X-ray irradiation of SARS-CoV-2 Omicron BA.4 and comparison of the sensitivity of live and irradiated virus in a lateral flow test assay format

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INTRODUCTION

The inactivation of virus through X-ray irradiation generates a non-replicating product, which is still detectable using molecular methods. Additionally, there is evidence that the irradiated virions not only retain envelope integrity, but also maintain the native protein quaternary structure of the antigenic epitopes.[1,2]

The 2020 COVID-19 pandemic demonstrated the need for inactivated SARS-CoV-2 virus for new Lateral Flow Device (LFD) test reference material, suitable for use in diagnostic assay development in Advisory Committee on Dangerous Pathogens (ACDP) Containment Level 2 (CL2) laboratories. As the pandemic continued and SARS-CoV-2 variants evolved, antigenic drift caused the original LFD tests to be no longer effective. By using non-infectious irradiated circulating variants, the development of new LFD assays was reliably facilitated. At the time the new variants evaded immunity provided by both previous infection and vaccination, requiring updated LFDs, vaccines and monoclonal antibody (mAb) therapeutics.[3,4,5,6,7]

This poster describes the irradiation process for SARS-CoV-2 Omicron BA.4 and the efficacy of Omicron BA.4 in comparison with live and other irradiated SARS-CoV-2 variants in an LFD assay format.

In collaboration with the Virology and Pathogenesis and Pathogen Characterisation Group, the National Collection of Pathogenic Viruses (NCPV) contributed to the development of X-ray irradiated SARS-CoV-2 Omicron BA.4, for distribution to clinical laboratories to help expediate the development of LFDs.

METHODS

SARS-CoV-2 Omicron BA.4 (NCPV 2209291) was grown in Vero/hSLAM cells (ECACC 04091501), and the titre determined to be 2 x 10^5 $TCID_{50}$/ml. Approximately 50 ml of the virus was X-ray irradiated to 9.7 kGy at 4 °C, using a 0.5 aluminum filter in a MultiRad350 irradiator (Precision X-Ray Irradiation, Madison, USA). This dose was calculated by determining the D-value (the dose required for a 1 log reduction in virus titre considering the specific matrix and packaging used).

To test that no viable virus remained after irradiation 10% of the pooled irradiated product was serial passaged three times in Vero E6 cells (ECACC 85020206). The flasks were observed daily under the microscope for signs of cytopathic effect (CPE). Samples were taken at the beginning and end of each passage and nucleic acids extracted for PCR analysis. The samples were inactivated with AVL buffer (Qiagen, Applied Biosystems, UK) and RNA extracted with Bio-Sprint All-For-One Vet Kit (Indical, UK) on the KingFisher Flex system platform (ThermoFisher, UK).

To compare recognition of viral epitopes of irradiated and live SARS-CoV-2 Omicron BA.4 and with other SARS-CoV-2 variants, samples were tested in triplicate using the Orient Gene Biotech Rapid Covid-19 (Antigen) Self-Test Lateral Flow Devices assay (Zhejiang Orient Gene Biotech, Zhejiang, China) according to manufacturer's instructions. The irradiated sample was also subject to whole genome sequence using a Sequence-Independent, Single-Primer Amplification (SISPA) protocol, libraries prepped using Nextera XT DNA library prep kit and run on Illumina NextSeq (Illumina, UK). The genetic analysis was carried out using the GISAID data base and the Nextclade platform.[8]

RESULTS

During the three weeks of serial passage no CPE was observed in any of the flasks inoculated with irradiated material. The positive control showed the expected CPE, and no CPE was observed in the negative control flask.

PCR analysis of the five replicates of irradiated virus showed a reduction of viral genome copy number between the beginning and end of each passage. The positive control showed an increase in genome copy number (Figure 1).

Serial dilutions of live and irradiated Omicron BA.4 samples were tested in triplicate using the Orient Gene's Rapid Covid-19 (Antigen) Self-Test Lateral Flow Devices assay (Figure 2). The live virus sample was detected down to the 10^{-4} serial dilution and the irradiated virus sample was detected down to the 10^{-3} serial dilution.

Previously irradiated SARS-CoV-2 variants, Wuhan-Hu-1, Alpha and Gamma showed equivalent sensitivity (Figure 3).

The irradiated sample was also subject to Transmission Electron Microscope (TEM) to visually confirm integrity and presence of virions as seen in Figure 4 a).

Finally, the Nextclade analysis demonstrates that the irradiated product retained the characteristic mutations of Omicron BA.4, **L**452**R, F**486**V** and **R**493**Q** shown in Figure 4 b) and c).

Sample ID / Dilution	Positive Control Interpretation		LF Test Band Intensity		LF Test Interpretation	
Live & Irradiated	**Live**	**Irradiated**	**Live**	**Irradiated**	**Live**	**Irradiated**
Neg. control	Positive	Positive	Negative	Negative	Negative	Negative
Neg. control	Positive	Positive	Negative	Negative	Negative	Negative
Neg. control	Positive	Positive	Negative	Negative	Negative	Negative
10^{-4}	Positive	Positive	Weak	Negative	**Positive**	Negative
10^{-4}	Positive	Positive	Weak	Negative	**Positive**	Negative
10^{-4}	Positive	Positive	Weak	Negative	**Positive**	Negative
10^{-3}	Positive	Positive	Weak	Negative	**Positive**	Negative
10^{-3}	Positive	Positive	Weak	Weak	**Positive**	**Positive**
10^{-3}	Positive	Positive	Weak	Weak	**Positive**	**Positive**
10^{-2}	Positive	Positive	Weak	Weak	**Positive**	**Positive**
10^{-2}	Positive	Positive	Weak	Weak	**Positive**	**Positive**
10^{-2}	Positive	Positive	Weak	Weak	**Positive**	**Positive**
10^{-1}	Positive	Positive	Strong	Strong	**Positive**	**Positive**
10^{-1}	Positive	Positive	Strong	Strong	**Positive**	**Positive**
10^{-1}	Positive	Positive	Strong	Strong	**Positive**	**Positive**

Figure 2 – Performance comparison of live and X-ray irradiated SARS-CoV-2 BA.4 in the Orient Gene's Rapid Covid-19 (Antigen) Self-Test Lateral Flow Device assay carried out in triplicate.

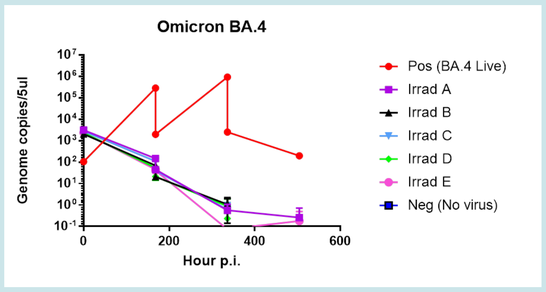

Figure 1 – PCR analysis of 10% of the X-ray irradiated SARS-CoV-2 Omicron BA.4 carried out in 5 replicates, Irrad A, Irrad B, Irrad C, Irrad D and Irrad E.

SARS-CoV-2 variant	Pre-irradiation titre PFU/ml	Sensitivity in an LFD
Wuhan-Hu-1	1.40 x 10^4	10^{-3}
Alpha	2.33 x 10^5	10^{-3}
Gamma	1.30 x 10^6	10^{-3}
BA.4	2.00 x 10^4	10^{-3}

Figure 3 – Comparison of the sensitivity in LFDs between irradiated SARS-CoV-2 variants.

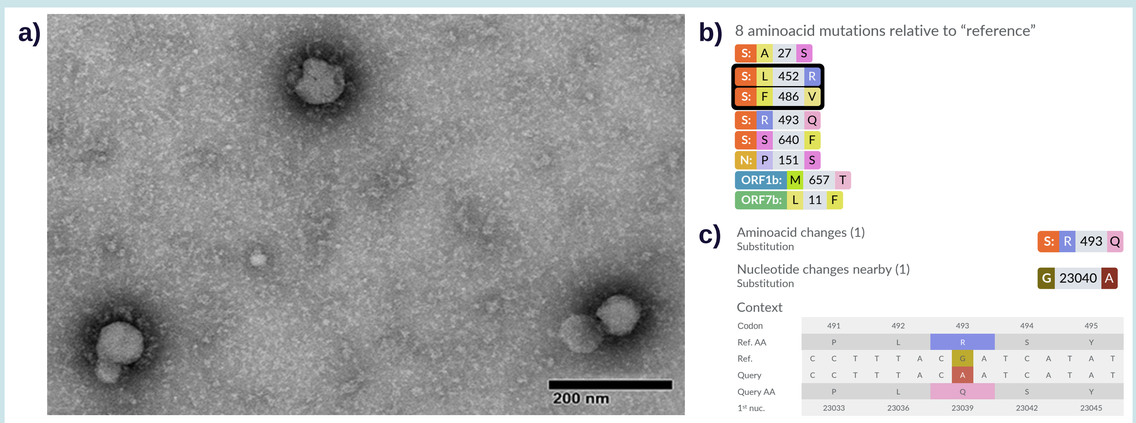

Figure 4 – a) Transmission electron micrograph of X-ray irradiated SARS-CoV-2 Omicron BA.4.
b) and **c)** Genetic analysis of the irradiated Omicron BA.4 through the Nexstrain, Nextclade web platform showing the amino acid changes of interest, **L**452**R, F**486**V** and **R**493**Q**.

DISCUSSION

From this study, it was confirmed that X-ray irradiation created a product that was non-replicating, yet still maintained antigenic structural features detected by the LFDs. This happens even when diluted to 1:1000 which is consistent with others previously X-ray irradiated SARS-CoV-2 variants. The TEM image shows the integrity of the virions, and the spike proteins are also visible.

The whole genome sequence analysis of this NCPV X-ray irradiated SARS-CoV-2 Omicron BA.4 batch has the defining amino acid changes: **L**452**R** and **F**486**V** in the spike receptor binding domain (RBD) as well as, a mutation reversing the Omicron arginine in position 493 to the wild type Wuhan-Hu-1 glutamine, i.e. **R**493**Q**, which made it far more transmissible than previous Omicron variants.[9]

The X-ray irradiated SARS-CoV-2 Omicron BA.4 (NCPV 2209291v) allows scientists to carry out work without higher hazard category concerns.

CONCLUSIONS

- X-ray irradiated BA.4 has shown to have a comparable sensitivity profile to other irradiated SARS-CoV-2 variants on LFDs showing antigen epitopes integrity.
- The availability of X-ray irradiated viruses will enable a broad range of work to be performed on high consequence pathogens by scientists in widely available ACDP CL2 and BSL 2 laboratories, increasing the speed and efficiency of reagent sharing, outbreak response and diagnostic testing as well as therapeutics development.
- X-ray irradiated viruses can be shipped as non-infectious. This reduces the requirements for transport licenses and reduces shipping costs.
- UKHSA's X-ray irradiation capabilities have been developed through a highly effective collaboration between UKHSA departments at Porton and Chilton.
- NCPV plans to expand the range of irradiated viruses available, to include other high consequence viruses.

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