A Rapid Antibody Screening Haemagglutination Test for Predicting Immunity to Sars CoV-2 Variants of Concern

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Abstract

Evaluation of susceptibility to emerging SARS-CoV-2 variants of concern (VOC) requires rapid screening tests for neutralising antibodies which provide protection. We developed a receptor-binding domain specific hemagglutination test (HAT) which correlated with neutralising antibodies (R=0.74-0.82) in two independent cohorts from 798 convalescents. Home-dwelling older individuals (80-99 years, n=89) had significantly lower antibodies after one dose of BNT162b2 vaccine than younger adult vaccinees (n=310) and naturally infected individuals (n=307). The second vaccine dose boosted and broadened the antibody repertoire to VOC in naïve but not previously infected, older and younger adults. >75% of older adults responded after two vaccinations to alpha and delta, but only 59-62% to beta and gamma, compared to 96-97% of younger vaccinees and 68-76% of infected individuals. Overall, the HAT provides a surrogate marker for neutralising antibodies, could be used as a simple inexpensive, rapid test, rapidly adaptable to emerging VOC for large-scale evaluation of potentially diminishing vaccine effectiveness.

Introduction

There is increasing evidence that neutralising antibodies to the receptor binding domain (RBD) on the severe acute respiratory syndrome coronavirus-2 virus (SARS-CoV-2) spike protein represent an immunological correlate of protection[1]. SARS-CoV-2 evolution has been rapid with the ancestral virus and emerging variants of concern (VOC) straining global health care systems. These VOC (alpha (B.1.1.7)[2], beta (B.1.351)[3], gamma (P.1)[4] and delta (B.1.617.1/B.1.617.2)[5] show increased transmissibility, can escape pre-existing immunity and reduce vaccine effectiveness[6-8], with breakthrough infections reported in COVID-19 vaccinees with low neutralising antibodies[9]. There is a need for a rapid low-cost surrogate neutralisation assay, which can be used at a low biosafety level. This assay could be used for large-scale screening to identify vaccinees potentially susceptible to emerging VOC and who would benefit from a booster vaccine dose.

The neutralisation assay with live native virus is the gold standard for evaluating antibodies to VOC[1]. However, neutralising assays are difficult to standardise across laboratories, are time consuming, expensive and require high containment. Therefore antibody binding and pseudotype virus assays are widely used to study antibody responses[10-12], but still require specialised laboratory facilities.

Here, we correlate the low-cost rapid hemagglutination test (HAT)[13] with neutralisation of the ancestral Wuhan-like strain in two large independent cohorts of infected patients. In the HAT assay, the RBD domain is linked to a monomeric anti-erythrocyte single domain nanobody. When polyclonal serum antibodies bind to the RBD they cross-link and agglutinate the erythrocytes, which can be read visually after one hour. The HAT has a specificity of >99% for detection of convalescent antibodies after polymerase chain reaction (PCR) confirmed infection[13,14]. For influenza, a correlate of protection (COP) has been defined as a haemagglutination inhibition (HAI) titre of 40 for 50% protection from infection. If a similar COP could be established for HAT, it would allow simple standardised evaluation of susceptibility to SARS-CoV-2 infection and waning vaccine responses to VOC to guide public health policies.

Initially, we established that HAT titres correlated with neutralising antibodies (Extended data Figure 1). We then used the HAT to investigate the antibody responses in 719 individuals consisting of home-dwelling older vaccinees (80–99-year-olds) and younger adults, in both those vaccinated with mRNA (BNT162b2) and in naturally infected individuals to the Wuhan-like virus. We developed VOC HAT and confirmed that HAT titres could be used as a surrogate marker for neutralising antibody titres in vaccinated or infected individuals. Finally, we showed that the HAT is readily adapted to finger prick testing.

Methods

Study participants

Norwegian Vaccine and infections cohorts

A cohort of convalescents 378 infected individuals was prospectively recruited during the first pandemic wave in Bergen, Norway to compare the serological assays used in this study as described in[17-19]. For the comparison of vaccine and infections cohorts in Bergen Norway, we prospectively recruited two different age groups (home dwelling older and healthy younger adults) who received two doses of BNT162b2 mRNA COVID-19 vaccine at a 3-week interval during January 2021, and compared them to a group of 307 naturally infected individuals infected (1-89, median 47 years) with the Wuhan-like virus (D614G spike mutation) in February to April 2020[17,18] (Extended data table 1). The older vaccinee group consisted of 96 home-dwelling elderly (80-90 years, median 86), 88 (92.6%) of whom were seronegative and 7 had previous SARS-CoV-2 infection with detectable pre-vaccination antibodies. The younger adult group consisted of 316 vaccinees (23-77 years, median 38) of whom 309 adults had no history of confirmed SARS-CoV-2 PCR test. Four younger vaccinees were not vaccinated on day 21; they received their second vaccination at day 19 (n=1), or day 23 (n=2) or day 24 (n=1). Seven younger individuals had previous SARS-COV-2 infection and pre-existing antibodies. This study is compliant with all relevant ethical regulations for work with humans and conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. All Bergen subjects provided written informed consent before inclusion in the study, which was approved by the Western Norway Ethics committee (#118664 and #218629, NIH ClinicalTrials.gov Identifier: NCT04706390). Demographics (gender, age), PCR test results and COVID-19-like symptoms were recorded in an electronic case report form (eCRF) in (REDCap® (Research Electronic Data Capture) (Vanderbilt University, Nashville, Tennessee). Clotted blood samples were collected on the day of vaccination, 3 weeks after receiving the first and 3-5 weeks (mean 55 days, standard deviation ± 5 days) after the second vaccine doses or 3-10 weeks after confirmed infection. Sera were separated and stored at -80°C and heat-inactivated for one hour at 56°C before use in the serological assays.

UK convalescent cohort.

Informed signed consent was obtained from 420 blood donor in the NHS Blood and Transplant cohort for purposes of clinical audit, to assess and improve the services and the research, and specifically to improve knowledge of the donor population. Use of these anonymised samples in this study was approved by NHS Blood and Transplant Research and Audit Committee (BS-CARE).

Finger-prick and venous blood comparison

For the comparison of finger-prick and venous blood, participants were recruited from Oxford University Hospitals NHS Foundation Trust when they were attending research clinic with the Oxford Protective T Cell Immunology for COVID-19 (OPTIC) Clinical Team. Written informed consent was obtained from participants with different past infection and vaccination status (extended data table 1). Seventy-eight paired finger-prick blood and venous blood in EDTA tubes were taken at the same time and analysed on the same day by point HAT assay. The rest of the plasma and dilutions were stored at 4°C. Human study protocols were approved by the research ethics committee at Yorkshire & The Humber—Sheffield (GI Biobank Study 16/YH/0247).

Haemagglutination test (HAT)

The haemagglutination test (HAT)[13] was used to investigate the SARS-CoV-2 specific antibodies to the RBD of the ancestral virus (Wuhan-like, pre alpha) and to the VOC alpha (B.1.1.7), beta (B.1.351),gamma (P.1) and delta (B.1.617.1/B.1.617.2). Briefly, codon optimised IH4-RBD sequences of VOC containing amino acid changes in the RBDs B.1.1.7 (N501Y), B.1.351 (K417N, E484K, N501Y), P.1 (K417T, E484K, N501Y) and B.1.617.2 (L452R, T478K). IH4-RBD were expressed in Expi293F cells and purified by their c terminal 6xHis tag using Ni-NTA chromatography.

The point HAT was performed in V-bottomed 96-well plate on the same day as the blood was collected. Whole blood was diluted 1 in 40 in Phosphate buffered saline (PBS) 50 µl of dilution was mixed with 50 µl 2 µg/ml IH4-RBD reagent in the test well. Anti-RBD monoclonal antibodies, EY-6A[33] or CR3022[34] (100ng) were positive controls and negative controls were whole blood dilution mixed with PBS. All sera were pre-screened at a dilution of 1:40 in PBS in 96 well V well plates. If HAT positive, serum was double diluted in duplicate from 1:40 in 50 µl PBS giving final dilutions of 1:40 to 1:40,960. Equal volumes of human O negative red blood cells (~1 % v/v in PBS)[13] and 2.5 µg/ml IH4-RBD of Wuhan-like or VOC (B.1.1.7, B.1.351, P.1 or B.1.617.2) (125 ng/well) were pre-mixed and 50 µl added per well. Negative controls (PBS) and positive controls (monoclonal antibodies CR3022 and EY-6A) were included in each run. Plates were incubated to allow red blood cells to settle for 1 hr and were read by tilting the plate for 30s and photographing. Positive wells agglutinated and the HAT titre is defined as the last well in which the teardrop did not form. Partial teardrops were scored as negative.

The IH4-RBD reagents for each VOC were standardised by showing that agglutination of red cells occurred at the same endpoint dilution (~16ng/well) of the well characterised human monoclonal antibody EY6A[13,33] for each VOC at a working dilution of IH4-RBD of 2ug/ml (100ng/well in 50ul). All of the RBDs of the VOC share the conserved class IV epitope recognised by EY6A.

Enzyme-linked immunosorbent assay (ELISA)

SARS-CoV-2 antibodies were detected using the ELISA in Bergen, Norway as described by Amanat et al. 2020[11] and Trieu et al 2020[19] (Extended data Figure 2). Sera were screened using the Wuhan receptor-binding domain (RBD) ELISA at 1:100 dilution and were tested in duplicate to detect IgG (Sigma-Aldrich) binding to the Wuhan-like RBD protein using 3,3´,5,5´-tetramethylbenzidine (TMB) (BDbiosciences) and the optical density (OD) read at 450/620nm. Sera were titrated for endpoint titres in the spike ELISA, starting from 1:100, to detect IgG (Sigma-Aldrich) binding to the Wuhan spike protein. The endpoint titres were calculated for each sample. Individuals with no antibodies were assigned a titre of 5 for calculation purposes.

Pseudotype-based neutralisation assay

The pseudotype-based neutralisation assay was performed in biosafety level 2 laboratory in Bergen, Norway. The SARS-CoV-2 pseudotype virus was generated by co-transfection lentiviral vectors pHR'CMV-Luc, pCMVRΔ8.2, and pCMV3 construct encoding the Wuhan spike protein into HEK293T cells as previously described[35]. The protease TMPRSS2 and human ACE2 encoding constructs were transfected into HEK293T to make target cells for the neutralisation assay. The lentiviral vectors and TMPRSS2-encoding constructs were a kind gift from Dr. Paul Zhou, Institute Pasteur of Shanghai, China. The ACE2-encoding construct was a kind gift from Dr. Nigel Temperton, University of Kent, UK. The SARS-CoV-2 spike-encoding construct was purchased from Sino Biological. Serum samples were heat inactivated at 56°C for 60 min, analysed in serial dilutions (duplicated, starting from 1:10). The SARS-CoV-2 pseudotype viruses corresponding to 20,000 to 200,000 relative luciferase activity (RLA) were mixed with diluted sera in 96-well plates and incubated at 37°C for 60 min. Afterwards, ACE2-TMPRSS2 co-transfected HEK293T cells were added into 96-well plates and cultured for 72 hours. RLA was measured by a BrightGlo Luciferase assay according to the manufacturer's instructions (Promega, Madison, WI, USA). The pseudotype-based neutralization (PN) titres (IC_{50} and IC_{80}) were determined as the reciprocal of the sera dilution giving 50% and 80% reduction of RLA, respectively. Negative titres (<10) were assigned a value of 2 for calculation purposes.

Virus strains

The Wuhan-like strain used in the microneutralisation and virus neutralisation assays in Bergen Norway was the clinical isolate; SARS-CoV-2/Human/NOR/Bergen1/2020 (GISAID accession ID EPI_ISL_541970) and at Public Health England, UK the isolate England/02/2020[20] (GISAID accession ID EPI_ISL_407073). At Oxford, UK[22] the Wuhan-like strain was

Victoria/01/2020 (GenBank MT007544.1, B hCoV-19_Australia_VIC01_2020_ EPI_ ISL_ 406844_ 2020-01-25, and alpha (B.1.1.72) virus was the H204820430, 2/UK/VUI/1/2020 and the beta (B.1.351) (20I/501.V2.HV001) isolate.

Microneutralisation assay

The microneutralisation (MN) assay was performed on 345 Bergen convalescent sera in a certified Biosafety Level 3 Laboratory in Norway[17-19] and for the 420 convalescent UK samples as previously described at Public Health England (PHE), UK[20]. Serum samples were tested against a clinically isolated virus: SARS-CoV-2/Human/NOR/Bergen1/2020 (GISAID accession ID EPI_ISL_541970) or England/02/2020 (GISAID accession ID EPI_ISL_407073). Briefly, serum samples were heat inactivated at 56°C for 60 min, analysed in serial dilutions (duplicated, starting from 1:20), and mixed with 100 $TCID_{50}$ viruses in 96-well plates and incubated for 1 hour at 37° C. In Bergen, mixtures were transferred to 96-well plates seeded with Vero cells. At PHE the cell suspension was added to the virus/antibody mixture[20]. The plates were incubated at 37° C for 22 hours at PHE, UK and 24 hours in Bergen Norway. Cells were fixed and permeabilized with methanol and 0.6% H_2O_2 and incubated with rabbit monoclonal IgG against SARS CoV2 nucleoprotein (NP) (Sino Biological). Cells were further incubated with biotinylated goat anti-rabbit IgG (H+L) (Southern Biotech), and Extravidin-peroxidase (Sigma-Aldrich). The reactions were developed with o-Phenylenediamine dihydrochloridec (OPD) (Sigma-Aldrich). The MN titre was determined as the reciprocal of the serum dilution giving 50% inhibition of virus infectivity. Negative titres (<20) were assigned a value of 5 for calculation purposes.

At Oxford, UK the detection of antibodies to the Wuhan-like VOC and (alpha, B.1.1.7.2 and beta, B.1.351) used the method described in[22]. Briefly quadruplicate serial dilutions of serum were preincubated with appropriate SARS-CoV-2 for 30 minutes at room temperature, then Vero CCL81 cells were added and incubated at 37°C, 5% CO_2 for 2 hours. A carboxymethyl cellulose-containing overlay (1.5%) was added, monolayers were fixed and stained for the nucleocapsid (N) antigen or spike (S) antigen using EY2A and EY6A monoclonal antibodies, respectively. After development the number of infectious foci were counted by ELISpot reader. Data were analysed using four-parameter logistic regression (Hill equation) in GraphPad Prism 8.3.

Virus neutralisation assay

The virus neutralisation (VN) assay was performed in a certified Biosafety Level 3 facility in Bergen, Norway[19]. Serum samples were tested against a clinically isolated virus: SARS-CoV-2/Human/NOR/Bergen1/2020 as previously described[19]. Briefly, serum samples were heat inactivated at 56°C for 60 min, analysed in serial dilutions (duplicated, starting from 1:20), and mixed with 100 TCID50 viruses in 96-well plates and incubated for 1 hour at 37° C. Mixtures were transferred to 96-well plates seeded with Vero cells. The plates were incubated at 37°C for 4-5 days, all wells were examined under microscope for cytopathic effect (CPE). The VN titre was determined as the reciprocal of the highest serum dilution giving no CPE. Negative titres (<20) were assigned a value of 5 for calculation purposes.

Statistical analysis

The Mann-Whitney U test was used to compare the older and adult vaccinees. The Spearman R correlation analysis was used to investigate the correlation between the antibody titres from different serological assays. All analyses were conducted in graph pad prism version 9.20.

Results

Previous studies have shown that the HAT titre correlates with SARS-CoV-2 RBD binding and ACE2 blocking antibodies[13,15,16], and identifies high titre (>100) neutralising sera with a sensitivity 76.5%[16]. Here, we investigated the relationship between endpoint HAT titres and neutralising antibodies using three neutralisation assays in convalescent

sera from SARS-CoV-2 infected individuals from the first pandemic wave (pre-alpha) in Bergen, Norway[17-19] and confirmed the results in an independent UK cohort[20].

Correlation of neutralising antibodies and HAT titres

In the Bergen cohort of 378 samples, microneutralization 50% inhibitory concentrations (IC_{50}) titres were significantly associated with HAT titres (Spearman's R=0.82, p<0.0001) (Figure 1C, Extended data Figure 1). A HAT titre³40 detected 99% of samples with MN IC_{50}³20, with positive predictive value (PPV) of 94%. A HAT titre>480 predicted MN titres>100 with a sensitivity 77% and PPV of 78%.

We extended these results by comparisons to a pseudotype neutralisation (PN) assay, and a classical live virus neutralisation (VN) assay with complete inhibition of Cytopathic Effect (100% CPE) as its endpoint (Figure 1A,B,D)[19]. The correlation of HAT and PN titres were significant (p<0.0001) at 50% (R=0.79) and 80% (R=0.78) IC (Figure 1A,B). Confirming our previous results, the VN titres correlated with HAT titres (R=0.72,p<0.0001) (Figure 1D). HAT titres ≥40 detected 100% of samples with VN titres≥20, but the PPV fell to 54% consistent with the classical VN assay having the more rigorous endpoint.

Confirmation of correlation between neutralisation and HAT titres

As interlaboratory variation has been reported for neutralisation assays, we confirmed the significant correlation between HAT and MN titres (R=0.88, p<0.0001) in an independent UK collection of 420 convalescent samples (Figure 1E, Extended data Figure 1). In close agreement with the Bergen cohort, a positive HAT³40 detected 98% of samples with MN IC_{50} titre ³20, with PPV of 87%. Similarly, for identification of high titre sera a HAT >480 identified 75% of sera with MN IC_{50} >100 with PPV 86%. In summary, the HAT titres highly correlated with neutralisation titres in two independent laboratories showing the utility of HAT as a rapid and inexpensive surrogate for the neutralisation test.

Evaluation of HAT antibody responses in older and younger vaccinees

Older adults have carried the burden of COVID-19 throughout the pandemic with increased risk of hospitalizations and death, and are prioritised for vaccination, although they were not included in vaccine licensure trials. As proof of principle, we used the HAT to investigate the Wuhan-like antibody responses in seronegative healthy younger adults (n=309, median 37years) and older home-dwelling adults (n=89, 80-99years, median 86years) after the BNT162b2 mRNA COVID vaccine and in individuals naturally infected with the Wuhan-like strain (n=307, median 47years)[18] (Table 1, Figure 2). A HAT titre of ³40 was used as a cut-off to assess the proportions of vaccine responders to the Wuhan-like virus[13]. Only 31% of older subjects responded after the first vaccination compared to 74% of younger vaccinees (Figure 2A, Extended data Table 2). After the second dose, 78% of the older vaccinees had HAT titre of ³40 compared to 94% of infected individuals and 99% younger vaccinees. Older people also had a significantly lower magnitude of response than younger adult vaccinees after both the first and second vaccine doses, with the exception after second dose against alpha (Table 3). In summary, the older adults had a blunted response after one dose of mRNA vaccine and required the second dose to increase the magnitude of the response.

Development of variant of concern reagents for HAT

Variants of concern have amino acid changes in their spike protein, and importantly in their RBD which may allow escape from neutralising antibodies. The alpha variant rapidly became the dominant strain in early 2021[2] with beta and gamma dominating in some geographical areas, and has since mainly been replaced by the highly transmissible delta variant[21]. We developed HAT reagents for the VOC as they arose and confirmed a strong correlation between the alpha (R=0.79,p<0.0001) and beta (R=0.89,p<0.0001) in a UK set of naturally infected and vaccinated donors[22] (Figure 1F).

HAT antibodies to variants of concern in older vaccinees

We then investigated the breadth of the VOC response in vaccinees and infected subjects. Older vaccinees had the lowest number of responders and lower cross-reactivity after both one and two vaccinations. The second vaccination boosted the number of responders in older adults, from 20-29% to 62-76% to VOC (Figure 2E,I,M,O). In older and younger vaccinees that responded to VOC, there was good cross-reactivity to alpha and delta, but less so to the beta and gamma in all groups after two doses of vaccine or infection. A similar but higher response pattern to different VOC was observed in infected individuals, with 82% to both alpha and delta, 75% to beta and 68% to gamma compared to responses in 96-100% of younger adult vaccinees. In summary, two doses of mRNA vaccine or natural infection induced higher responses in younger adults to VOCs than in older vaccinees.

Vaccine response in previously infected younger and older individuals

Natural infection induces higher titres of SARS-CoV-2 specific antibodies in older individuals than in younger adults[17]. Previously infected older subjects (n=7, median age 87years), none of whom had been hospitalised, had higher pre-vaccination HAT titres to the Wuhan-like virus than previously infected younger adults (n=7, median age 38years). Previously infected older and younger adults developed high Wuhan-like and alpha cross-reactive antibody titres after one vaccine dose (Figure 2C,D), although lower responses to other VOC. Cross-reactive titres were boosted in some of the older and healthy vaccinees after the second vaccination (Figure 2G,H,K,L,O,P,S,T). In summary, previously infected older individuals develop high antibody titres after one vaccine dose comparable to healthy younger adults, which contrasts with the suboptimal antibody responses in SARS-CoV-2 naïve older vaccinees.

The use of HAT as a point of care fingerprick test

For the HAT to be implemented at low biosafety level and in resource limited settings, a fingerprick test using autologous patient erythrocytes could be used to rapidly identify populations with low titres to Wuhan-like and VOC SARS Cov-2 viruses. As advised by the Infectious Diseases Society of America guideline on serological testing[23], we confirmed the comparability of fingerprick and venous blood for reactivity to all VOC (Figure 3). The HAT can thus be rapidly adapted to test for antibodies to emerging VOC for large-scale screening of fingerprick blood samples with autologous erythrocytes.

Discussion

The rapid evolution of SARS-CoV-2 VOC with increased transmissibility and the possibility of escape from vaccine induced immunity represents a considerable threat. There is a need for a low-cost rapid serological assay which can be used for large-scale screening globally without requiring specialised laboratory equipment to rapidly identify populations susceptibility to VOC. HAT is simple to perform, requires no special equipment, and can be done at the point-of-care in virtually any setting using a fingerprick sample. The HAT IH4-RBD VOC reagents are freely available for research[13]. Inter-laboratory comparability can be guaranteed by including HAT titrations on WHO approved standard sera (as shown in Extended data Table 1).

We demonstrate the versatility of the HAT in analysing susceptibility to VOC in home-dwelling older vaccinees showing the importance of two vaccine doses to achieve good cross-reactive antibody titres in older adults who have not been previously infected. Older and high risk individuals were prioritised in the very first rounds of vaccination early in 2021 in Europe and America. Depending upon the decay in antibody titres over time, the HAT could be used to rapidly identify individuals who may need a booster vaccine dose to mount efficient antibody responses to VOC. We found the oldest age group had a decreased breadth of cross-reactive antibodies to VOC after the first vaccine dose, particularly to the beta and gamma viruses, in agreement with escape from neutralising antibodies[8]. Although vaccination induced cross-reactive antibodies against delta, milder break through infection with this variant in vaccinated subjects is becoming an increasing

problem[9]. Reports of very high viral load during delta infections[24,25] may necessitate higher antibody titres to provide sterilising immunity and prevent infection.

In previously infected adults, only one dose of vaccine seems to be required to produce high levels of cross-reactive antibodies against the VOC[14,15]. Extending these findings, we found that in previously SARS-CoV-2 infected older adults, only one dose of vaccine was required to mount strong anamnestic responses, similar to younger vaccinees[26-32].

Caveats to our study are that convalescent blood samples from our naturally infected cohort were only collected during the first SARS-CoV-2 Wuhan-like wave. However, the strengths are that we have confirmed the relationship between HAT and several neutralisation assays in two large cohorts in independent laboratories, shown that relationship holds for VOCs, and included 719 individuals either infected and/or vaccinated, aged up to 99years old. To our knowledge, this is the first study reporting antibody cross-reactivity to four VOC in this older age group.

Neutralising titres of between 10-30 in humans[1], depending upon the assay, have been reported to predict 50% protection from symptomatic infection, and much lower levels to protect against severe infection. Although the absolute HAT titres correlating with protection are not yet known, we demonstrated that the HAT titres correlated with neutralisation titres, and thus provide a surrogate test for neutralising antibodies. We suggest that a positive HAT titre of 40–80, equivalent to 1:40 dilution of whole blood obtained by fingerprick, would correlate with neutralising titres 10-30, and would predict protection. A prospective study to test this predicted relationship between HAT titres and protection is now warranted. The HAT may also aid in evaluating and licensing of new COVID vaccines.

We predict that the lower HAT titres against VOC will lead to a more rapid decline in protective efficacy against variants, thus requiring booster vaccinations. The emergence of the highly infectious and transmissible delta VOC which has caused breakthrough infections in vaccinees highlights the importance of real-time cross-reactivity studies. Monitoring of population susceptibility of both previously infected subjects and vaccinees to VOC with increased transmissibility through simple serological assays can guide public health policy.

Declarations

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Author contribution

RJC, NL and AT designed the study, and together with NUE and JX analysed the results and wrote the paper. NUE, JX and SL conducted the HAT assays. FZ ran all neutralisation assays in Bergen, Norway. HS, SLL, LH, MS, LH, AM, KGIM, EF JSO KAB recruited and followed up 345 SARS CoV-2 positive individuals and 412 vaccinees in Bergen and ran the lab assays. TKT, PR, and LS developed and standardised the VOC HAT. SD and AJ recruited and collected fingerprick and venous blood samples. DR, WSJ and ACH recruited the infected subjects and vaccinees in Oxford and ran the neutralisation assays for the VOC. HH and MZ collected and tested 420 convalescent samples at Public Health England Colindale UK for neutralising antibodies. SØ recruited and vaccinated the older patients. All authors reviewed the manuscript and approved the final version for publication.

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Tables

Table 1. The demographics of the mRNA old and healthy adult vaccinees and naturally infected subjects

Characteristics	Bergen cohort				PHE cohort
	Vaccinated		Infected[&]	Convalescents[$]	Convalescents
	Old (n=96)	**Adult (**n=316)	(n=307)	(n=378)	(n=420)
Age (median (age range))	86 (80-99)	38 (23-77)	47 (1-89)	45 (1-89)	44 (19-65)
Sex (Female)	57 (64%)	209 (67%)	159 (51.8%)	216 (57.1%)	114 (27.2%)
Comorbidity[*]	71 (79%)	21 (7%)	136 (44.3%)	145 (38.4%)	-
Immunosuppression[#]	14 (16%)	3 (1%)	12 (3.9%)	11 (2.9%)	-

*Diabetes, chronic respiratory diseases, chronic heart diseases, neurological diseases, chronic kidney, or liver diseases, dementia, rheumatologic diseases, active cancer

[#]Inherent immunosuppressive disease, HIV, organ transplant, chemotherapy, other immunosuppressive treatment/drugs

[§]In correlation analysis, Figure 1A-D

[&]In HAT analysis, Figure 2

- Information was not available

Table 2. Comparison of Wuhan-like and variant of concerns haemagglutination test responders after one and two doses of mRNA vaccine in younger and older adults, as well as in previously infected with SARS CoV-2.

Responders n/N (%)					
	Vaccinated				Infected[3]
	Older[1]	Younger[2]	Older[1]	Younger[2]	
Virus[#]	Post 1st dose		Post 2nddose		
Wuhan-like	28/89 (**31**)	228/309 (**74**)	70/89 (**78**)	308/309 (**100**)	289/307 (**94**)
Alpha,B.1.1.7	26/89 (**29**)	177/309 (**57**)	68/89 (**76**)	307/309 (**99**)	284/307 (**82**)
Beta, B.1.351	18/89 (**20**)	168/309 (**54**)	55/89 (**62**)	298/309 (**96**)	233/307 (**76**)
Gamma, P.1	22/89 (**25**)	171/309 (**55**)	52/89 (**58**)	299/309 (**97**)	209/307 (**68**)
Delta B.1.617.2	21/89 (**24**)	204/309 (**66**)	64/89 (**72**)	309/309 (**100**)	253/307 (**82**)

[1]89 seronegative older vaccinees 3 weeks after 1st and 3-5 weeks after 2nd dose of mRNA vaccine

[2] 309 younger adult vaccinees 3 weeks after 1st and 3-5 weeks after 2nd dose of mRNA vaccine

[3] 307 SARS-CoV-2 infected individuals with convalescent sera collected 4-6 weeks after infection

[#]The viruses tested are the founder virus (Wuhan-like) and variant of concern (B.1.1.7 alpha; B.1.351 beta; P.1 gamma and B.1.617.2 delta) viruses. The data is presented as the subjects with HAT titre over 40 against the different variants, n/N and as percentage (%) of the whole group.

Table 3. The HAT antibody response in GMs and fold-changes after one and two doses of mRNA vaccine and after SARS-CoV-2 infection in seronegative younger and older adults tested against the Wuhan-like virus and variants of concern.

	Vaccinated										Infected[3]
	Older[1]		Younger[2]		Older vs. younger	Older[1]		Younger[2]		Older vs. younger	
	Post 1st dose					Post 2nd dose					
Virus[#]	GM*	Fold-change[$]	GM	Fold-change	P value[#]	GM	Fold-change	GM	Fold-change	P value[#]	GM
Wuhan-like	14	2.8	60	11.9	**<0.0001**	104	7.3	262	4.4	**<0.0001**	438
Alpha B.1.1.7	13	2.7	31	6.2	**<0.0001**	101	7.6	175	5.6	0.3323	292
Beta B.1.351	10	2.0	30	5.9	**<0.0001**	45	4.5	133	4.5	**<0.0001**	82
Gamma P.1	11	2.2	25	5.1	**<0.0001**	44	4.0	116	4.6	**0.0036**	64
Delta B.1.617.2	10	2.0	41	8.3	**<0.0001**	59	5.7	223	5.4	**<0.0001**	141

[1] 89 seronegative older vaccinees 3 weeks after 1st and 3-5 weeks after 2nd dose of mRNA vaccine

[2] 309 younger adult vaccinees 3 weeks after 1st and 3-5 weeks after 2nd dose of mRNA vaccine

[3] 307 SARS-CoV-2 infected individuals with convalescent sera collected 4-6 weeks after infection

[#]The viruses tested are the founder virus (Wuhan-like) and variant of concern (B.1.1.7 alpha; B.1.351 beta; P.1 gamma; and B.1.617.2 delta) viruses.

* The data is presented as the geometric mean (GM) of the HAT titres. Negative values were assigned a value of 5 for calculation purposes.

[$] The fold change is shown in the vaccinated individuals from pre to post 1st dose and from post 1st to post 2nd vaccine dose. All individuals were seronegative (HAT <40) at baseline.

[#] The Mann-Whitney U test was used to compare differences in HAT titres between the adults and the older vaccinees, with P values <0.05 considered significant.

Figures

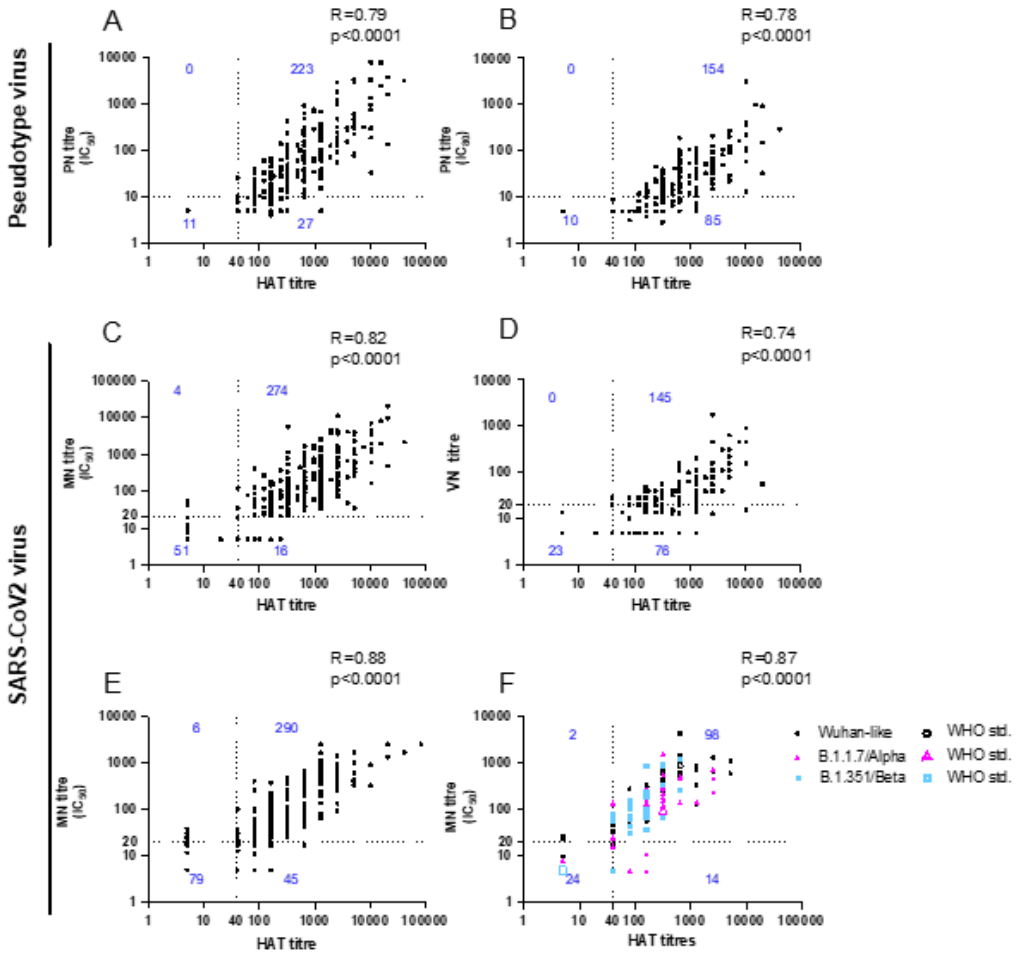

Figure 1

The correlation between haemagglutination test and SARS-CoV-2 neutralising antibodies Correlation of endpoint HAT titres with neutralising antibody titres. In a cohort of infected individuals from the first pandemic wave (diagnosis by PCR from nasopharyngeal swabs or serology in Bergen, Norway) (A-D, G), Wuhan-like neutralising antibodies were measured using the pseudotype (PN) neutralisation assay at 50% (PN50) (A) (R= R=0.79, 95% confidence interval (CI): 0.73-0.83) and 80% (PN80) (B) (R=0.78, 95% CI: 0.72-0.82) inhibition of pseudotype virus infectivity, the microneutralisation (MN) 50% inhibitory concentration (C) (R=0.82, 95% CI: 0.78-0.85) and virus neutralization (VN) 100% inhibition of cytopathic effect (D) (R=0.74, 95% CI: 0.68-0.80) assays. Convalescent sera from 420 infected individuals in UK for whom neutralising antibody and HAT titre were measured (E) (R=0.88, 95% CI: 0.86-0.90). The correlation between the HAT and 50% inhibition of neutralising antibody titres for Wuhan-like, and B.1.1.7 and B.1.351 VOC antibody titres performed at Oxford, UK (F) (R=0.87, 95% confidence interval: 0.82-0.90). HAT titres were measured in a set of donors either infected or vaccinated with one or two doses of the Pfizer BNT162b2 mRNA vaccine who had neutralising antibody levels to ancestral Wuhan, B.1.1.7 and B.1.351 live viruses. Open symbols represent the positive anti SARS-CoV-2 WHO standard (20/130). The Spearman R correlations and significant values are shown. In the microneutralisation (MN) assay, virus infectivity was measured by detecting the amount of nucleoprotein and also spike after 22-24 hours incubation in Vero cells. In Bergen (C,D) the Wuhan-like local D614G virus hCoV-19/Norway/Bergen-01/2020 (GISAID accession ID EPI_ISL_541970) was used in a certified Biosafety Level 3 Laboratory. The dotted lines show the lowest detectable titre in each assay, all negative values were assigned the number 5 for consistency, and the numbers in blue are the number of samples in each quadrant.

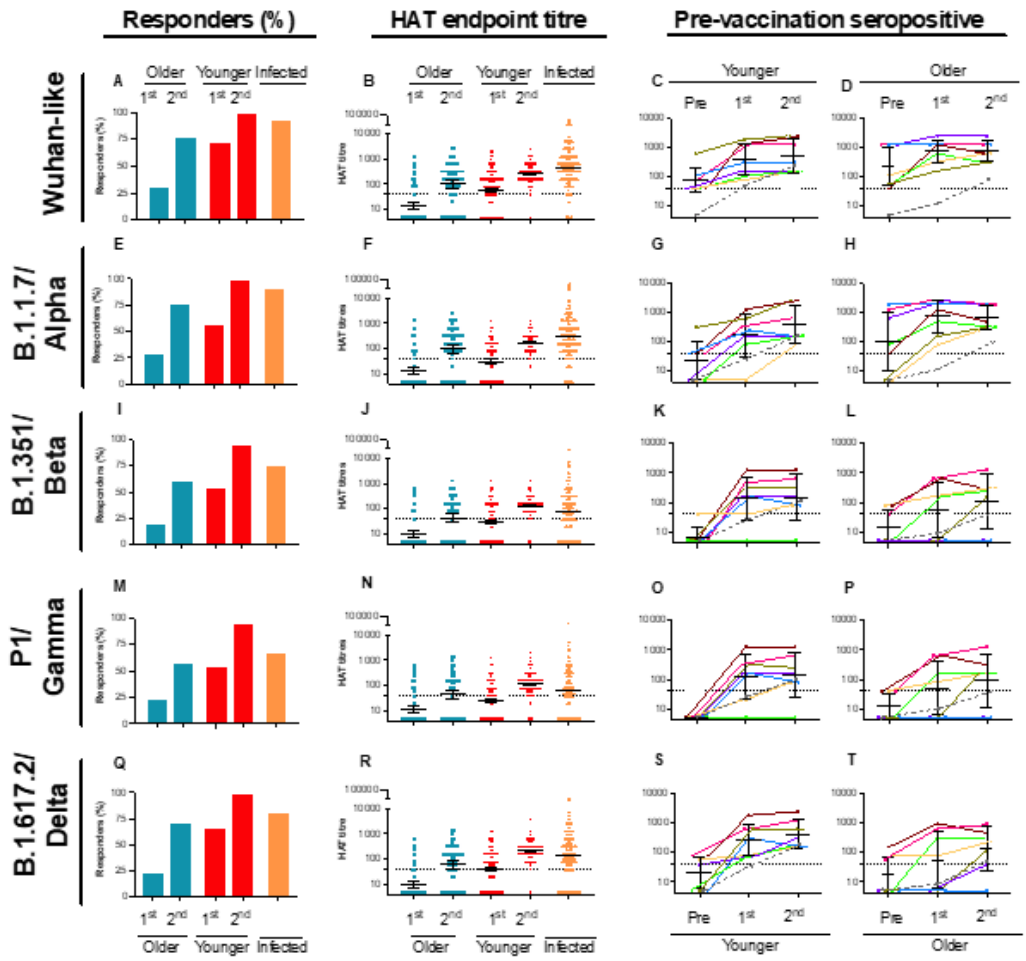

Figure 2

Haemagglutination test antibodies to the ancestral natural SARS-CoV-2 virus and variants of concern in older and younger adult vaccinees and after natural infection. Haemagglutination test (HAT) antibodies to the SARS-CoV-2 virus receptor binding domains of homologous founder virus (A-D: Wuhan-like) and variants of concern (E-H B.1.1.7 alpha; I-L: B.1.351 beta; M-P: P.1 gamma and Q-T: B.1.617.2 delta). Endpoint HAT titres are presented in B-D, F-H, J-L, N-P, R-T. The percentage of responders with haemagglutination test (HAT) titre 40 (A, E, I, M, Q) and endpoint HAT titres (B, F, J, N, R) in seronegative older (n=89) and seronegative adults (n=309) post 1st dose (3 weeks) and post 2nd dose (6-8 weeks after 1stdose) mRNA BNT162b2 COVID-19 vaccination. In infected individuals, convalescent serum was collected 3-10 weeks after SARS-CoV-2 confirmed infection (infected, n=307) with D614G virus during the first pandemic wave (A-B, E-F, I-J, M-N, Q-R). HAT endpoint titres to Wuhan-like and VOC in previously infected older individuals (n=7) and adults (n=7) who were vaccinated are shown in different colours, with the gray dashed line showing comparison of the geometric mean HAT titres for the corresponding seronegative (not previously infected) old (n=89) and adult (n=309) vaccinees (C-D, G-H, K-L, O-P, S-T). For endpoint HAT titres (B-D, F-H, J-L, N-P, R-T), negative values were assigned a value of 5. The geometric mean titres (GMT) with 95% confidence intervals are shown in black and each symbol represents one subject (B, F, J, N, R).

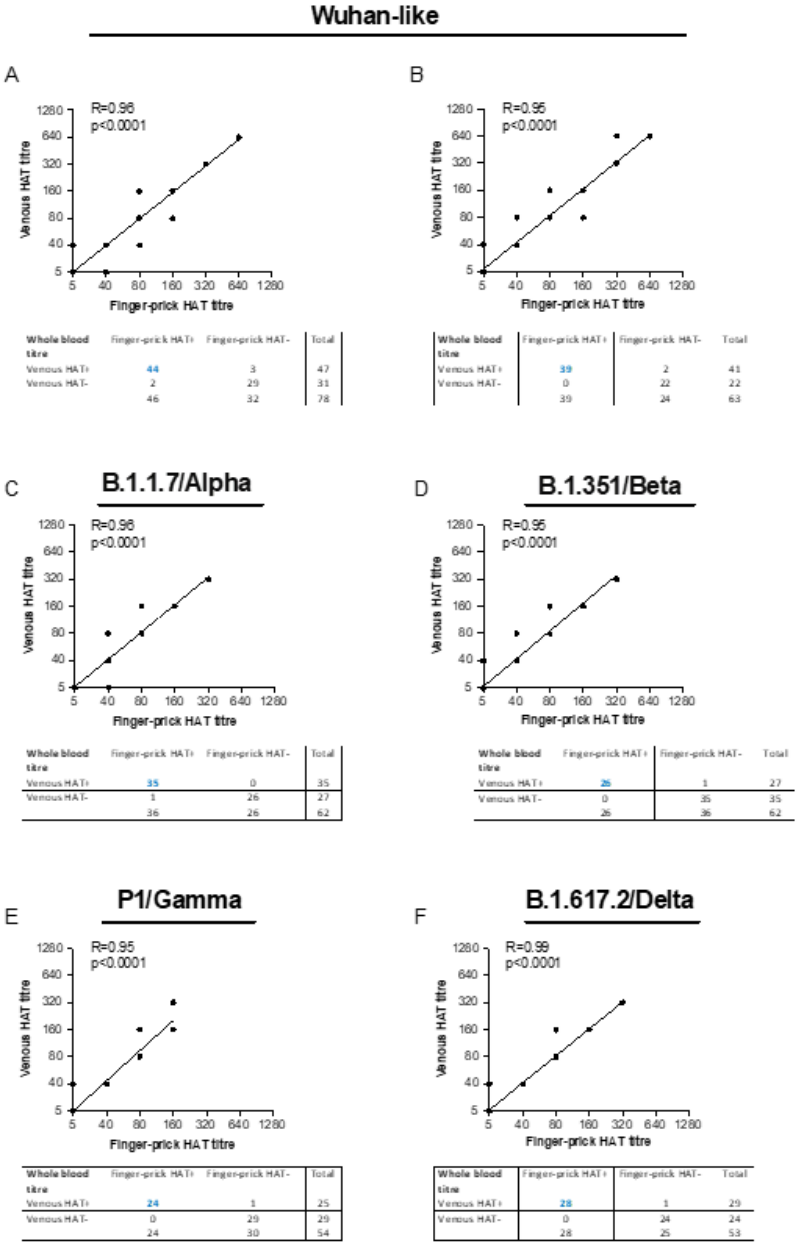

Figure 3

Comparison of finger-prick and venous blood samples using the haemagglutination test (HAT) on variants of concern Seventy-eight paired finger-prick and venous blood samples were taken from vaccinated healthcare workers. A) Correlation of finger-prick and venous whole blood samples in point HAT assay (n=78). Whole blood was diluted 1 in 40 in PBS and mixed with 50 µl 2 µg/ml IH4-RBD reagent for 1 hour and scored as shown in the contingency table. HAT + means sample resulted in haemagglutination and HAT – refers to no haemagglutination. Diluted finger-prick or venous whole blood samples (1 in 40 in PBS) were centrifuged and the supernatant was titrated in HAT assay using WT IH4-RBD and autologous RBC (washed and diluted 1 in 40 in PBS). Plates were scored after 1 hour incubation and HAT titre is the highest titration of haemagglutination. HAT titre from paired finger-prick and venous samples were plotted and the number of samples at each titre combination is reflected by the size of the purple bubbles. B-F) Finger-prick and paired venous blood were diluted 1:40, centrifuged and the supernatant was titrated in the haemagglutination test (HAT) using IH4-RBD of the ancestral Wuhan and different variants. (B: Wuhan-like (n=63), C: B1.1.7/Alpha (n=62), D: B.1.351/Beta (n=62), E:

P1/Gamma, (n=54), and F: B.1.617.2/Delta (n=53) and indicator O negative red blood cells from one donor (washed and diluted 1 in 40 in PBS as described9). HAT end point titres (the highest titration of haemagglutination) were scored after 1 hour incubation. Sample was assigned a value of 5 if there was no haemagglutination with undiluted supernatant; HAT+ is HAT titre >5; HAT- no detected haemagglutination HAT titre = 5. Correlation of HAT titres from paired finger-prick and venous samples were plotted and analysed by linear regression showed in each graph. A summary table of assay correlation between finger-prick and venous samples for each variant is shown below each graph

Supplementary Files

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