# Study of Viral Load Reduction in Disposable Face Masks Upon UV-C Exposure Using RT-qPCR

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

School of Pharmacy
Brac University
October 2022

## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

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# Approval

The thesis titled "Study of Viral Load Reduction in Disposable Face Masks Upon UV-C Exposure Using RT-qPCR", submitted by A.A.M Sharfuddin (18346053) of Summer 2018 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy on November 2022.

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## Ethics Statement

Consent was collected from each participant before this research was conducted and none of their information has been disclosed.

## Abstract

This research is designed to promote the long-term usage of surgical masks, proper disposal, environmental protection, controlled production and the developed UV-C chambers validation. Moreover, we sought to evaluate the effectiveness of our developed decontamination technique for surgical masks using UV-C irradiation, as there is no established guideline for the safe reuse of surgical masks. Masks were collected from the patients and included in the experiment if the presence of the Covid-19 virus was ensured. These used masks were treated with UV-C to decontaminate them and RT-qPCR analysis was used to determine whether the Covid-19 virus was present. The final evaluation was done by comparing the RT-qPCR results of before and after UV-C exposed samples. The established results are quite preferable and consistent with the hypothesis and the UV-C exposure rendered the Covid-19-causing genes inactive. Using the UV-C chamber to reuse the masks could be a new addition of technology in recent eras, which may ensure the rational use of masks.



**Keywords:** RT-qPCR; UV-C Exposure; Sterilization; RNA Extraction; UV-C Chamber; Disinfection; Viral Load; CT Value.

## Dedication

*Dedicated to my parents*

## Acknowledgment

I would like to proceed by thanking Almighty Allah to give me the knowledge, strength and patience I needed to accomplish my research. Also, I am grateful to my parents for supporting me throughout this journey.

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# Table of Contents

**Declaration**.....**ii**

**Approval** ..... Error! Bookmark not defined.

**Ethics Statement**.....**iv**

**Abstract**.....**v**

**Dedication** .....**vi**

**Acknowledgment**..... Error! Bookmark not defined.

**List of Figures**.....**x**

**List of Acronyms** .....**xi**

**1. Introduction**.....**12**

1.1 UV-C Disinfectant Chamber.....13

1.2 Effects of the UV-C Chamber.....14

1.3 RT-qPCR Technique.....15

**2. Methodology** .....**15**

2.1 Sample Collection and Preparation.....16

2.2 RNA Extraction Procedure .....17

2.3 Master-mix Preparation .....19

2.4 Sample Run in RT-qPCR for amplification.....21

2.5 UV treatment of the used masks .....21

**3. Results** ..... **21**

3.1 RT-qPCR results for Sample 1 ..... 23

3.2 RT-qPCR results for Sample 2 ..... 24

3.3 RT-qPCR results for Sample 3 ..... 25

3.4 RT-qPCR results for sample 4 ..... 26

3.5 RT-qPCR results for Sample 5 ..... 27

3.6 Positive Control ..... 28

3.7 Negative Control ..... 28

**4. Discussion** ..... **29**

**5. Conclusion:** ..... **32**

**6. References** ..... **33**

# List of Figures

Figure 1: UV-C Chamber, Laboratory, EEE Department, Brac University. ..... 13

Figure 2: Pyrimidine dimer formation after UV-C exposure..... 14

Figure 3: Illustration of Complete workflow followed during the experiment. ..... 16

Figure 4: RNA extraction protocol followed during the experiment..... 19

Figure 5: Master Mix Preparation Protocol followed during the experiment..... 20

Figure 6A: Illustrated graph and CT values of Sample 1 (Before UV-C Exposure)..... 23

Figure 6B: Illustrated graph and CT values of Sample 1 (After UV-C Exposure)..... 23

Figure 7A: Illustrated graph and CT values of Sample 2 (Before UV-C Exposure)..... 24

Figure 7B: Illustrated graph and CT values of Sample 2 (After UV-C Exposure)..... 24

Figure 8A: Illustrated graph and CT values of Sample 3 (Before UV-C Exposure)..... 25

Figure 8B: Illustrated graph and CT values of Sample 3 (After UV-C Exposure)..... 25

Figure 9A: Illustrated graph and CT values of Sample 4 (Before UV-C Exposure)..... 26

Figure 9B: Illustrated graph and CT values of Sample 4 (After UV-C Exposure)..... 26

Figure10A: Illustrated graph and CT values of Sample 5 (Before UV-C Exposure)..... 27

Figure 10B: Illustrated graph and CT values of Sample 5 (After UV-C Exposure)..... 27

Figure 11: Illustrated graph and CT values of Positive Control ..... 28

Figure 12: Illustrated graph and CT values of Negative Control..... 28

Figure13:Comparison between the effect of N gene in before and after UV-C exposed samples 30

Figure 14: Comparison between the effect of ORF1ab gene in before and after UV-C exposed samples..... 30

Figure15: Comparison between the effect of IC gene in before and after UV-C exposed samples ..... 31

## List of Acronyms

UV-C: Ultraviolet C (Germicidal UV)

RT-qPCR: Reverse Transcription Quantitative Polymerase Chain Reaction

BSL: Biosafety Level

Covid-19: Coronavirus Disease of 2019

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

VNE Buffer: Viral Nucleic Acid Extraction Kit

RNase-free Water: Nuclease-Free Water

IPA: Isopropyl Alcohol

VTM: Viral Transport Medium

CT values: Cycle of threshold values

RFU: Relative Fluorescence Units

N gene: Nucleocapsid gene of Covid-19 Virus

ORF1ab: The largest polyprotein gene of Covid-19 Virus

IC: Internal Control (Normal human Cellular gene)

# 1. Introduction

A discernible increase in the usage and manufacture of face masks has been reported worldwide since the Covid-19 pandemic emerged. The global market for disposable masks has expanded from $0.73 billion to $22 billion annually due to governments mandating the wearing of face masks in public places (Ahmed & Lim, 2022; Atilgan Turkmen, 2022; Z. Chen et al., 2022; Rathinamoorthy & Raja Balasaraswathi, 2022; Yan et al., 2020). The enormous use of face masks has drawn attention recently due to waste generation, which endangers the environment as it is used a million of times each day globally, required more energy and raw materials to manufacture (Atilgan Turkmen, 2022; Chowdhury et al., 2021; Hui Li et al., 2022; Morganti, 2020; Muhyuddin et al., 2022; Torres & De-la-Torre, 2021).

Moreover, these used masks are discarded in public spaces that impacts the environment and threatens the ecosystem due to improper and mismanaged disposal (Ferronato & Torretta, 2019; Nzediegwu & Chang, 2020). Following soil exposure, the ecotoxicological impacts of these wastes on immunologic parameters, survival, reproduction, and energy-related parameters were investigated. (Ahmed & Lim, 2022; Aragaw, 2020; H. Chen et al., 2022; Z. Chen et al., 2022; Delgado-Gallardo et al., 2022; Du et al., 2022; Jemec Kokalj et al., 2022; Liang et al., 2022; Rathinamoorthy & Balasaraswathi, 2022).

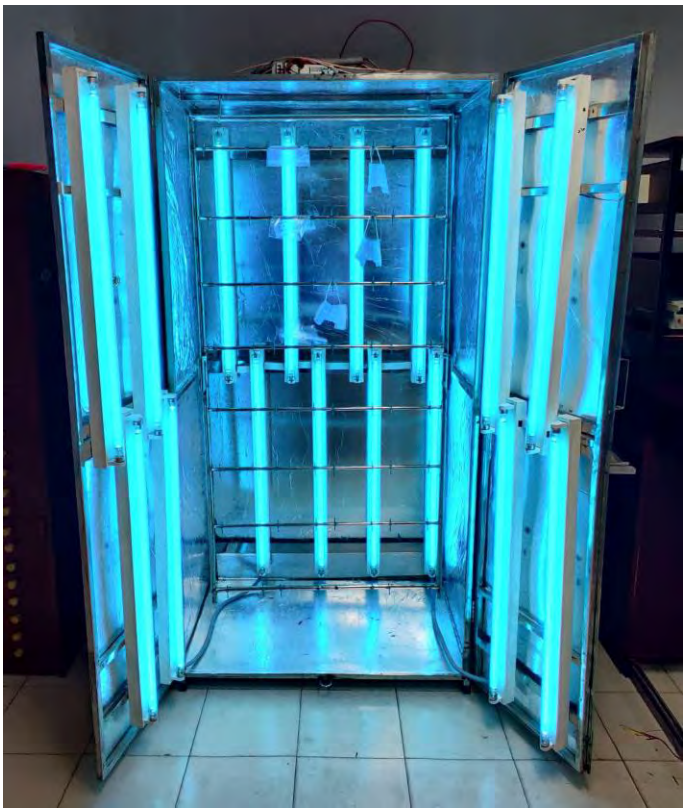
Furthermore, health experts warn that improperly discarded masks can spread viral diseases throughout a community (Ferronato & Torretta, 2019; Mol & Caldas, 2020; Organization, 2017; Sangkham, 2020; Shiferie, 2021; *Solid Waste Management*, n.d.; Yong et al., 2009). With this in mind, face mask disposal is an uppermost concern in the post-pandemic stage. Recent waste disposal techniques include burning and landfilling, which are inappropriate public health

solutions. Some other prevention methods are heat sterilization, filtering, chemical disinfectant agents, and ultraviolet irradiation, which might reduce the risk of viral infection. UV-C technology is used to reuse masks after disinfection, which might reduce pollution and save money worldwide (Ahmed & Lim, 2022; Raeiszadeh & Adeli, 2020; Teo et al., 2021; van Straten et al., 2021).

This research is designed to promote the long-term usage of surgical masks, proper disposal, reduce waste generation, environmental protection, controlled production and the developed UV-C chambers validation.

## 1.1 UV-C Disinfectant Chamber

The UV-C disinfectant chamber used here was developed by the students of the EEE Department at Brac University following the established model made by Stanford University, USA.



Size: H-72”, W-36”, D-24”

Fluence Level (Dosage): 1 J/cm2

Lamp: 16 lamps (11W each),

Lamp spacing :23 cm/ 9.05 in

Throughput/per day:5000

UV-C emission inside the chamber:

254 nm

Duration: 5 minutes

*Figure 1: UV-C Chamber, Laboratory, EEE Department, Brac University.*

Firstly, validation test was performed for this chamber and then used it to disinfect masks. The wavelength emitted inside the chamber is 254nm, which is standard to kill germs. There are 16 UV lamps, each of which is 11 watts. The fluence level is the product of UV radiation's intensity and the duration of exposure to an organism. Moreover, the throughput estimated per day is five thousand masks for this chamber.

## 1.2 Effects of the UV-C Chamber

UV disinfection is a chemical-free method for disinfecting surfaces, air, and water. UV-C is a germicidal ultraviolet spectrum range between 200-280nm. UV-C light inhibits microorganism growth in any medium (Raeiszadeh & Adeli, 2020; Spencer et al., 2017; Teo et al., 2021; Yang et al., 2019). UV-C photons absorbed by nucleic acids damage microorganisms' ability to replicate due to photochemical changes (Raeiszadeh & Adeli, 2020).

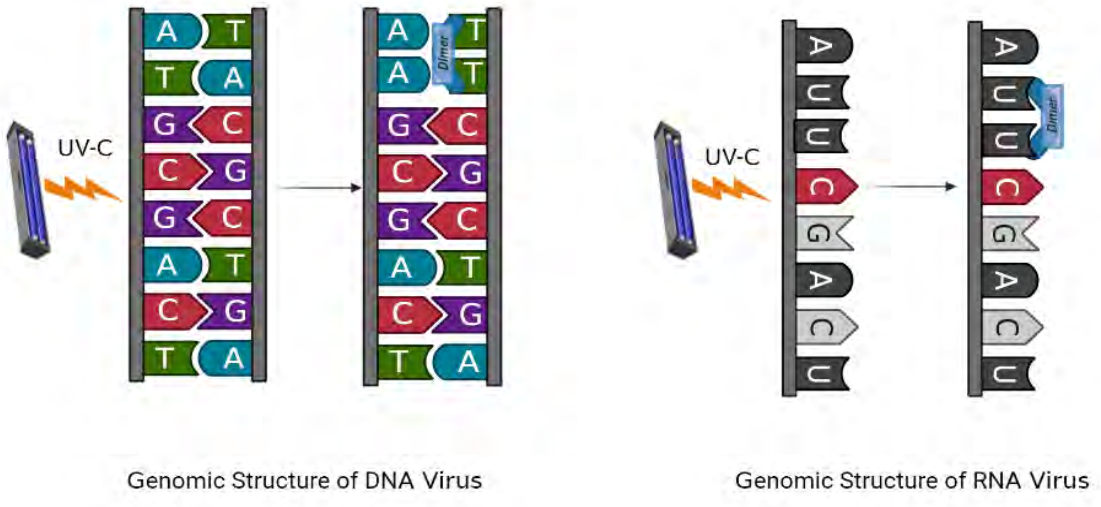



*Figure 2: Pyrimidine dimer formation after UV-C exposure.*

UV-C photons break the adenine-thymine bond and form a pyrimidine dimer, which prevents cell replication. In RNA virus, pyrimidine dimers were formed between uracil instead of thymine. Moreover, UV-C light is more destructive to single-stranded viruses because the second strand lacks genetic information that helps double-stranded viruses to fix damages. (Kowalski, 2009; Rauth, 1965).

### 1.3 RT-qPCR Technique

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is considered to be the most efficient and precise ways to detect and investigate the Covid-19 virus. This is a nuclear-derived method that uses fluorescent dyes to identify specific genetic substances in any disease, even a virus. (Jawerth, 2020; Rannan-Eliya et al., 2021; Wang et al., 2020). This technology enables scientists to observe the outcomes in near-real-time while the process is running (Heid et al., 1996; Vaerman et al., n.d.; Wittwer et al., 1997). In this experiment, we used the BIO-RAD CFX96TM Real-Time System (C1000TM Thermal Cycler), which is a sophisticated, accurate, and detects real-time PCR results. This 6-channel (5 color and 1 FRET channel) RT-qPCR apparatus incorporates advanced optical technology with precision in thermoregulation (*CFX96 Touch Real-Time PCR Detection System*, n.d.).

## 2. Methodology

The research plan was divided into "before UV-C exposure" and "after UV-C exposure" categories. For this study, the sample were chosen to include data from 5 participants (n = 5). These procedures were done in the Bio Safety Cabinet's Level 2+ laboratory. The Cabinet was cleaned with IPA and the UV was switched on for 5 minutes to prevent contamination from the previous users. The workflow followed during the experiment is attached below:

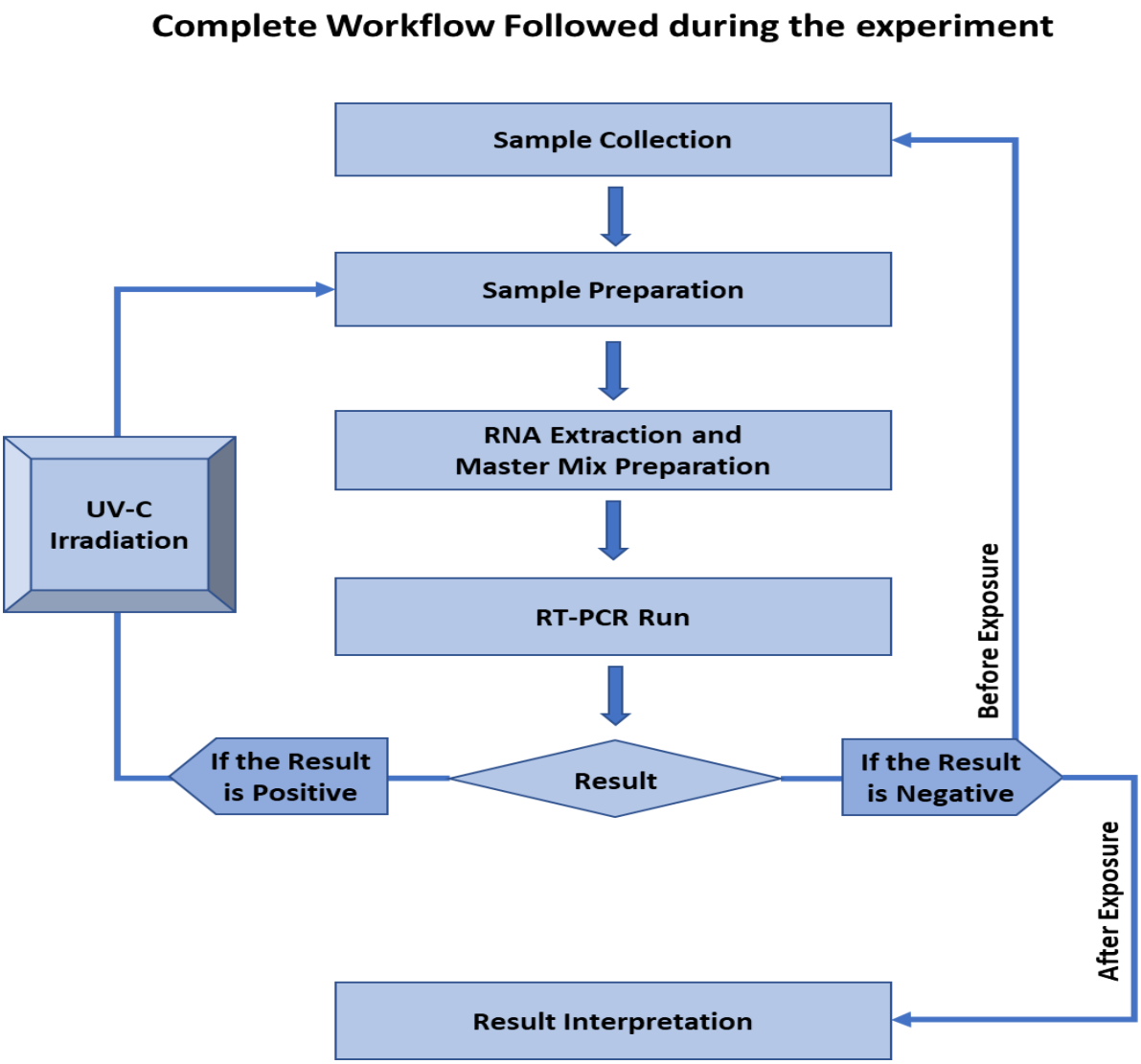



*Figure 3: Illustration of Complete workflow followed during the experiment.*

## 2.1 Sample Collection and Preparation

Covid-19-positive individuals around Dhaka city consented to the collection of their samples. Collected masks were sealed in zip-lock bags and kept in an icebox to prevent the virus from spreading. We only included the surgical masks from the collected samples and excluded other masks manufactured with different materials. The samples were most likely taken from the doctors and health workers as they always kept in contact with the patients. Moreover, we also ensured

that the patients wore the masks for as long as possible. Also, the mask wasn't taken from the patient who was no longer Covid-19 positive or stored for a very long time.

The collected masks were marked and cut into 1 cm × 1 cm pieces to add in the Viral Transport Medium (VTM). Then the VTM was vortexed to thoroughly mix with the portion of the mask before being stored in a -80°C refrigerator for RNA extraction. The VTM was needed to retain the viral integrity of the sample which was necessary for culture, isolation and further sequence analysis (McAuley et al., 2021; Syrmis et al., 2004; Zhou et al., 2021).

## 2.2 RNA Extraction Procedure

FAVORPREP™ Viral DNA/RNA Kit was used to extract the RNA and the protocol provided by the company was followed to run the experiment. These steps were conducted under the Bio Safety Cabinet to prevent any kind of contamination.

**The following protocol was:**

1. **Sample:** Sample in VTM was vortexed and centrifuged to mix, then 140µL sample was transferred to a tube with a micropipette.
2. **Sample Lysis:** Then 560µL of VNE and carrier RNA were added, vortexed, and kept 10 minutes of incubation at room temperature.
3. **Optimization of binding conditions:** 560µL ethanol was added to the sample and vortexed to mix.
4. **Bind Viral RNA to Column Membrane:** Kit collection tube had a VNE column attached. 700µL of the sample mixture was then pipetted into the VNE column and

centrifuged at 8000 RPM. The liquid was discarded when it was stored in the collection tube and then reattached to the VNE column.

The rest of the sample mix was put into the VNE column, centrifugation was done at 8000 RPM, and the residue was discarded. After that, a new collection tube was attached to the VNE column.

5. **Wash column membrane by Wash Buffer 1:** Ethanol-containing wash buffer 1 was put into the VNE column in a quantity of 500µL and centrifugation was done at 8000 RPM, discarding the residual liquid. The VNE column was reattached to the collection tube.

6. **Wash column membrane by Wash Buffer 2:** Ethanol-containing wash buffer 2 was put into the VNE column in a quantity of 650µL and then centrifugation was done at 8000 RPM, discarding the residual liquid. The VNE column was reattached to the collection tube. Repeat this step.

7. **Dry membrane:** The VNE column was centrifuged at 18000 RPM to dry it, and the remaining liquid and collection tube were discarded.

8. **Elution of viral RNA:** 40µL of RNase-free water was lastly added to the VNE column. The drop was placed in the VNE membrane center for better absorption. Then the VNE column was centrifuged at 18000 RPM to elute RNA.

9. The viral RNA in the elution tube could be stored in a -80°C refrigerator if necessary.

10. Finally, 10µL from each sample was transferred to the prepared master mix PCR tube carefully along with the positive control.

**The protocol followed for RNA Extraction:**

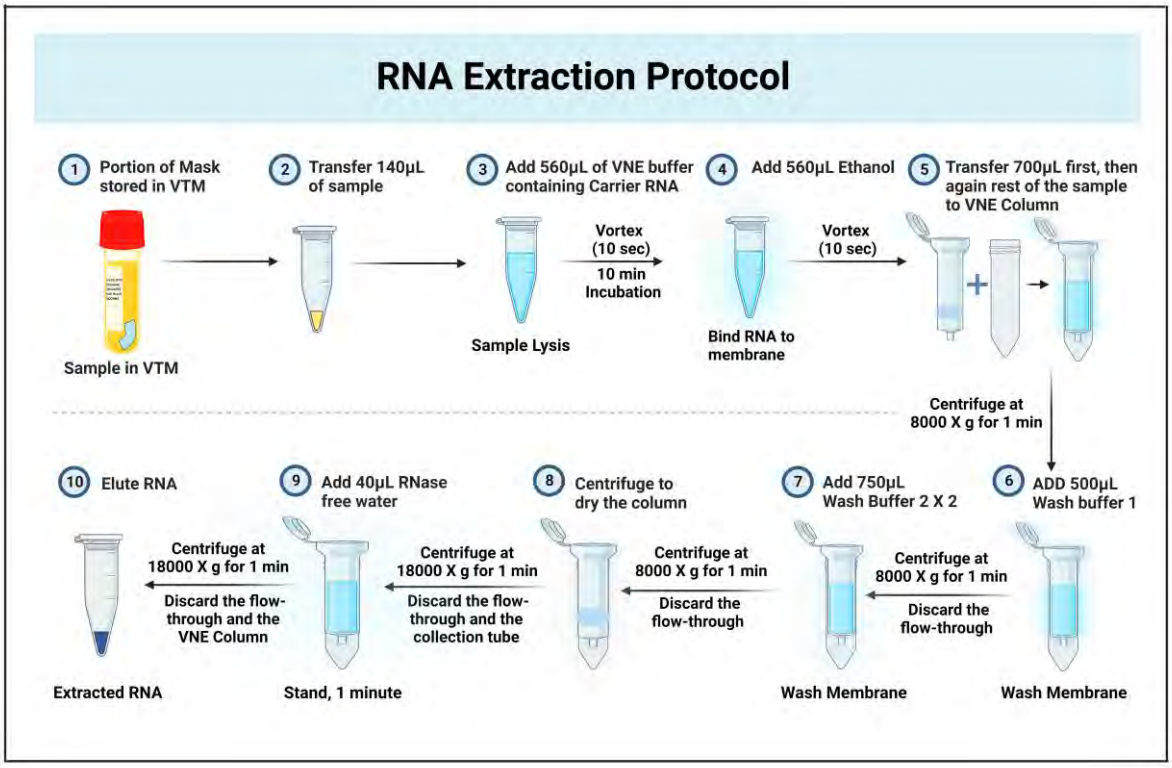



*Figure 4: RNA extraction protocol followed during the experiment*

## 2.3 Master-mix Preparation

The laminar airflow was cleaned with IPA and the UV was switched on for 5 minutes to prevent contamination from previous users. Then the sample release reagent, negative control and master mix were brought to room temperature (25°C). The master mix was prepared by adding 4µL of a 2019-nCoV-PCR-Enzyme Mix with a 26µL of 2019-nCoV-PCR Mix in a PCR tube. The same preparation was made for the positive and negative controls, which was necessary for the RT-qPCR run. Then the mixture was pipetted 5-6 times and needed to vortex for 1 minute. After that the mixture needed to spin for 5 seconds in a centrifuge machine. Then 10µL sample release reagent was added to the testing PCR tube and 10µL negative control to the negative control tube. Finally, the caps needed to be closed tightly in the tube and spun for 5

seconds again. Lastly, 10µL of positive control was added to the PCR tube separately in the sample extraction room to prevent contamination of other sample PCR tubes. In addition, the RT-qPCR enzyme mix contains Taq DNA polymerase, reverse transcriptase, RNase Inhibitor and PCR Mix contains primers and probes for RNase P and SARS-CoV-2 which are necessary to run the RT-qPCR. These oligonucleotide primers or probes for SARS-CoV-2 identification are chosen from ORF1ab and the nucleocapsid gene (N). This pack contains primers for SARS-ORF1ab CoV-2's along with N genes. This kit uses internal control of primers from the human RNase-P gene for sample integrity, multiplication, nucleic acid extraction and analysis (Bioperfectus, n.d.; Mascuch et al., 2020; van Kasteren et al., 2020).

**The following is the Master Mix Preparation Protocol:**

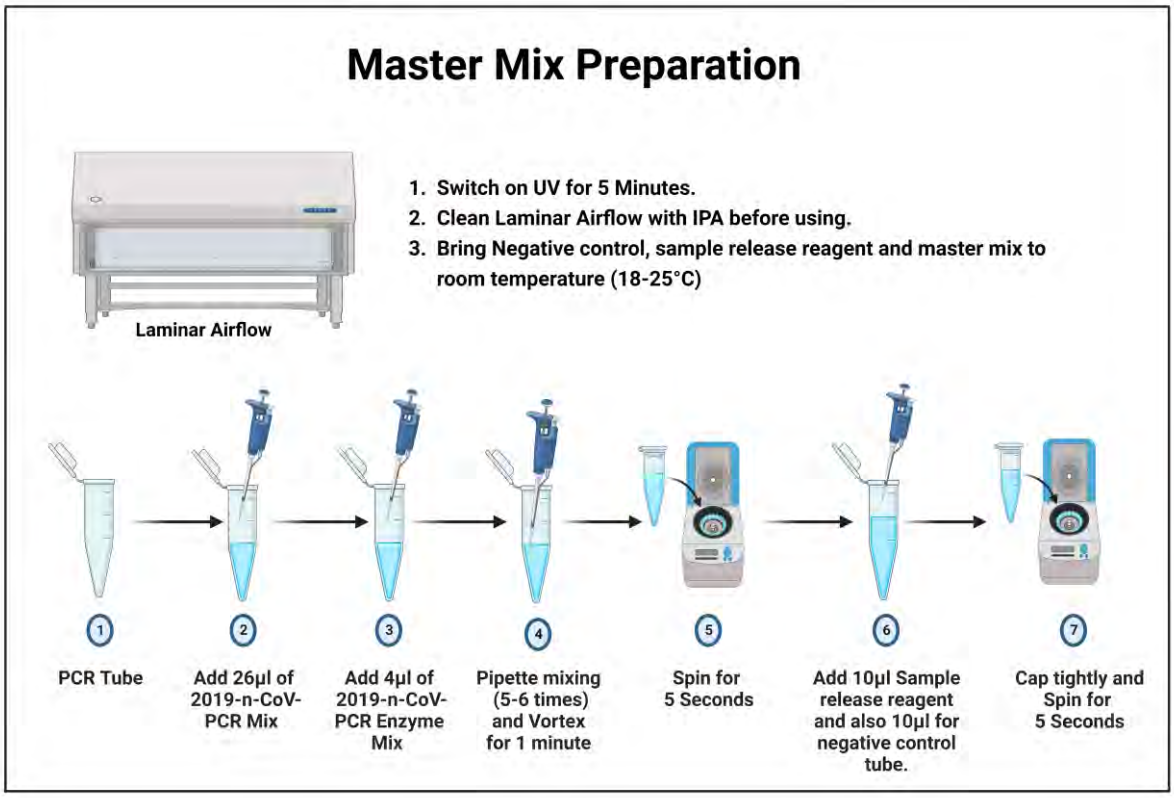



*Figure 5: Master Mix Preparation Protocol followed during the experiment*

## 2.4 Sample Run in RT-qPCR for amplification

Firstly, the RT-qPCR machine was switched on and the software needed a quick set-up to run a new experiment. Proper naming of the file is important to check the results later. Moreover, wells need to select carefully for corresponding samples and put the samples in the respective well. Every run needs a positive and a negative control each time (Moldovan & Moldovan, 2020). The other setups were different stages and temperatures based on reagent, detection format, cycle range, and specific channel and then saving the changes to run the machine. After 1 hour 20 minutes and 46 cycles, Relative Fluorescence Units (RFU) and Cycle of threshold (CT) values, as well as curves, were displayed in the software (Bioperfectus, n.d.). The RT-qPCR method included cell lysis and RNA extraction, target selection, reaction configuration, detecting and analysis. (Barra et al., 2020; Nyaruaba et al., 2021).

## 2.5 UV treatment of the used masks

After confirmation of the Covid-19 responsible virus's existence, the stored masks were placed in the UV-C chamber. The masks were separately hung on the chamber hook to dispose properly. Then the UV-C machine was powered on for 5 minutes and after completing UV-C treatment, these masks were stored in a new zip-locked bag and carried to the laboratory again to run the RT-qPCR test. The previous steps were done before UV-C treatment were followed again accordingly to get the results after UV-C treatment.

# 3. Results

We assessed the RT-qPCR results to compare differences between before and after UV-C exposed samples. The Covid-19-causing genes are N gene and ORF1ab proteins, which are the primary targets of RT-qPCR to detect them as Covid-19 positive. In addition, statistics show that the N gene was the most commonly observed gene in the analyzed samples (Zhang

et al., 2020). Also, a normal human cellular gene called IC is found in the test, and this curve determines whether or not the amplification was sufficient (Loying et al., 2020; Lu et al., 2021; Rosenstraus & Wang, 1998; van Kasteren et al., 2020; Wang et al., 2020).

Moreover, IC gene displays a purple line, the ORF1ab gene shows a blue line and the N gene shows a yellow line in the RT-qPCR result curve, which is depicted below with the indicated color. The N gene is primarily responsible, and the outcome is mostly determined by its CT value (Gill et al., 2021; Waller et al., 2020).

Furthermore, the X axis indicates the CT value, representing the cycle necessary to finish the procedure, and the Y axis displays the RFU value, representing the fluorescence required for detection. CT values are mostly used to determine whether or not a sample is positive or negative (Rabaan et al., 2021; Waller et al., 2020).

RT-qPCR usually sets for 46 thermal cycles. If the test is positive, Covid-19 genes were found with less than 40 cycles of amplification. This value depends on many factors (Rabaan et al., 2021; Tang et al., 2020).

The software illustrates real-time amplification using a curve for each gene to predict its existence. Below are the final result and explanations.

## 3.1 RT-qPCR results for Sample 1

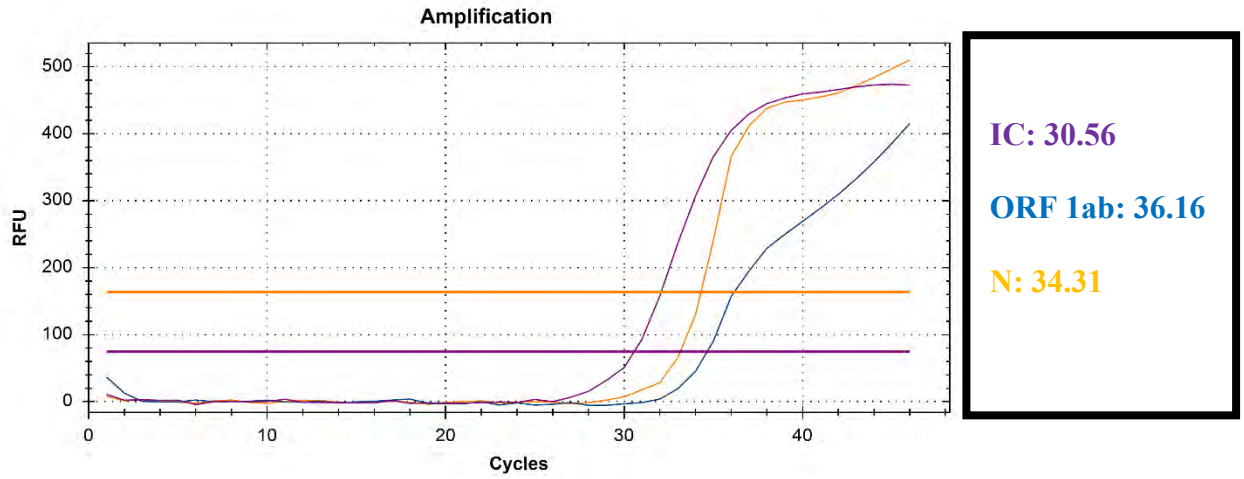



*Figure 6A: Illustrated graph and CT values of Sample 1 (Before UV-C Exposure)*

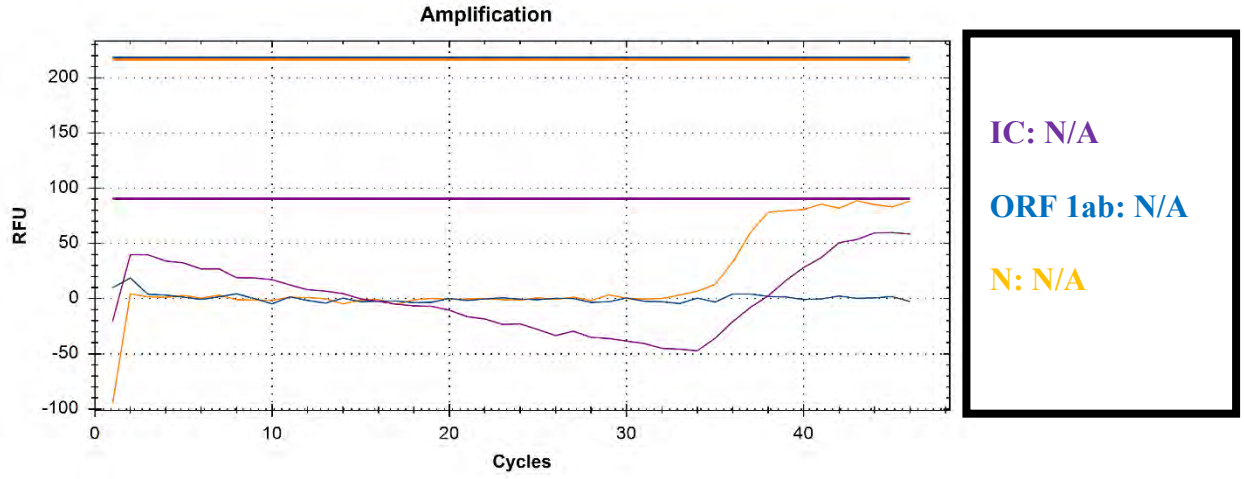



*Figure 6B: Illustrated graph and CT values of Sample 1 (After UV-C Exposure)*

The figure of Before UV-C exposure shows the existence of the ORF1b gene, N gene which are responsible for Covid-19 and normal cellular IC gene. After UV-C irradiation, the genes are responsible for Covid-19 (ORF1b and N) and IC disappear.

## 3.2 RT-qPCR results for Sample 2

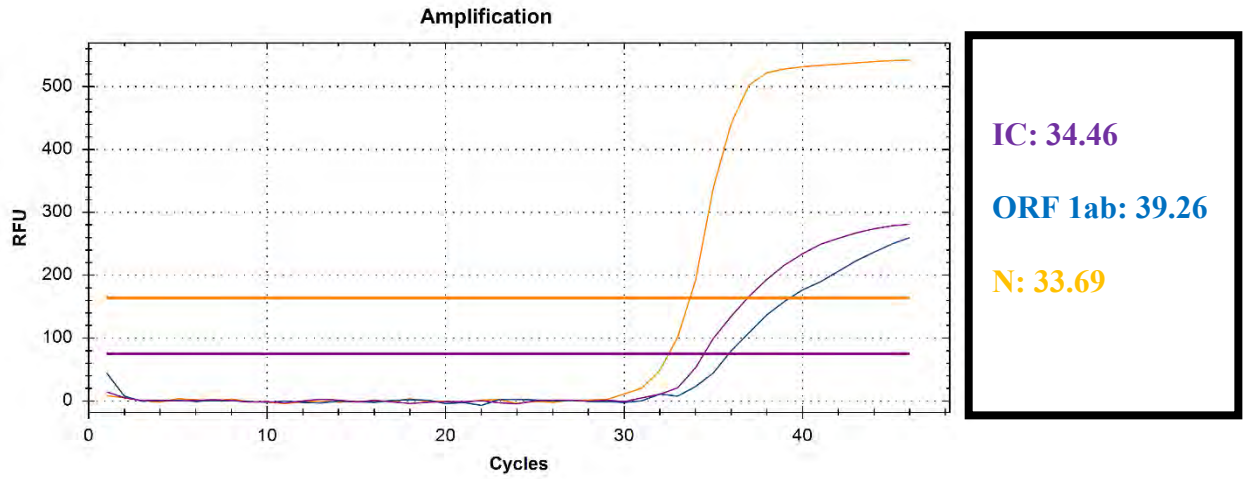



*Figure 7A: Illustrated graph and CT values of Sample 2 (Before UV-C Exposure)*

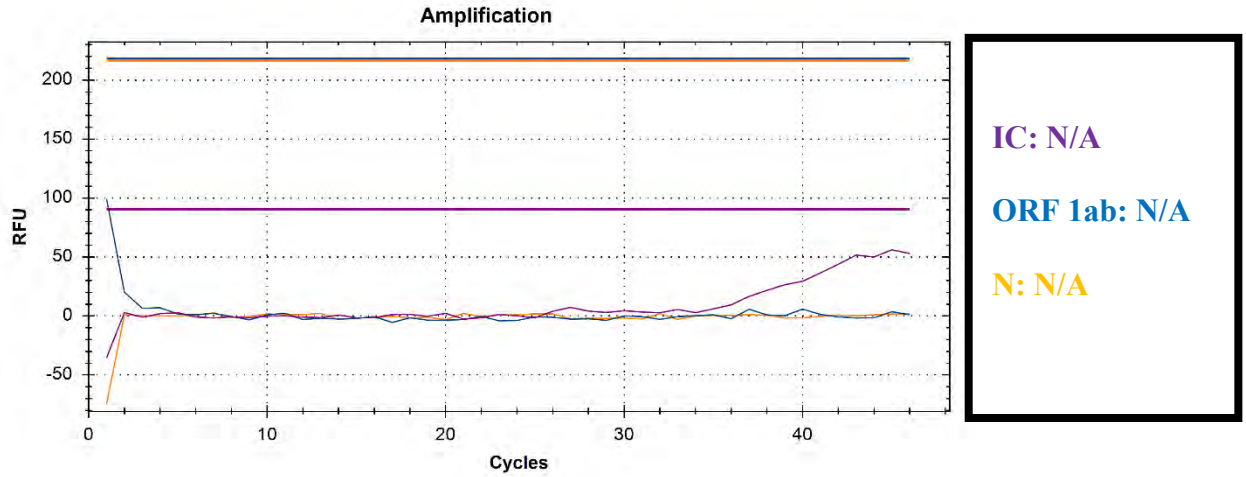



*Figure 7B: Illustrated graph and CT values of Sample 2 (After UV-C Exposure)*

The figure of Before UV-C exposure shows the existence of the ORF1b gene, N gene which are responsible for Covid-19 and normal cellular IC gene. After UV-C irradiation, the genes are responsible for Covid-19 (ORF1b and N) and IC disappear.

## 3.3 RT-qPCR results for Sample 3

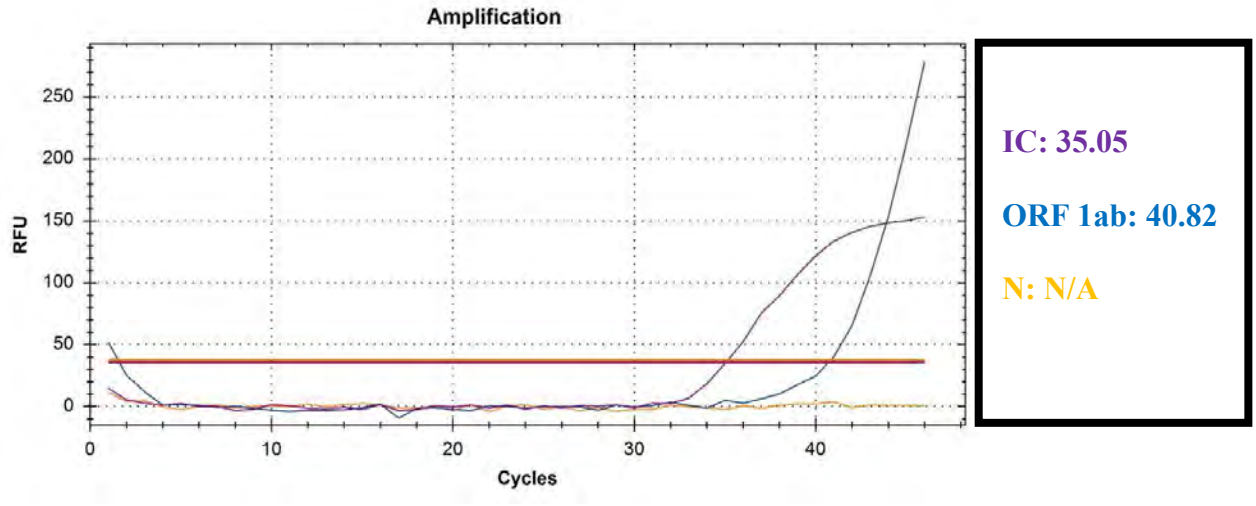



*Figure 8A: Illustrated graph and CT values of Sample 3 (Before UV-C Exposure)*

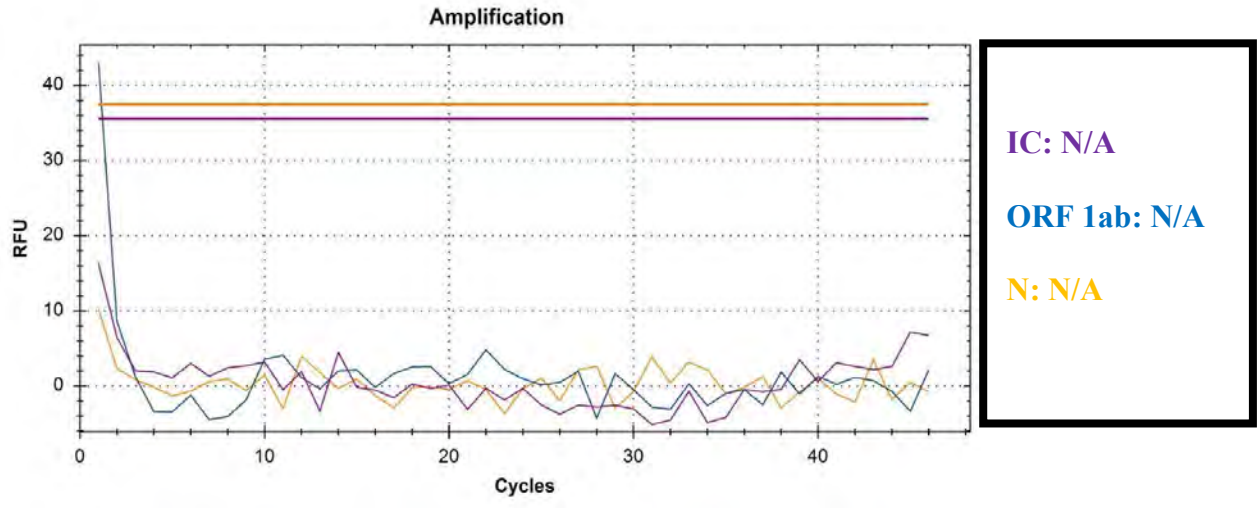



*Figure 8B: Illustrated graph and CT values of Sample 3 (After UV-C Exposure)*

The figure of Before UV-C exposure shows the existence of the ORF1b gene, which is responsible for Covid-19 and normal cellular IC gene. After UV-C irradiation, the genes responsible for Covid-19 (ORF1b) and IC disappear.

## 3.4 RT-qPCR results for sample 4

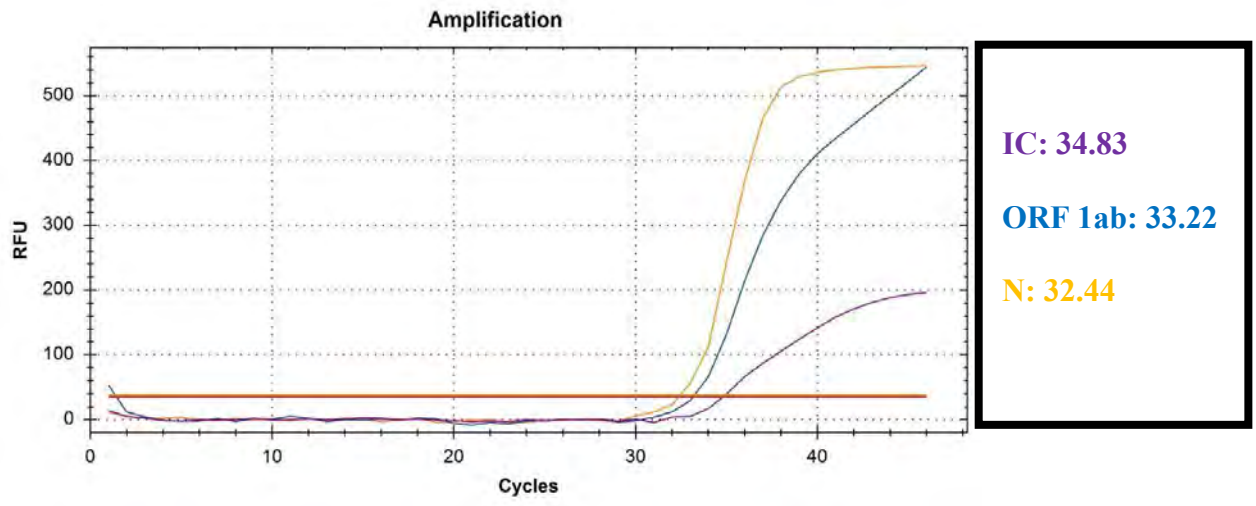



*Figure 9A: Illustrated graph and CT values of Sample 4 (Before UV-C Exposure)*

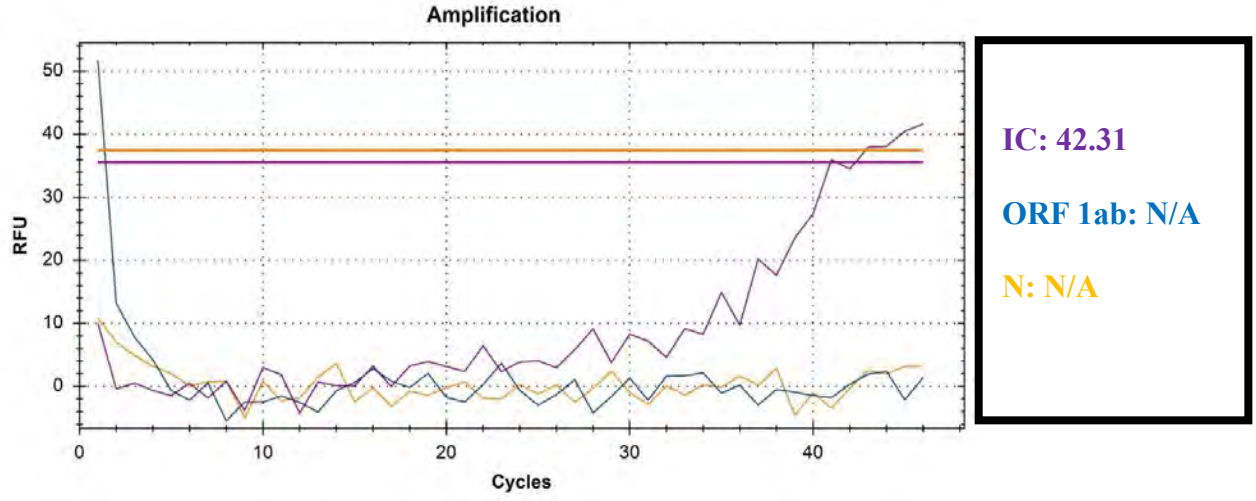



*Figure 9B: Illustrated graph and CT values of Sample 4 (After UV-C Exposure)*

The figure of Before UV-C exposure shows the existence of the ORF1ab and N gene, which are responsible for Covid-19 and normal cellular IC gene. After UV-C irradiation, the genes responsible for Covid-19 (ORF1ab and N) disappear along with the significant decrease in IC.

## 3.5 RT-qPCR results for Sample 5

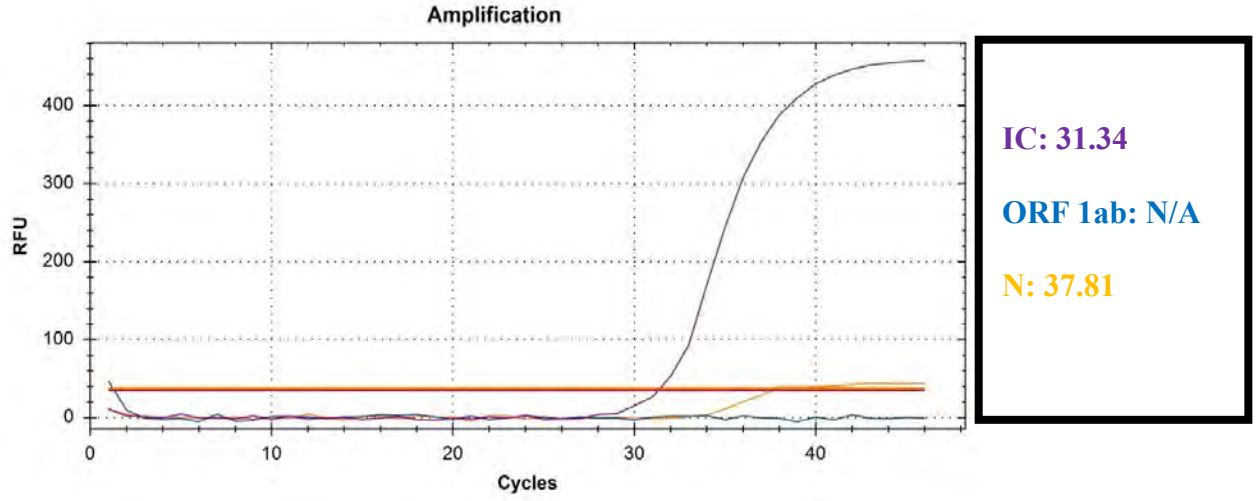



*Figure10A: Illustrated graph and CT values of Sample 5 (Before UV-C Exposure)*

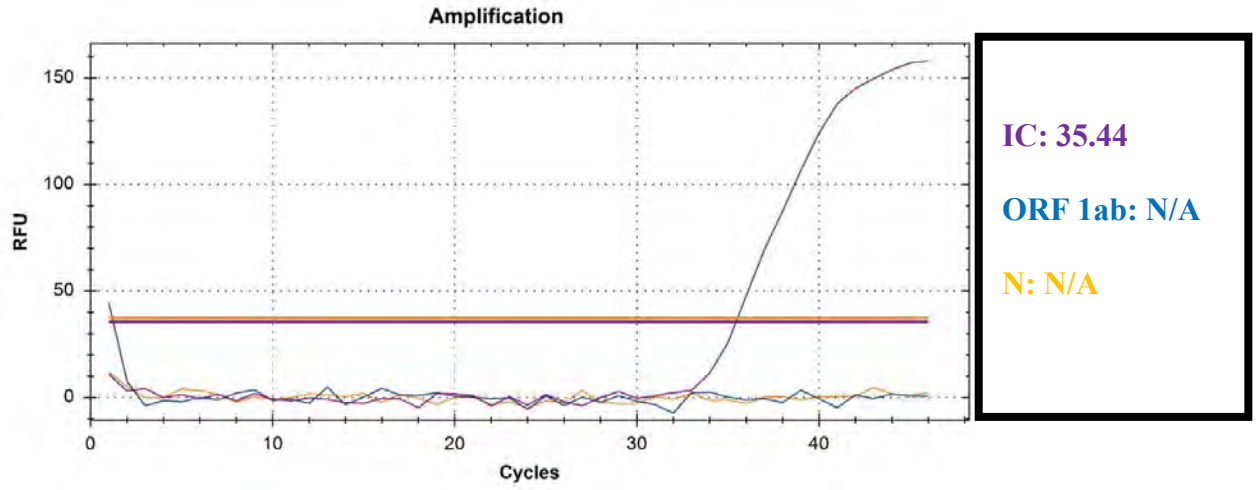



*Figure 10B: Illustrated graph and CT values of Sample 5 (After UV-C Exposure)*

The figure of Before UV-C exposure shows the existence of the N gene, which is responsible for Covid-19 and normal cellular IC gene. After UV-C irradiation, the gene responsible for Covid-19 (N) disappears along with the significant decrease in IC.

## 3.6 Positive Control

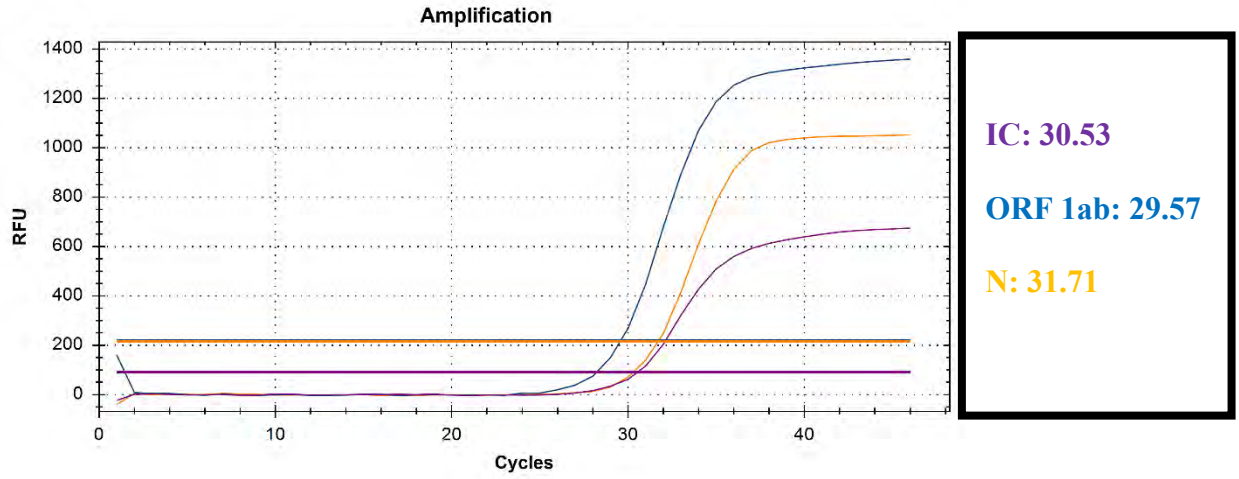



*Figure 11: Illustrated graph and CT values of Positive Control*

The figure shows that all the CT values are in a suitable range, indicating that the reagents worked perfectly during the cycle.

## 3.7 Negative Control

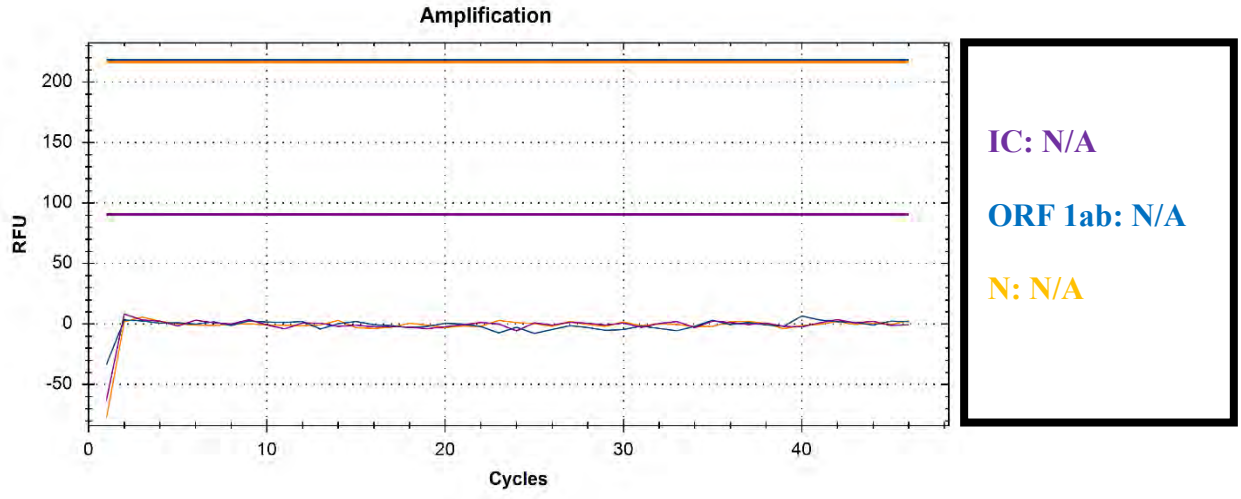



*Figure 12: Illustrated graph and CT values of Negative Control*

The figure shows no amplification indicating no contamination took place and didn't form any primer dimers, which helps to consider the results accurate.

# 4. Discussion

The information from the current study is pretty comparable to the earlier published data. According to some studies, UV-C decontamination system removes almost all viruses from the surfaces of filtering facepiece respirators (N95 masks) (Bentancor et al., 2021; Kayani et al., 2021; Purschke et al., 2020; Seresirikachorn et al., 2021; Weaver et al., 2021). Additionally, UV-C irradiation is utilized mostly in hospitals to cleanse the air, surfaces, clinical equipment and numerous forms of microorganisms (Anderson et al., 2013; Moore et al., 2012; Rutala et al., 2010; Santos & de Castro, 2021). Moreover, the N95DECON organization stated in their UV-C disinfection study, "we observe that a UV-C irradiation dose of ≥1.0J/cm2 at the N95 respirator surface deactivates SARS-CoV-2 analogues (≥3-log diminution) on the majority of experimented N95 facepieces."(*N95DECON*, n.d.; Wilde et al., 2020).

In this experiment, surgical masks infected with Covid-19 were decontaminated using UV-C radiation at a dosage of 1.0 J/cm2 for 5 minutes. The goal was to ascertain virucidal ability of UV-C in contrast to its microbicidal performance, which has already been tested in several fields. Additionally, after completing UV-C irradiation, promote the reusability of used or infected masks and assure its safety.

Based on the findings, the genes responsible for Covid-19 such as N, ORF1ab and the normal cellular gene IC are no longer found in the after exposed mask samples. The result is considered accurate because the positive and negative controls showed a standard result that means the reagents worked properly and no contamination occurred during the test. It indicates that the UV-C light used in the chamber possesses the ability to eradicate the Covid-19 virus and the duration of exposure time is also enough. The comparison between the effects of the N gene, ORF1ab gene and IC gene in before and after UV-C exposed samples are illustrated with the CT value differences in Figure 13, 14 and 15.

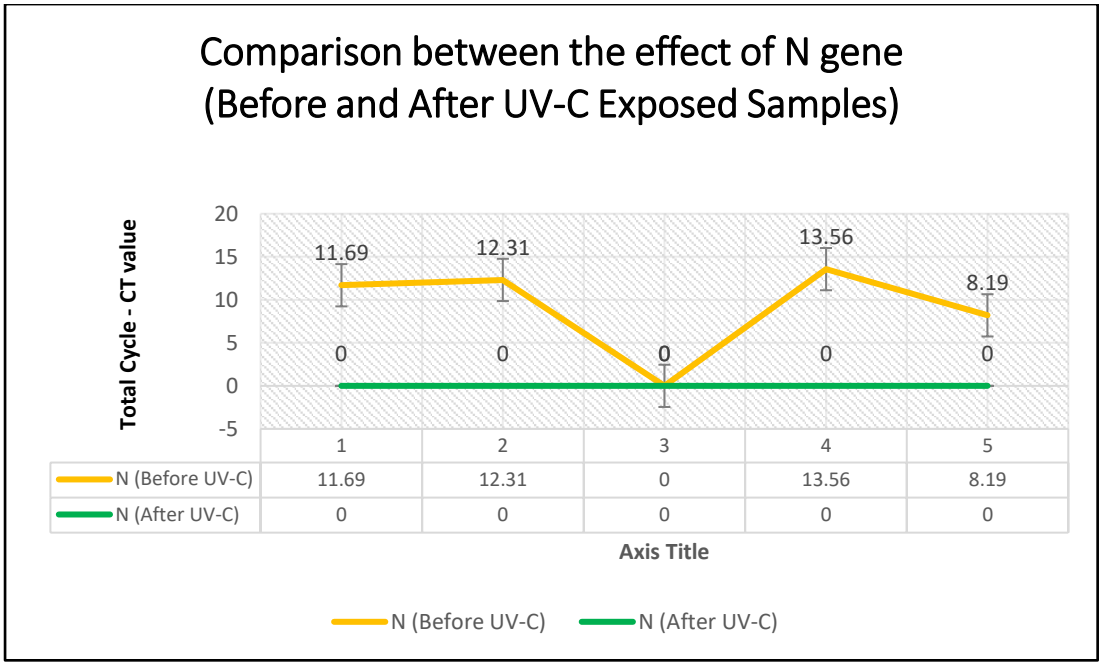



*Figure 13: Comparison between the effect of N gene in before and after UV-C exposed samples*

The yellow line depicts the CT value differences of Covid-19 specific N gene in before UV-C samples and the green line depicts the same gene in after UV-C exposure. It shows that the gene is disappeared in all the samples after UV-C irradiation.

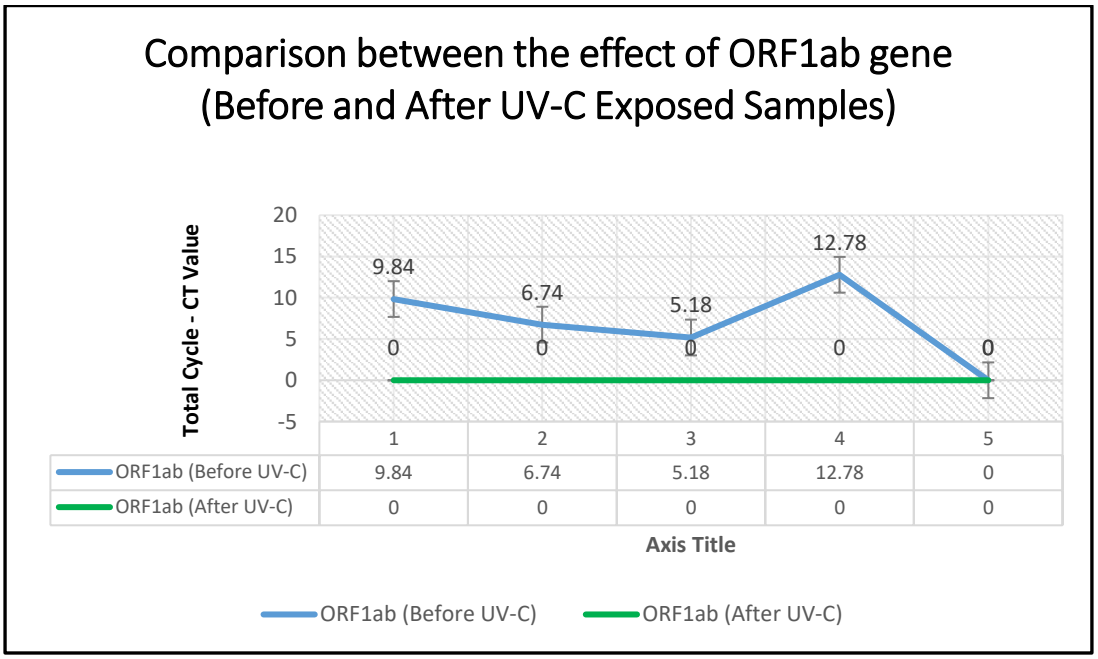



*Figure 14: Comparison between the effect of ORF1ab gene in before and after UV-C exposed samples*

Moreover, the blue line depicts the CT value differences of Covid-19 specific ORF1ab gene in before UV-C samples and the green line depicts the same gene in after UV-C exposure. It shows that this gene is also disappeared in all the samples after UV-C irradiation.

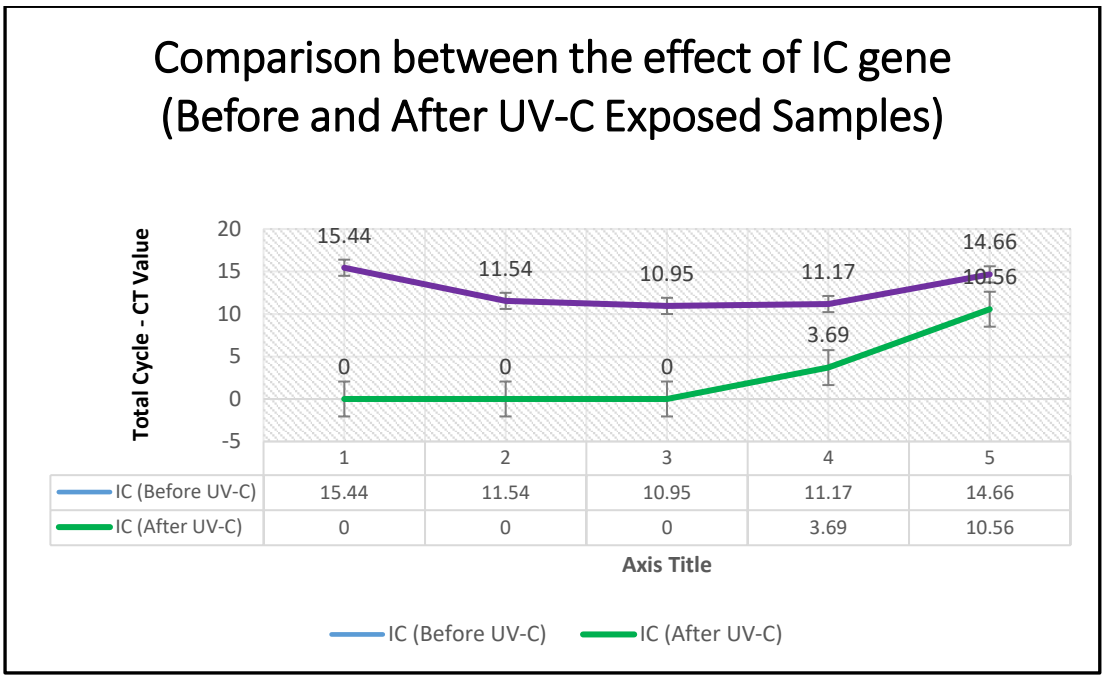



*Figure 15: Comparison between the effect of IC gene in before and after UV-C exposed samples*

Also, the purple line depicts the CT value differences of IC gene in before UV-C samples and the green line depicts the same gene in after UV-C exposure. It shows that this gene is disappeared and significantly decreased in these samples after UV-C irradiation.

Besides, normal cellular IC gene was detected in two samples despite of using the UV-C irradiation. This may have occurred during the handling of the samples after completing UV-C irradiation. The presence of these IC genes is deemed harmless since they do not cause or spreads illness.

This research also has a few limitations. Firstly, detecting Covid-19 responsible genes from the collected mask samples was not conducted following a fixed timeframe. It enhanced the chance

of missing a particular gene in the RT-qPCR analysis. Furthermore, the sample size was inadequate for the experiment. Due to resource constraints, only surgical face masks were used in this experiment, rather than other forms of PPE.

Furthermore, there were no previous related publications that contained any significant data on a SARS-CoV-2 decontamination procedure that is risk-free and simple to carry out. Depending on the results, it ensures that there is no risk of contamination if these masks are used again as all the infective genes responsible for Covid-19 are disappeared. Also, it seemed that the decontamination method using the UV-C chamber would be quite easier for some target health service sectors. Lastly, continuous monitoring and research are still required to improve this chamber's performance.

## 5. Conclusion:

In conclusion, the primary objective of this research is to promote the proper use of masks for a long time, maintain proper disposal, protect the environment and decrease the uncontrolled production. The established results are quite preferable and consistent with the hypothesis and the UV-C exposure rendered the Covid-19-causing genes inactive. Moreover, based on the study it can be assumed that similar personal protective equipment can be decontaminated along with the masks by employing the UV-C chamber which would be beneficial for the diagnostic centers. Furthermore, infected masks used by the patients can be disposed safely after using this chamber. Subsequently, this UV-C chamber could be the newest addition to the decontamination techniques in the diagnostic centers and other health service sectors.

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