

# THE LANCET

## Supplementary appendix

This appendix formed part of the original submission. We post it as supplied by the authors.

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## Supplementary file

Table S1 Baseline characteristics by vaccine schedules

	AZD1222/AZD1222 N=22	BNT162b2/BNT162b2 N=21
Interval between 1 <sup>st</sup> and 2 <sup>nd</sup> dose, Median(range), weeks	9.5 (8-11)	9 (8-10)
Interval between 2 <sup>nd</sup> dose and blood sample	28 (27-42)	29 (28-40)
Age, Median(range), years	61.9 (51.6-69.2)	60.8 (51.2-69.5)
Sex, female	10 (45.4%)	7 (33.3%)
Ethnicity, white	17 (77.2%)	19 (90.5%)

## Methods

### *The Com-COV2 study*

Com-COV2 is an ongoing, multi-centre, single-blinded UK study enrolling individuals aged 50 years or older previously immunized with a single dose of AZD1222 or BNT162b2 at least 8 weeks previously. Participants were randomized on a 1:1:1 basis to receive the same dose again, or a COVID-19 vaccine manufactured by Novavax or Moderna (Ethics reference 21/SC/0119). Samples were selected for the AZD1222/AZD1222 and BNT162b2/BNT162b2 groups from an immunology cohort (25 per group), pre-defined at the point of enrolment.

### *Viral culture and isolation*

Omicron virus was cultured from a routine diagnostic throat swab (IRAS Project ID: 269573, Ethics Ref: 19/NW/0730). Briefly, VeroE6/TMPRSS2 cells (NIBSC) were maintained in Dulbecco's Modified Eagle Medium (DMEM) high glucose supplemented with 1% fetal bovine serum, 2mM Glutamax, 100 IU/ml penicillin-streptomycin and 2.5ug/ml amphotericin B, at 37 °C in the presence of 5% CO<sub>2</sub> before inoculation with 200ul of swab fluid. Cells were further maintained at 37°C with daily observations for cytopathic effect (CPE). Virus containing supernatant were clarified at 80% CPE by centrifugation at 3,000 r.p.m. at 4 °C before being stored at -80 °C in single-use aliquots. Viral titres were determined by a focus-forming assay on Vero CCL-81 cells (ATCC). Sequencing of the Omicron isolate shows the

expected consensus S gene changes (A67V, Δ69-70, T95I, G142D/Δ143-145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F), an intact furin cleavage site and a single additional mutation A701V

#### *Focus reduction neutralisation test*

The focus reduction neutralisation test (FRNT) was carried out in order to determine the neutralisation potency of tested sera using passage 1 virus with the sequence above. Tissue culture 96-well, flat-bottom microplates were seeded with Vero CCL-81 cells 24 h prior to use and were used at around 90-95% confluent monolayer. A three-fold dilution of heat-inactivated serum samples, starting from 1:20, were pre-mixed with an equal volume of viral solution containing 100 foci. The controls included virus alone layered on top of Vero CCL-81 cells. After 1 hour of incubation at 37°C, the serum-virus mixture at each dilution was added in duplicate to Vero cells monolayer and incubated for 2 hrs followed by the addition of 1.5% semi-solid carboxymethyl cellulose (CMC) immobilizing medium to each well to prevent virus spread. After addition of the overlay, cells were further incubated for 20 hrs.

The viral foci were visualized by focus-forming assay. Briefly, cells were fixed and permeabilized with 4% PFA and 2% triton-X 100, respectively. This was followed by the staining with human anti-N mAb (mAb206) and peroxidase-conjugated goat anti-human IgG (A0170; Sigma). Finally, TrueBlue Peroxidase substrate was used to develop the plates followed by imaging and counting the foci under ELISPOT reader. The FRNT50 titer is calculated using the probit program from the SPSS package.

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*Declarations of interest:*

G.R.S sits on the GSK Vaccines Scientific Advisory Board and is a founder member of RQ Biotechnology. MDS acts on behalf of the University of Oxford as an investigator on studies funded or sponsored by vaccine manufacturers, including AstraZeneca, GlaxoSmithKline, Pfizer, Novavax, Janssen, Medimmune, and MCM Vaccines. He receives no personal financial payment for this work. AJP reports grants from UKRI, CEPI and NIHR, during the conduct of the study. AJP is Chair of DHSC's Joint Committee on Vaccination & Immunisation (JCVI), but does not participate in discussions on COVID19 vaccines, and is a member of the WHO's SAGE. The views expressed in this article are those of the authors and do not necessarily represent the views of DHSC, JCVI, NIHR or WHO. The University of Oxford has entered into a partnership with AstraZeneca for the development of a coronavirus vaccine. JSN-V-T is seconded to the Department of Health and Social Care, England (DHSC). The views expressed in this manuscript are those of its authors and not necessarily those of DHSC.

*Contribution*

GRS and JM designed the experiment, which was performed by WD, PS and CL. WD and PS isolated the Omicron strain. DC sequenced the Omicron virus. WD, PS and CL performed and analysed the neutralising experiments. MDS is the chief investigator for Com-COV2. MDS, JVT, ASVS, RHS, TL and XL contributed to the protocol and design of Com-COV2. ASVS, RHS and RW led the implementation of Com-COV2. XL did the statistical analysis and has verified the underlying data. GRS, JM, AJP, XL and MDS drafted the manuscript, with contributions from DIS. All authors reviewed and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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