**Report**

# Testing UVC machine (Quantum Innovations) as a potential sterilizer for masks contaminated with Coronavirus.

## Background and objectives:

Validation of a UVC cabinet, manufactured by Quantum Innovations, for sterilization of N95 masks contaminated with avian infectious bronchitis virus (IBV) as a surrogate for the SARS COV-2 virus causing the current COVID-19 pandemic, was performed in the Gerhold laboratory at the College of Veterinary Medicine at the University of Tennessee (UTCVM). Successful sterilization of the masks will allow health care workers to reuse their masks at least twice, which is crucial to mitigate the current shortage of facemasks and protective clothing.

## Introduction:

Due to the rapid spread of SARS COV-2 virus and its high infectivity rate, there was a need for wearing personal protective equipment (PPE) by health care workers which led to a shortage in this equipment (WHO, 2020). Reusing facemasks is one of the proposed methods to overcome this shortage; however, there are no recommendations or suggestions on the methods that can be used for disinfection (CDC, 2020). Several methods were investigated for their ability to inactivate various viruses and, among them, using UV irradiation is promising (Tseng and Li, 2007; Lore et al., 2012; Mills et al., 2018). A group in Nebraska described a detailed protocol for disinfecting facemasks via hanging them in a room occupied by a UV tower on each side of the room, for a total of two towers (Lowe et al., n.d.). Another study investigated the use of biosafety cabinets for mask disinfection (Card et al., 2020). In the current study, we investigated the use of a UVC machine designed specifically to disinfect masks contaminated with avian coronavirus. Validating UV irradiation for inactivation of corona virus will allow for numerous applications, such as disinfecting PPE, hospital rooms, and equipment and disinfecting public spaces like trains and metro stations.

## Materials and methods:

### UV source

The UVC machine was sent to our lab by Norm Kester from Quantum innovations (Figure 1). The machine was used in a series of experiments according to the company instructions.

### Virus strain

Infectious bronchitis virus (IBV) (i.e. avian coronavirus) was used in the current study. The virus strain used was Ga-08-IBV (Goraichuk et al., 2020) with a concentration of ($7.3X10^4$ virions /ml).

The virus was kept in -80C and thawed on ice just before dilution with nuclease free water (NFW) to the mentioned concentration and then used in the experiments.

Experiment design

A volume of 200 µl of IBV was deposited on an N95 mask (provided by Quantum Innovations) and the mask was inserted inside the machine. The mask rested on two quartz rods that were stabilized on two black plastic brackets (Figure 2).


Figure 1. UV machine by Quantum Innovations for disinfection of face masks.

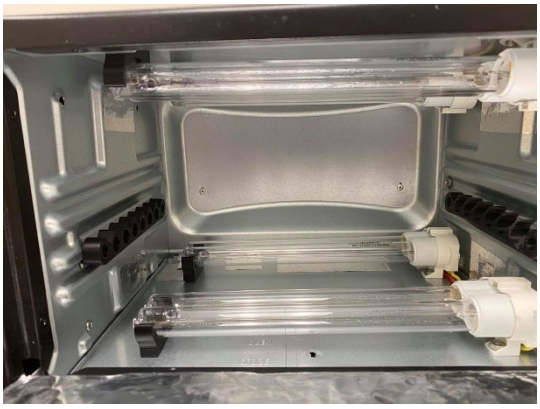

Figure 2. Inside of UV machine.

A N95 mask, provided by the UT CVM, was used as a positive control. The virus was deposited on this mask and the positive control mask was placed on the lab benchtop away from the UV machine. For the experimental masks, the virus was deposited on two separate spots on each mask followed by placement in the UCV machine. The UV exposure periods (minutes during which the mask was exposed to UV light inside the UV machine) were 1, 3, 10, 15, and 20 minutes. After the specified time, the machine automatically turned off. The virus droplets were collected from the mask surface and placed in 1.5ml tubes (about 200 µl per tube).

RNA extraction

Viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer instructions with minor modifications. Extracted RNA was eluted using 50µl of NFW. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed directly after the RNA extraction or on the following day. In case of inability to run qRT-PCR on

the same day, the extracted RNA was kept in -20C until next day. A negative extraction control was included for each extraction to ensure sample contamination did not occur.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

A 143-bp fragment of the 5`- untranslated region (5`-UTR gene) of IBV was targeted using forward primer 5 ` -GCT TTT GAG CCT AGC GTT-3 ` , reverse primer 5 ` -GCC ATG TTG TCA CTG TCT ATT G-3 ` and a Taqman® labeled probe 5 `-FAMCAC CAC CAG AAC CTG TCA CCT C-BHQ1-3 ` (Callison et al., 2006). The reaction mixture contained 0.5µl of SuperScript III RT/Platinum Taq Mix (Invitrogen), 1.5µ each of forward primer, reverse primer, probe and NFW and 12.5 µl of 2X Reaction Mix (Invitrogen). RNA template (5µl) was added and the qPCR was performed on StepOne Real-Time PCR system, Applied Biosystems thermocycler. Initial cycle of 42C for 30 minutes followed by 95C for 2 minutes for the reverse transcription was followed by 40 cycles of 95C for 10 seconds, 50C for 40 sec and 72C for 30 seconds for RT-PCR. A positive and negative PCR control was included with each run. The cycle threshold value (Ct) was determined for each sample.

**Results:**

Results are represented by Ct values and not virus concentrations. A higher Ct value indicates a lower amount of viral RNA present on the masks following the various treatments. There is an increase in the Ct values with the increase in exposure time to the UVC, indicating the longer UVC exposure leads to lower residual viral RNA. The targeted virus gene was undetected by PCR at 15 and 20 minutes of UVC exposure, indicating that no residual viral RNA was detected. The viral sample that was not exposed to UVC (positive control) remained relatively stable with a Ct value ranging from 23.5 to 19.0 (Figure 3).

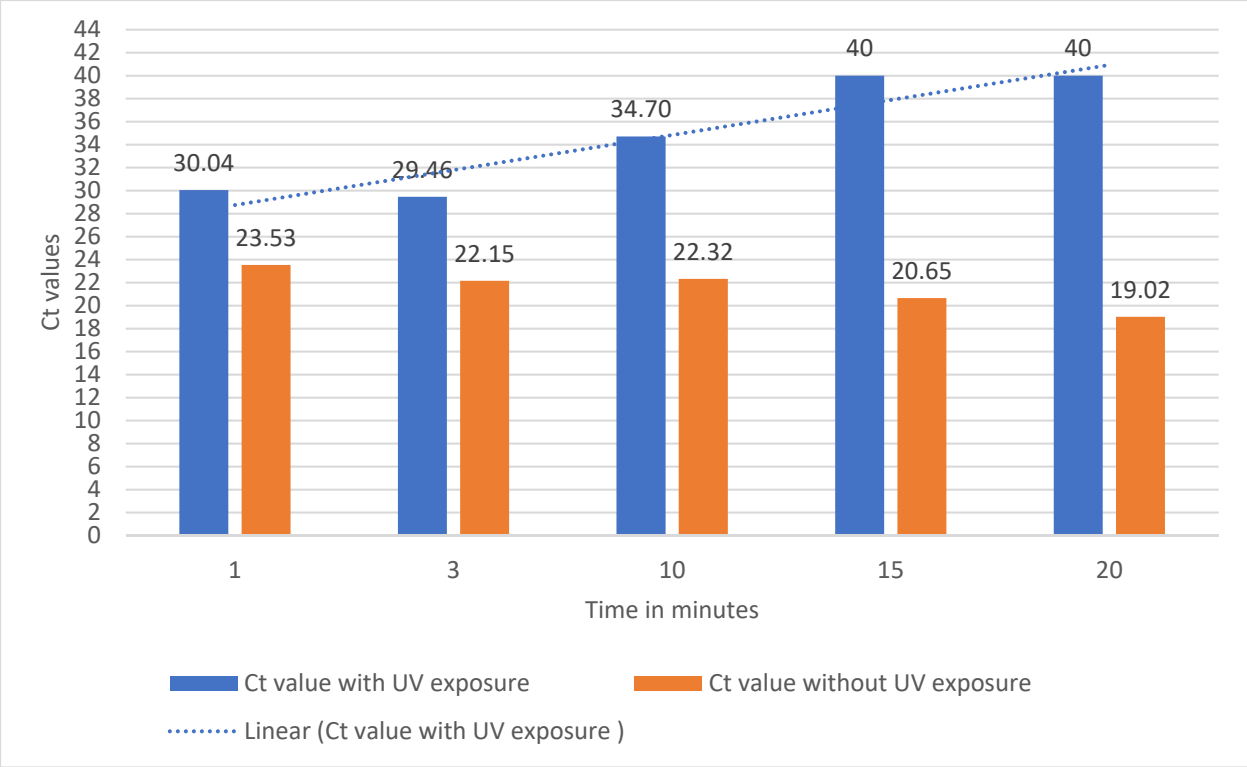


Figure 3. A graph showing the cycle threshold (Ct) values of infectious bronchitis virus (i.e. avian coronavirus) at different time points. Experiments were performed in the Quantum innovations machine using N95 masks. 7.3 X $10^4$ virions/ml were used on both control and experimental masks.

**Discussion:**

The present study was conducted to estimate the time required to eradicate viral RNA of infectious bronchitis virus (IBV) after exposure to UVC light generated by UVC machine manufactured by Quantum Innovations. From the data presented, UVC light exposure for 15 minutes under the mentioned experimental setting can inactivate the virus to the degree that the targeted gene is not detected by RT-PCR machine. However, the UVC may have inactivated the virus and rendered it incapable of inducing infection earlier than 15 minutes. The approach used in this study is detection of the viral genetic material (i.e. RNA) via qRT-PCR which indicates the presence or absence of the viral RNA via the relative quantity of the targeted gene (Ct values) at different time points of UVC exposure. It is important to note that to determine if the virus is capable of inducing infection after exposure to UVC light, a plaque assay or production of cytopathogenic effect (CPE) of the virus on cell culture (i.e. virus isolation) is a more sensitive method. That approach was not possible in the present study, which was performed in BSL-2 level lab.

Data represented here are Ct values and a generation of a standard curve to estimate virus concentrations are required to better interpret the data. Ct values increase with decrease in amount of the gene targeted because the RT-PCR machine requires more cycles to detect the RNA in the tested sample. Although we did not detect viral RNA after 15 minutes of UVC exposure, this conclusion applies only for the used experimental settings. For this reason, our results will vary from previous studies due to the difference in UV light source, temperature, humidity, virus dose applied, and test method used for detection of the virus (i.e. qRT-PCR vs CPE).

Quantum Innovations provided the facemasks tested in this study. There are various models of N95 masks. These masks differ greatly in the style, materials used, and filters. A previous study on 15 different N95 face respirators found that a mask could be sterilized at least three times before any change in its physical integrity (Heimbuch and Harnish, 2019). That may decrease the stress on masks production and ease the challenges for this industry sector.

We used IBV as a surrogate for the SARS COV 2 virus. IBV is a single stranded RNA enveloped virus that belongs to family Coronaviridae and genus *Gammacoronavirus*. The SARS COV-2 virus that is causing the current pandemic belongs to the same family as IBV but to the genus *Betacoronavirus* (Lu et al., 2020). Although IBV and SARS COV-2 belong to the same family, there may be some differences in their resistance to UVC light. Further research is needed to investigate the difference in resistance between the two viruses and the susceptibility of the SARS COV-2 to UVC light used in this study.

In conclusion, UVC light is a promising method for sterilizing N95 face masks, which is crucial during the ongoing pandemic. Exposure to UVC light under these experimental conditions caused decrease in the virus RNA to undetectable levels by the RT-PCR machine after 15 minutes.

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