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Correspondence

Inactivation of SARS-CoV-2 by gamma irradiation

Sir,

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most impactful pandemic of the 21st century affecting millions of persons within a short span of time[1]. Several protective and control measures have been implemented to control the rapid spread of the disease. However, universal mask usage, social distancing and maintaining hand and surface hygiene seem to be the most useful methods to contain the transmission of the virus[2].

The World Health Organization (WHO) has recommended and approved SARS-CoV-2-specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) as the optimal method for the diagnosis of COVID-19[3]. Serological assays are also being utilized in monitoring and surveillance activities to determine the new case frequency during outbreaks and the true infection rate of the disease. The development of such serological assays requires the production of authentic SARS-CoV-2 virus stock for antigen preparation. Considering the high risk associated with SARS-CoV-2 infection, the WHO has advised that the propagation of the virus be conducted within the Biosafety level-3 (BSL-3) laboratories. To protect laboratory personnel from SARS-CoV-2 infection, the developed serological assay reagents should be non-infectious and therefore, safe to be used in resource-limited settings. Hence, it is necessary to inactivate the virus so that it can be handled within the BSL-2 laboratory for developing serological assays.

Gamma irradiation is a commonly employed method for inactivation of viruses. The method inactivates virus mainly by cross-linking of genomic material or radiolytic cleavage[4,5], resulting in the loss of infectivity of viruses without affecting the structural integrity of the antigenic proteins[6]. Darnell *et al*[7] studied the gamma inactivation of SARS-CoV at a maximum irradiation dose of 15,000 rad (0.15 kGy), a dose not expected to inactivate the virus. There is no information available on gamma inactivation of SARS-CoV-2. Considering the need to develop non-infectious diagnostic reagents for use in serological assays, the present study was carried out to evaluate the inactivation of SARS-CoV-2 using gamma irradiation.

The study was conducted at the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), Pune, India, and approved by the Institutional Biosafety Committee. SARS-CoV-2 clinical virus isolate (passage-2) obtained from the throat/nasal swab of a COVID-19 patient was used in all the experiments[8]. The SARS-CoV-2 was freshly propagated in Vero CCL-81 cells using Eagle's minimum essential medium (MEM, GIBCO, Thermo Fisher Scientific, USA) supplemented with two per cent fetal bovine serum (HiMedia, Mumbai), penicillin (100 U/ml) and streptomycin (100 mg/ml)[8]. On the 4th day post-infection, cells were observed for cytopathic effects. The supernatant of the infected Vero CCL-81 cells was harvested after a single freeze-thaw cycle. After centrifugation, the clear supernatant was aliquoted in a 1.5-ml/tube and stored at −80°C until further use. This virus stock was titrated in duplicate using the median tissue culture infectious dose ($TCID_{50}$) assay in a 96-well plate[8]. Subsequently, the virus titre was calculated using the Reed and Muench method[9]. The SARS-CoV-2-infected cells produced a titre of $10^{6.5}$ $TCID_{50}$/ml.

Duplicate vials of each gamma irradiation experimental set and virus control vials were prepared from the virus stock. All the cryovials were sealed using a Parafilm, packed in a biohazard bag and thoroughly surface disinfected. The experimental sets of the cryovials were sent out from the containment laboratory to a BSL-2 laboratory for gamma irradiation. Non-irradiated virus control vials were stored at −80˚C until further use.

A gamma chamber (GC-5000) with a cobalt-60 source [Board of Radiation and Isotope Technology (BRIT), India] was used for gamma irradiation experiments. Annually, the gamma dosimetry of GC-5000 is established by the authorities of BRIT, Mumbai, at ICMR-NIV, Pune. Uncertainty in the absorbed dose was determined using a dosimeter during GC-5000 calibration. This process validated the actual gamma radiation dose imparted to the specimens in GC-5000. Virus stock vials for gamma irradiation experiments were thawed in an ice bucket. The vials were placed in the biohazard bag containing ice flakes. The sample bucket was taken out of the sample chamber by turning the slide locking arrangement of the removable half door. The biohazard bag with the specimens was then loaded in the bucket and the bucket was again fixed in the sample chamber. After closing the sample chamber, the system was operated on auto mode. The gamma-irradiated specimens were removed after the completion of each irradiation dose of 1, 5, 10, 15 and 25 kGy. Two vials of each irradiated specimen were tested in duplicates using the $TCID_{50}$ assay to determine the inactivation of the irradiated virus specimens.

The $TCID_{50}$ assay of the irradiated specimens (preliminary study) demonstrated that SARS-CoV-2 was inactivated by gamma irradiation at the 10 kGy dose (data not shown). This confirmed the complete inactivation of SARS-CoV-2 at this dose. All the gamma doses above 10 kGy (15, 20 and 25 kGy) were also found to inactivate the virus completely. A dose of 5 kGy failed to completely inactivate the SARS-CoV-2 with a remaining titre of 10^1 $TCID_{50}$ observed.

Another experiment was carried out in duplicate with gamma irradiation doses of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 kGy to determine the dose-response kinetics for inactivation of SARS-CoV-2. The results of this study revealed the complete inactivation of SARS-CoV-2 at doses of 6 kGy and above. The non-irradiated virus control specimens of both the experiments did not show any reduction in the original virus titre with a standard error of ± 0.5 $\log_{10}\times10^3$ $TCID_{50}$/ml (Fig. 1). The D_{10} value is the ability of gamma radiation to reduce an exposed microbial population 90 per cent (one $\log_{10}$) under standard conditions of time, temperature and dose. The D_{10} values were calculated using the inverse of the slopes of the regression lines (1/slope) of gamma irradiation dose against log virus titre using Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA, USA). The D_{10} was found to be 1.09 kGy. This corresponds to a 0.92 $\log_{10}$ reduction in titre per kGy (Fig. 2).

Feldman *et al*[6] reported inactivation of another betacoronavirus, SARS-CoV, with gamma irradiation dose of 10 kGy. The present study demonstrated the inactivation of SARS-CoV-2 by gamma irradiation at doses expected to inactivate viruses. Understanding of the gamma inactivation dose and optimization of gamma inactivation procedure for SARS-CoV-2 should help in developing non-infectious and safe viral stocks for use in preparing diagnostic reagents. The data generated from this study can also be utilized

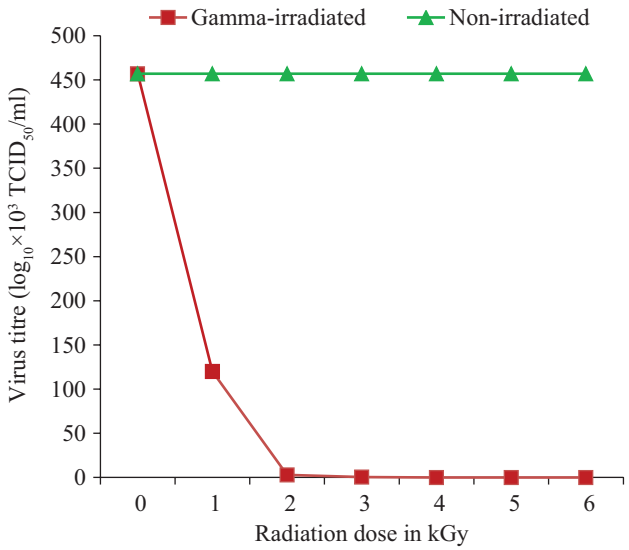

Fig. 1. Effect of gamma radiation on the infectivity of SARS-CoV-2. $TCID_{50}$, median tissue culture infectious dose.

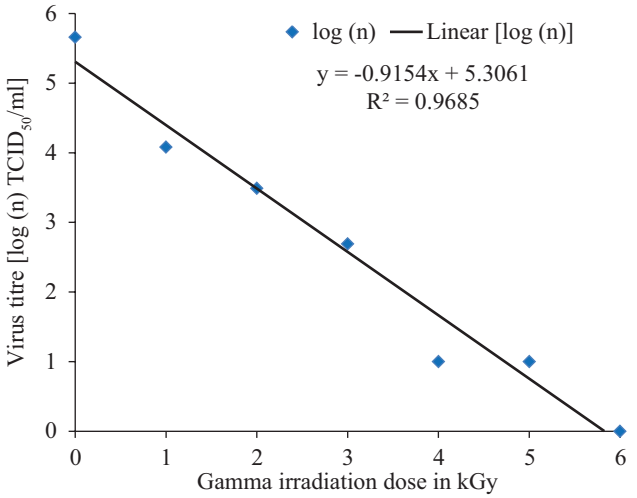

Fig. 2. Plot used for calculating the gamma radiation dose required to reduce infectivity of SARS-CoV-2 by 90% (D_{10} value) in kGy.

further for the gamma inactivation of quality control panels of diagnostic kits and clinical specimens of SARS-CoV-2.

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