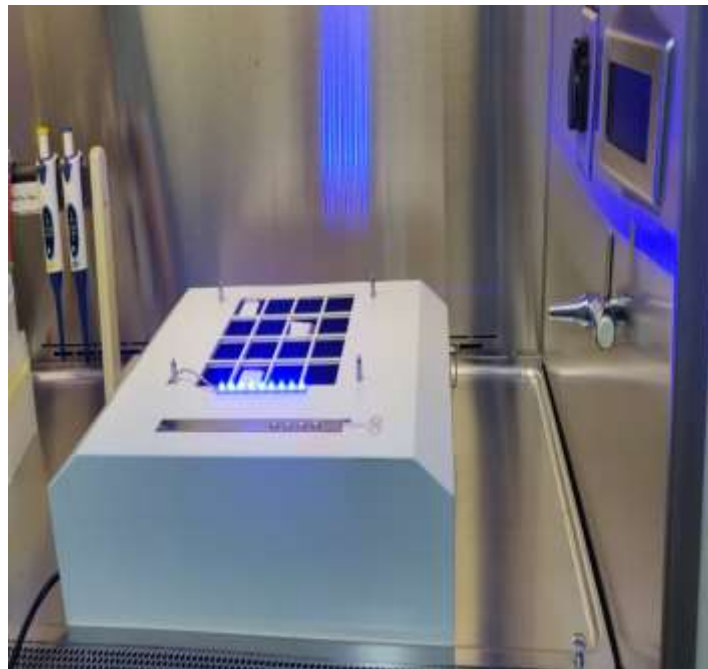


FIGURE 4 – VirusKiller device in Bio Safety Cabinet

### C) Spray Gun for Aerosol Generation

Spray gun was used to generate aerosols from a solution with a mixture of pathogens. These aerosols were introduced at the inlet of the device.



**FIGURE 5 – Spray gun for aerosol generation**

### D) Filter Membrane

This is used to collect Nucleic Acid and other micro-organisms used in the tests. These membranes were placed at various locations on the VirusKiller device. The filter membrane used is of Whatman make.



**FIGURE 6 – Membrane used at inlet and outlet of the device**

### E) Refrigerated Centrifuge

A refrigerated centrifuge delivering up to 16,000 rpm was used.



**FIGURE 7 – Refrigerated Centrifuge**

F) Micropipette Sets



**FIGURE 8 – Micropipette Sets**

G) Real Time PCR

The following model of Real Time PCR detection system was used:



**FIGURE 9 – BIO RAD CFX96**



The CFX96 Touch System is a powerful, precise, and flexible real-time PCR detection System. This six-channel real-time PCR instrument combines advanced optical technology with precise temperature control to deliver sensitive, reliable detection for single or multiplex reactions.

#### H) Automated Nucleic Acid Extraction

The following device was used for extraction of Nucleic Acid:



**FIGURE 10 - NEXTRACTOR NX-48 from GENOLUTION INC.**

Nextractor is automatic extraction system for rapid isolation of DNA or RNA from a variety samples.

### 3.0 TEST MATERIALS

The following reagents were used in the test:

- a) Positive Control for SARS CoV2 & Respiratory Pathogen**
- b) Nuclei Acid Extraction reagents – Genolution – Lot No. – CVN143-200714**
- c) Amplification or Real Time PCR Kits. – Altona Lot No. -025971**

## 4.0 TEST APPROACH

The entire test design was prepared by keeping in mind following:

- a) Safety
- b) Proper aerosol generation
- c) Trapping of exhausted air to demonstrate if killing is effective.

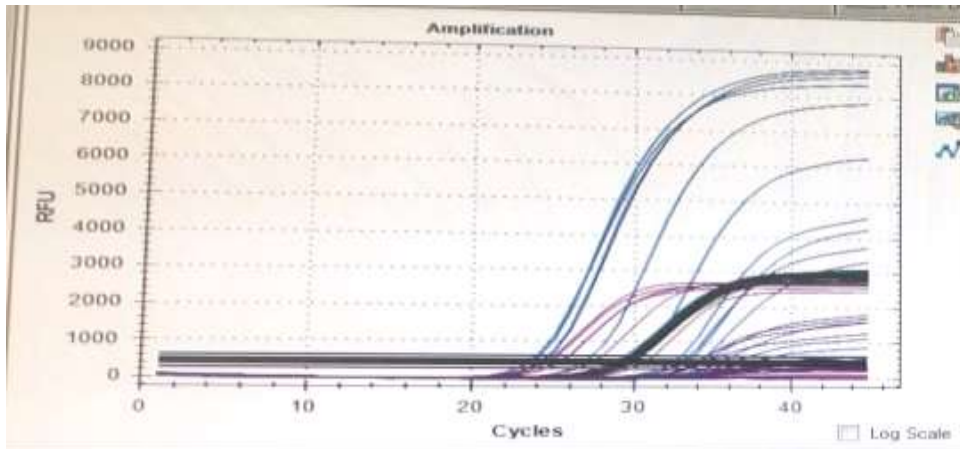
### Initial Verification

To verify the experiment whether viral nuclei acid amplifies on the filter membrane, Ultra-pure water was spiked with standard solution of synthetic SARS-CoV-2 from Altona Diagnostics GmbH (Invitro Transcribed) RNA. On one filter membrane – spiked sample was sprayed and on other filter membrane – unspiked buffer solution was sprayed. Both the filter membranes were put in Viral Transport Media and RNA Extraction was done. Sample from spiked positive sample show amplification and sample from unspiked VTM showed no amplification (Negative).

The analysis was performed using RT-PCR tests in accordance with WHO recommendations. The test kits included a one-step real-time RT-PCR Tests for two target genes on the SARS-CoV-2 virus genome and control samples.

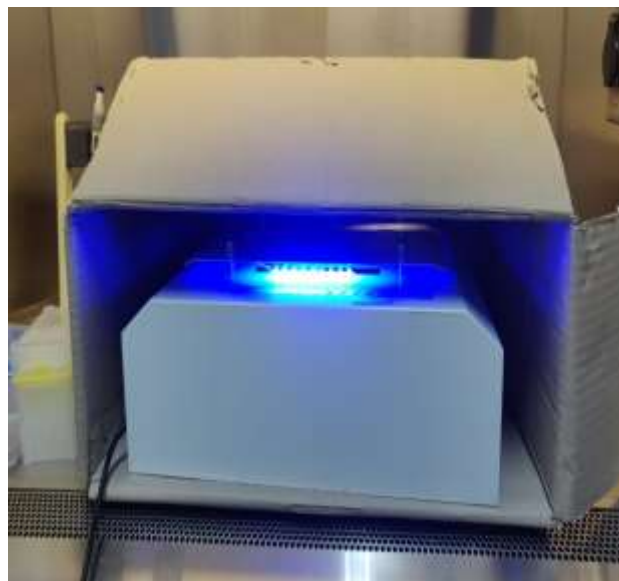
Currently available CE reagents and kits for SARS CoV2 in market is primarily meant for Human Sample testing. However, Primers for various genes for SARS COV2 – E-Gene; N-Gene; S-Gene; RdRp Gene or ORF1-gene used in currently available kits are specific to sarbecovirus family and SARS2 CoV2.

The signal obtained from a PCR analysis is a fluorescent intensity translated to a Ct value. The Ct value or cycle threshold value also called cycle quantification value (Cq) is the PCR cycle number at which the sample reaction curve intersects the threshold line. This line is the point at which a reaction reaches a fluorescent intensity above background levels and functions as a level of detection indicator. The Ct value is the number of cycles required to detect a discernible signal from the sample. The greater the Ct value the more cycles are required to achieve detection, which implies a lower RNA content in the specimen. This principle is represented in the figure below, which shows the amplification plots from a test performed.



**FIGURE 11 – Amplification Plot Showing Ct Threshold & Threshold Line in Different Coloured Lines. Represents Fluorescent Intensity from Different Samples.**

Sample testing was carried out in a Biosafety level 2 cabinet using IC (Internal Control) as a process control to validate that the method can extract the virus from the samples.



**FIGURE 12- Virus Killer instrument Inside sampling Box.**

After the initial verification, two tests were performed using the Virus Killer device:

### Test 1

The real-world scenario might present a mixture of various pathogens present the air simultaneously. Therefore, the device was tested for its effectiveness against a mixture of pathogens. The following respiratory pathogens were used in the test:

**BACTERIAL PATHOGEN:**

1. Chamydophila pneumoniae
2. M. pneumoniae
3. L. pneumophilia
4. B. pertussis
5. B. parapertussis
6. S. pneumoniae
7. H. Influenzae

**VIRAL PATHOGEN**

1. Influenza A Virus
2. Influenza B Virus
3. Respiratory Syncitial Virus A
4. Respiratory Syncitial Virus B
5. Influenza A Virus Strain H1
6. Influenza A Virus Strain H3
7. Pandemic Influenza A Virus Strain H1
8. **SARS CoV2**

**Test 2:**

The second test was conducted specifically for the device's effectiveness against SARS CoV2. Both Genes were included and demonstrated following Ct Values at different concentrations.

<b>Copies/ul</b>	<b>10<sup>4</sup></b>	<b>10<sup>3</sup></b>	<b>10<sup>2</sup></b>	<b>10<sup>1</sup></b>
<b>S-Gene</b>	23.44 Ct	26.43 Ct	29.70 Ct	33.03 Ct
<b>E-Gene</b>	23.94 Ct	27.31 Ct	30.42 Ct	33.63 Ct.
Ct values of S-Gene and E-Gene for Various Concentration				

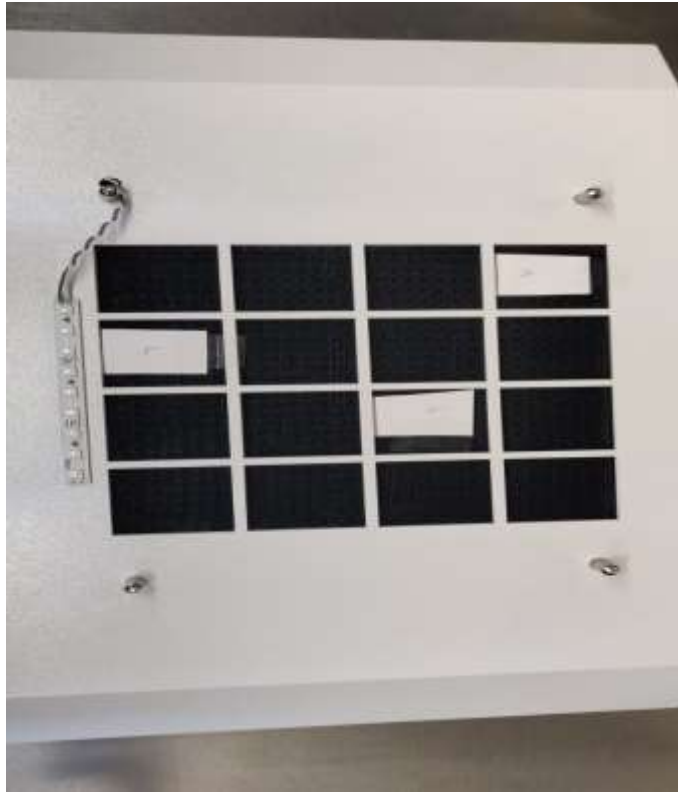
NOTE: Infectivity examination was not conducted using the device as SARS Virus Induction & culture is not possible.

In both tests, the VirusKiller instrument was placed inside the carton box and a small inlet created in it for injecting the aerosolized positive control. Filter membranes were placed at different locations on the device for collecting the aerosols prior to filtering. Filter membranes were placed at the outlets to collect the aerosols.

The positive control was added in Viral transport medium and an aerosol of the organisms was sprayed with the device plugged in and functioning.

All these filter paper samples which were collected were subjected to RNA Extraction protocol and subsequently SARS CoV2 RT PCR & Respiratory Pathogen Multiplex PCR.

Results were consistent with both experiment combination. This proved that result is robust with different extraction – either automated or manual extraction kit.



**FIGURE 13 - Virus Killer Front (Without Panel) with Sampling points.**



**FIGURE 14 - Virus Killer Side Panel (Exhaust) - Opened.**



FIGURE 15 - Spray Ionization of Positive control



FIGURE 16 - Virus Killer (with Sampling locations- Front and Side)

## 5.0 RESULTS

### A) Results of Test 1

**TABLE-1: RESPIRATORY PATHOGEN.**

Sr. No.	Sample No.	Parameters	Results
1	Outer Surface Location 1	Bacterial & Viral Pathogen	POSITIVE
2	Outer Surface Location 2	Bacterial & Viral Pathogen	POSITIVE
3	Outer Surface Location 3	Bacterial & Viral Pathogen	POSITIVE
4	Outer Surface Location 4	Bacterial & Viral Pathogen	POSITIVE
5	Inner Surface (On filter) 1	Bacterial & Viral Pathogen	POSITIVE
6	Inner Surface (On Filter) 2	Bacterial & Viral Pathogen	POSITIVE
7	Inner Surface (On Filter) 3	Bacterial & Viral Pathogen	POSITIVE
8	Outlet (Exhaust) 1	Bacterial & Viral Pathogen	NEGATIVE
9	Outlet (Exhaust) 2	Bacterial & Viral Pathogen	NEGATIVE
10	Filter Paper (Positive)	Bacterial & Viral Pathogen	POSITIVE
11	Filter Paper (Negative Control)	Bacterial & Viral Pathogen	NEGATIVE
12	Swab Test On Surface	Bacterial & Viral Pathogen	POSITIVE

\* **BACTERIAL PATHOGEN** - i) Chlamydia pneumoniae ii) M. pneumoniae iii) L. pneumophila iv) B. pertussis v) B. parapertussis vi) S. pneumoniae vii) H. Influenzae

\* **VIRAL PATHOGEN** - i) Flu A ii) Flu B iii) RSV A iv) RSV B v) Flu A-H1 vi) FluA-H3 vii) Flu-A-H1 pdm09



## B) Results of Test 2

**TABLE-2: RESULTS WITH CT VALUES FOR E-GENE AND S-GENE FOR SARS CoV2**

Sr. No.	Sample No.	Results	Ct Value
1	Outer Surface Location 1	POSITIVE	E-Gene – 27.47 S-gene – 22.36
2	Outer Surface Location 2	POSITIVE	E-Gene – 21.73 S-gene – 24.05
3	Outer Surface Location 3	POSITIVE	E-Gene – 25.86 S-gene – 25.74
4	Outer Surface Location 4	POSITIVE	E-Gene – 23.10 S-gene – 23.24
5	Inner Surface (On filter) 1	POSITIVE	E-Gene – 28.86 S-gene – 28.74
6	Inner Surface (On Filter) 2	POSITIVE	E-Gene – 26.95 S-gene – 26.72
7	Inner Surface (On Filter) 3	POSITIVE	E-Gene – 28.19 S-gene – 28.13
8	Outlet (Exhaust) 1	NEGATIVE	E-Gene – 33.36 S-gene – 33.01
9	Outlet (Exhaust) 2	NEGATIVE	E-Gene – 33.36 S-gene – 33.01
10	Filter Paper (Positive)	POSITIVE	E-Gene – 30.88 S-gene – 30.67
11	Filter Paper (Negative)	NEGATIVE	Not Amplified.
12	Swab Test On Surface	POSITIVE	E-Gene – 30.94 S-gene – 31.07

Sample analysis was performed using CE marked RNA Extraction Protocol and CE Marked SARS CoV2 Real time Amplification methodology.

Appropriate negative, positive controls were run & found satisfactory.

## 5.0 CONCLUSIONS

Virus Killer Model VK-401 from Radic 8 marketed by M/s Oorja Energy Engineering Services Pvt Ltd was tested using respiratory pathogens. It was verified that the DNA/RNA of respiratory pathogens in air becomes negative at the exhaust when it passes through the device.

Following pathogens were tested for this purpose:

- 1) Chlamydomophilia pneumoniae
- 2) Mycoplasma pneumoniae
- 3) Legionella pneumophila
- 4) Bordetella pertussis
- 5) Bordetella parapertussis
- 6) Streptococcus pneumoniae
- 7) Haemophilus influenza
- 8) Influenza-A
- 9) Influenza -B
- 10) Respiratory Syncytial Virus-A
- 11) Respiratory Syncytial Virus -B
- 12) Influenza -A Strain- H1
- 13) Influenza -A Strain- -H3
- 14) Influenza -A Strain-H1pdm09
- 15) SARS CoV2 – (Novel Corona Virus)

Since device comprises of multiple filters (Pre-Filter, HEPA Filter and Carbon Filter) and a reactor chamber comprising of nano tubes coated with TiO<sub>2</sub> & UVC Light, it offers barrier by not allowing nuclei acid particles to pass through as noticed in the exhaust air.

## 6.0 REFERENCES

- 1) Sharp G: The lethal action of short ultraviolet rays on several common pathogenic bacteria, *Journal of Bacteriology*, 1939;37:447-59
- 2) Duan et al., 2003: Stability of SARS corona virus in human specimens and environment and its sensitivity to heating and UV irradiation. *Biomed. Environ. Sci.*, 16(2003), p246-255
- 3) High-efficiency particulate air (HEPA) filters in the era of COVID 19): function and efficacy, David A Christopher, William C Yao, MD, Minming Lu, PhD. <https://doi.org/10.1177/0194599820941838>

## 7.0 DISCLAIMER

1. The kit and the extraction method used have been optimized for testing the matrix provided. However, the LODs have not been verified for the current sample matrices.
2. This test detects the E gene and the S gene of SARS-COV2 from the RNA extracted but does not determine the viral activity.
3. The content of the report should not be interpreted as endorsement of the product by Vimta Labs. The name and logo of Vimta Labs should not be used without written consent from Vimta Labs.
4. This test is not covered under the scope of NABL testing. This test was performed in the Molecular Biology department of Clinical Reference Laboratory.
5. The samples have been processed with all the PPE and procedures required as per the BSL 2 requirements.
6. Method: Realtime Reverse Transcriptase PCR, Instrument used: BioRad CFX96
7. Other consumables: Buffers, pipettes, aerosol barrier, pipette tips etc.