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TITLE PAGE

Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study

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ABSTRACT

Introduction

Neutralizing antibodies (NAbs) have been shown to be predictive of immune protection against SARS-CoV-2. We report the protocol for a national longitudinal study to assess and compare the level of NAb generated in response to COVID-19 vaccines in Brunei Darussalam in adults 2 to 6 weeks post-primary series (BBIBP-CorV, AZD1222, or mRNA-1273 vaccines) and their subsequent follow-up after administration of a third (booster-1) dose (BBIBP-CorV, mRNA-1273, or BNT1626b).

Methods and analysis

Participant data will be extracted and processed from the national electronic health record system ('Bru-HIMS') and the national mobile health application ('BruHealth') into a research data platform. Eligible adults who have received their primary or booster vaccine will be invited using a stratified random sampling strategy based on age, gender and vaccine type (baseline target population, n=3,000; 2 to 6 weeks post last dose). Blood serum will be isolated, and NAb level assessed using the cPass™ surrogate virus neutralization test. Baseline participants will then be screened for eligibility for subsequent longitudinal analysis. Those who have received a third dose will be followed up at 1, 3, 6, 9, and up to 12 months. NAb levels will be evaluated across the participant population according to vaccine platform/booster type, time since the last dose and correlated with demographic data. The study period is from December 2021 to January 2023 and aims to evaluate how NAb levels wane following a third vaccine dose across different vaccine platforms and determine the impact and rate of breakthrough infections.

Ethics and dissemination

This study has been approved by the Medical and Ethical Research Committee (MHREC) of the Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each participant by SMS. The findings from this study will help policymakers in Brunei develop future vaccination strategies and establish regulations across multiple agencies.

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- Three vaccine platforms used for primary series vaccination can be compared (BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT1626b (mRNA) with and without a third (booster-1) dose.
- Stratified random sampling recruitment targeting adults across all age groups (18-30; 31-40; 41-50; 51-60; 61 and above) with a target of 3,000 participants across vaccine platforms and controlled sampling time points (2 to 6 weeks post-primary series, then longitudinal follow-up at 1, 3, 6, 9, 12 months ± 1-week post the booster dose).
- Data extraction and integration from national electronic health record databases to facilitate participant recruitment and communication.

Limitations

- The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit only measures the ability of participant's antibodies to inhibit the binding of receptor-binding domain (RBD) from the ancestral SARS-CoV-2 strain (Wu01) to its ligand, human angiotensin-converting enzyme 2 (hACE2). Further, neutralizing antibodies alone are not absolute correlates of protection against infection, disease severity and death.
- Breakthrough infections are determined according to either positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) result and not individually sequenced.

INTRODUCTION

Background and rationale

Vaccination has been instrumental in emerging from the restrictions introduced globally in response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented breakthroughs in vaccine technology[1] and new platforms, e.g., viral vector (AZD1222 developed by Oxford-AstraZeneca[2]) and mRNA vaccines (mRNA-1273 from Moderna[3], and BNT1626b developed by Pfizer-BioNTech[4]) have been approved for human use.[5] Traditional whole inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by Sinopharm[6]). Immunity develops following exposure to a pathogen, and vaccines aim to mimic this by inducing a primary immune response. This results in a rapid secondary immune response to the wild-type pathogen which prevents the development of infection and/or serious illness.[7 8]

It is, therefore, of great interest to monitor immune responses to the various vaccine preparations in populations to evaluate the effectiveness of these vaccination programmes.[9] There is evidence that antibody measurements can be used as a correlate of protection post-vaccination.[10] Antibodies can be isolated from whole blood and subsequently analyzed for specificity and functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2 weeks after vaccination and can persist for several months but are likely to wane over time.[11-14]

Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein and block viral entry to host cells. High level of NAb have been correlated with survival[16 17] and appear to be the best measure of vaccine efficacy.[18] Surrogate virus neutralization assays in the form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed and validated to determine specific functional antibodies that can inhibit the interaction of viral

receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2) receptors.[15] Commercially available NAb preparations have been authorized for treatment and are particularly useful in immunocompromised individuals.[19] There is much interest in whether NAb generated by one variant (or vaccine type) can protect against future variants of concern.[20]

The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct waves. The first wave, in March 2020, was largely import-driven and successfully contained within a month. This was followed by a period of relative stability, with no domestic cases reported for over a year due to strict international travel restrictions.[21] The second wave commenced in August 2021 and was predominantly caused by the Delta variant with more than 15,000 local cases with only a small proportion of imported cases reported from August until December 2021. Subsequent waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.

Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with vaccine administration implemented in three phases. The first phase covered healthcare and frontline workers, and individuals aged 60 years and above; the second phase for individuals with chronic diseases; and the third phase for the general public comprising adults aged 18 years and above. Three different vaccine types were distributed for the primary immunization series: BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT1626b (mRNA) administered at least three months following completion of the primary series. Vaccination for adolescents aged 12 years and above commenced in November 2021 using the BNT162b2 vaccine and they are not included in this study population.

The pandemic catalyzed the development of a national mobile health application, BruHealth (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in May 2020. This provides a virtual health management platform with digital contact tracing, a premise scan QR code function in public spaces, self-test reporting and vaccination appointment capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which links primary and secondary health care data across the entire government health network. Both platforms will be utilized accordingly for the conduct of this study.

Aims and objectives

Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines in Southeast Asian populations. Thus, our study aims to assess the level of NAbs generated by primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated Bruneian population and to compare their immunogenicity. In addition, this study captures participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or BNT1626b. Durability of NAbs and rate of breakthrough infections will also be investigated over a period of one year. Furthermore, our study will allow the evaluation of the effects of a heterologous booster dose on NAb levels in the study cohort. This will provide new information on immune responses in a Southeast Asian population and provide data on any differences in NAb levels across different age groups, gender and vaccine types. This is the first instance where the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth application for the evaluation of NAb levels across the Bruneian population.

METHODS

Study design

This study is designed to assess the immune response generated in adult Bruneian population who have been vaccinated with BBIBP-CorV, AZD1222, or mRNA-1273 (primary series), and BBIBP-CorV, mRNA-1273, or BNT1626b (booster-1). NAb levels will be analyzed across the participant population according to vaccine platforms and associated with socio-demographic data. There are two phases to this study: Phase 1 is a baseline study to compare the levels of NAb induced by each primary series vaccine platform with and without a booster dose across the Bruneian population; Phase 2 is a longitudinal study to evaluate the potential effects of waning and breakthrough infection following primary series plus one booster dose on NAb levels over a period of a year. Individuals who had a confirmed COVID-19 diagnosis prior to the first blood withdrawal will be excluded from the study. The overview of the participant events and timeline is summarized in Table 1.

Study settings

Vaccinated members of the general adult population will be recruited to this study. Blood sampling and serum isolation will take place in PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam for Phase 1 (baseline) of the study and Phlebotomy Services, Central Specimen Receiving Area (CSRA), Department of Laboratory Services (DLS) at RIPAS Hospital, Brunei Darussalam for Phase 2 (longitudinal follow-up). NAb level measurement will be conducted in Duke-NUS Medical School, Singapore for Phase 1, and DLS, RIPAS Hospital, Brunei Darussalam for Phase 2.

Table 1. Participant event and timeline.

Timepoint (post-last dose)	Phase 1	Phase 2				
	2 to 6 weeks	1 month	3 months	6 months	9 months	12 months
Eligibility screening	x	x	x	x	x	x
Allocation	x	x	x	x	x	x
Informed consent	x	x	x	x	x	x
Sociodemographic data	x					
COVID-19 vaccination history	x	x	x	x	x	x
Medical history	x					
Drug history	x					
Blood samples	x	x	x	x	x	x
COVID-19 breakthrough infection history	x	x	x	x	x	x

Eligibility criteria

Inclusion criteria

Participants will be included in the study if they meet the following criteria:

1. Aged 18 years and above.
2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an additional booster dose (BBIBP-CorV, mRNA-1273, or BNT1626b) for Phase 1. Phase 2 will follow up on participants who have received a booster dose over a period of one year.

Exclusion criteria

Participants will be excluded from the study if they meet any of the following criteria:

1. History of travel between the first dose and first blood withdrawal in Phase 1.
2. History of COVID-19 infection before the first blood withdrawal in Phase 1.

Outcomes

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Presence of NAbs can be categorized as either positive (> 30%) or negative (< 30%) based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be analysed across the participant population according to vaccine platform/booster type, time since the last dose and associated with socio-demographic data. This analysis will advise policymakers in Brunei on future vaccination strategies and establishing regulations across multiple agencies.

Sample size

The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222, and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly selected, stratified by four categories: (1) the three primary vaccination series platform; (2) gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after receiving the primary or booster doses. Considering the outcome variable for NAb measurement as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an expected positive rate of 88%, though the positive rate is expected to be much higher. The sample size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate, a sample size of 100 could detect a difference of 5% between two groups, with a power of 99% for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher positive rate e.g., at least higher than 80%, the targeted sample size is considered more than adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine category will achieve a precision of at least 20% of standard deviation (a small effect size) in estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose). In comparing groups of n = 160 for each category, a significant result can be obtained with a mean difference of at least 36% of standard deviation (small to medium effect size), with a power of 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software (version 3.1.6).

Recruitment and allocation

Eligible participants who are within 2 to 6 weeks after their last vaccination will be identified from the BruHealth database and invited on a weekly basis in the Phase 1 study. The Phase 2 study will follow up on participants who had received the third booster dose and sampled at 1, 3, 6, 9, and up to 12 months following the booster dose. The number of participants invited for both study phases is dependent on the target recruitment according to the maximum capacity of the blood sampling center, with an assumed response rate evaluated on a weekly basis. A balance in sample size for each stratum by age group, gender and vaccine platform will be included in the weekly target.

Potential participants will be sent three text messages for recruitment into the study in both English (Table 2) and native Malay languages. The first text message will be an invitation to participate in the study. The second text message acts as a reminder and will be sent the following day to prompt response to the invitation. Both text messages will contain a blood withdrawal appointment date and time with a link to an online form (Qualtrics XM, Qualtrics International Inc., USA), participant information sheet (PIS) and an option to agree or disagree to join the study. Those who agree will be sent a third text message 24 hours before their allocated appointment date. Individuals who do not agree will not be included in the study.

Table 2. Text message template for recruitment of study participants.

Text Message Type	Text content
First (Invitation)	[MOH] IC:01234567. You are invited to the next phase of the vaccine research study on dd/mm/yy at hh:mm - hh:mm. This will measure the status of your antibody level after vaccination. Please respond at bit.ly/abcdefg by Wednesday, 5:00 PM. Thank you.
Second (Invitation Reminder)	[MOH] IC:01234567. dd/mm/yy hh:mm - hh:mm. An antibody test is reserved for you next week. Please confirm your attendance at bit.ly/abcdefg by Wednesday, 05:00 PM. Thank you for your support. Please disregard this message if you have already responded.
Third (Appointment Reminder)	[MOH] IC:01234567. Reminder for your appointment tomorrow at Central Receiving Area (CSRA), RIPAS Hospital on dd/mm/yy at hh:mm - hh:mm. Location: bit.ly/tuvwxyz.

MOH, Ministry of Health; IC, Identity Card number; dd/mm/yy, allocated date; hh:mm, allocated time; bit.ly/abcdefg, custom link to online form; bit.ly/tuvwxyz, custom link for directions to blood sampling center.

Blinding mechanism

All data will be anonymized and each participant will be given a unique identifier following agreement to participate and recruitment. Identifying information is required to verify participant details at the time of blood sampling only. Otherwise, this information will not be included in any sample labels or data sharing.

Data collection

Study procedures and evaluations

Participant identities will be verified on-site at the blood sampling center. The PIS containing the study design and purpose will be explained to all participants. Written consent will be subsequently obtained prior to sampling. Socio-demographic data including age, gender, ethnicity, co-morbidities and immunosuppressive drug use will be enquired and recorded in a secured online platform (EVYDResearch, EVYD Technology Limited, Singapore). For Phase 2, participants with history of COVID-19 breakthrough infection (determined via either positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) results), if any, will be documented.

Blood sampling and serum analysis

A whole blood sample of 5 mL will be drawn from each participant by venipuncture and allowed to incubate at room temperature for 15 to 30 minutes to allow clotting. Serum will be separated by centrifugation at 1,000 – 2,000 x g for 10 minutes. Serum samples will be isolated and stored at -20°C.

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Briefly, 20 µL of serum sample, positive and negative controls will be diluted in the buffer (volume ratio of 1:9) and an equal volume of horseradish peroxidase (HRP)-RBD solution will be added. The mixture will be incubated at 37°C for 30 minutes before being added to a capture plate which will be further incubated for 15 minutes at 37°C. The well plate will be washed using a diluted washing buffer solution four times and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) solution will be added to each well. The well plate will be incubated in the dark at room temperature for 15 minutes, before the addition of 50 µL of stop solution to each well to stop the enzymatic reaction. The absorbance value (optical density, OD) of each well will be read using a microplate reader at 450 nm wavelength. The level of NAb in the serum samples will be measured by calculating the percentage inhibition as shown below. Serum samples with inhibition of at least 30% will be considered as positive for the presence of NAbs,[15] as stated in the kit instructions. Percentage inhibition will be converted to international units per milliliter (IU/mL) as previously described.[22]

$$\% \text{ Signal inhibition } = \left(1 - \frac{\text{OD value of serum sample}}{\text{OD value of negative control}}\right) \text{x } 100\%$$

The results of the NAb measurement as positive or negative and in IU/mL will be disseminated to all participants via a text message. A custom link for additional information on the interpretation of NAb levels will be included. Contact details of a medical doctor will be provided should the participant require further information and to address any enquiry.

Retention

All data recorded and stored on the online platform (EVYDResearch) will only be stored for the duration of the study. Following cessation of the study, data will be securely deleted and disposed of in accordance to EVYD Technology Limited's Data Retention Framework.

Data management and confidentiality

Data confidentiality will be maintained through the online platform and only the research team will have access to full participant information. MOH, Brunei Darussalam will retain ownership of the participant data on Bru-HIMS and BruHealth and hold the rights to grant access to authorized personnel for the purposes of conducting the research. EVYDResearch platform follows the data security and privacy requirement as set forth by the MOH, Brunei Darussalam. There is a management system for the platform administrator to distribute or withdraw permission for authorized users. The platform administrator will grant access to the relevant fields as permitted by MOH, Brunei Darussalam. Authorized users will be required to sign the Officials Secret Act and to maintain participant confidentiality at all times. The platform strictly abides by the Data Privacy Policy in the collection, usage and disclosure of participant data. In accordance with Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, all identifiers of the individual will be removed using the Safe Harbor method.

Statistical methods

Socio-demographic variables, medical and drug history will be used to produce visualization plots and descriptive statistics and simple univariate analyses will be performed. Statistical software including RStudio (RStudio, MA, USA) and GraphPad Prism (GraphPad, CA, USA) may be used when necessary for advanced statistical analyses and data visualization.

The NAb level elicited by each vaccine platform or subgroup (e.g., by post-vaccination week, by gender, by age group) will be presented with 95% confidence interval (CI) of proportion analysis. Comparison of these positive rates will be analyzed by binomial regression using binreg R package (RStudio) and presented with positive rate ratios, their 95% CIs and P values. P value of less than 0.05 will be considered statistically significant.

Patient and Public Involvement

No patient involved.

ETHICS AND DISSEMINATION

Research ethics approval

This study is approved by the Medical and Ethical Research Committee (MHREC) of the MOH, Brunei Darussalam (Reference No: MHREC/MOH/2021/14(1) dated 18th November 2021). The study will be conducted in conformance with the Good Clinical and Laboratory Practice.

Informed consent process

All participants will give written informed consent to participate in this study. PIS will be provided to all participants in their preferred language (English, Malay or Chinese). The PIS will contain all relevant information relating to the study, potential risks and benefits, confidentiality and contact details should further information be required. The PIS will also be explained verbally on-site by research assistants and queries will be addressed. Participants will acknowledge that they

understand the PIS and are free to withdraw from the study at any time. They will agree to their medical records being accessed and their blood being withdrawn for the purpose of this study. In addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for better antibody measurement techniques or against future emerging SARS-CoV-2 variants. Participants may agree to the first (current study) but not the second (future relevant studies), if they wish. A physical, hard copy signed consent form will be collected from each participant prior to blood withdrawal. No remuneration will be provided in exchange for their participation.

Declaration of interests

CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other authors declare no conflict of interests.

Access to data

Collected data will be secured against unauthorized access and will be stored securely on the online platform.

Dissemination

Study findings will be disseminated to all participants. The results of the study will be submitted for publications in peer-reviewed journals as they become available. The data will also be presented internally to MOH, Brunei Darussalam, universities and in national and international scientific conferences and seminars.

STUDY ADMINISTRATION

Funders

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Roles and responsibilities

Protocol contributors

All authors have contributed to the study design and conceptualization, writing and critical revision of the manuscript. HG, LA, HS, MFA, SB, ST, CYS, YW, SYC, YW, JW, FI, LN, and ACC will manage the recruitment of study participants, data coordination and blood withdrawal. CWT, XMO, FZ, and LFW will manage NAb measurement. All authors read and approved the final manuscript.

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Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study

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TITLE PAGE

Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study

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ABSTRACT

Introduction

Neutralizing antibodies (NAbs) have been shown to be correlative of immune protection against SARS-CoV-2. We report the protocol for a national longitudinal study to assess and compare the level of NAb generated in response to COVID-19 vaccines in Brunei Darussalam in adults 2 to 6 weeks post-primary series (BBIBP-CorV, AZD1222, or mRNA-1273 vaccines) and their subsequent follow-up after administration of a third (booster-1) dose (BBIBP-CorV, mRNA-1273, or BNT162b2).

Methods and analysis

Participant data will be extracted and processed from the national electronic health record system ('Bru-HIMS') and the national mobile health application ('BruHealth') into a research data platform. Eligible adults who have received their primary or booster vaccine will be invited using a stratified random sampling strategy based on age, gender and vaccine type (baseline target population, n=3,000; 2 to 6 weeks post last dose). Blood serum will be isolated, and NAb level assessed using the cPass™ surrogate virus neutralization test. Baseline participants will then be screened for eligibility for subsequent longitudinal analysis. Those who have received a third dose will be followed up at 1, 3, 6, 9, and up to 12 months. NAb levels will be evaluated across the participant population according to vaccine platform/booster type, time since the last dose and correlated with demographic data. The study period is from December 2021 to January 2023 and aims to evaluate how NAb levels wane following a third vaccine dose across different vaccine platforms and determine the impact and rate of breakthrough infections.

Ethics and dissemination

This study has been approved by the Medical and Ethical Research Committee (MHREC) of the Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each participant by SMS. The findings from this study will help policymakers in Brunei develop future vaccination strategies and establish regulations across multiple agencies.

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- Three vaccine platforms used for primary series vaccination can be compared (BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT162b2 (mRNA) with and without a third (booster-1) dose.
- Stratified random sampling recruitment targeting adults across all age groups (18-30; 31-40; 41-50; 51-60; 61 and above) with a target of 3,000 participants across vaccine platforms and controlled sampling time points (2 to 6 weeks post-primary series, then longitudinal follow-up at 1, 3, 6, 9, 12 months ± 1-week post the booster dose).
- Data extraction and integration from national electronic health record databases to facilitate participant recruitment and communication.

Limitations

- The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit only measures the ability of participant's antibodies to inhibit the binding of receptor-binding domain (RBD) from the ancestral SARS-CoV-2 strain (Wu01) to its ligand, human angiotensin-converting enzyme 2 (hACE2). Further, neutralizing antibodies alone are not absolute correlates of protection against infection, disease severity and death.
- Pre-vaccination blood serum sampling was not performed to indicate potential neutralizing antibodies present in participants. Moreover, comparisons of homologous vaccinations using AZD1222 and BNT162b2 were limited due to the difference in vaccine platforms distributed in the primary series and booster vaccinations.
- Breakthrough infections are determined according to either positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) result and not individually sequenced. Hence,

there will be limited information on the specific SARS-CoV-2 variants in the breakthrough infection cohort.

INTRODUCTION

Background and rationale

Vaccination has been instrumental in emerging from the restrictions introduced globally in response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented breakthroughs in vaccine technology [1] and new platforms, e.g., viral vector (AZD1222 developed by Oxford-AstraZeneca [2]) and mRNA vaccines (mRNA-1273 from Moderna [3], and BNT162b2 developed by Pfizer-BioNTech [4]) have been approved for human use [5]. Traditional whole inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by Sinopharm [6]). Immunity develops following exposure to a pathogen, and vaccines aim to mimic this by inducing a primary immune response. This results in a rapid secondary immune response to the wild-type pathogen which prevents the development of infection and/or serious illness [7], [8].

It is, therefore, of great interest to monitor immune responses to the various vaccine preparations in populations to evaluate the effectiveness of these vaccination programmes [9]. There is evidence that antibody measurements can be used as a correlate of protection post-vaccination [10]. Antibodies can be isolated from whole blood and subsequently analyzed for specificity and functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2 weeks after vaccination and can persist for several months but are likely to wane over time [11], [12], [13], [14].

Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein and block viral entry to host cells. High levels of NAb have been correlated with survival [15], [16] and appear to be the best measure of vaccine efficacy [17]. Surrogate virus neutralization assays

in the form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed and validated to determine specific functional antibodies that can inhibit the interaction of viral receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2) receptors [18]. Commercially available NAb preparations have been authorized for treatment and are particularly useful in immunocompromised individuals [19]. There is much interest in whether NAb generated by one variant (or vaccine type) can protect against future variants of concern [20].

The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct waves. The first wave, in March 2020, was largely import-driven and successfully contained within a month. This was followed by a period of relative stability, with no domestic cases reported for over a year due to strict international travel restrictions [21]. The second wave commenced in August 2021 and was predominantly caused by the Delta variant with more than 15,000 local cases with only a small proportion of imported cases reported from August until December 2021. Subsequent waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.

Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with vaccine administration implemented in three phases. The first phase covered healthcare and frontline workers, and individuals aged 60 years and above; the second phase for individuals with chronic diseases; and the third phase for the general public comprising adults aged 18 years and above. Three different vaccine types were distributed for the primary immunization series: BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT162b2 (mRNA) administered at least three months following completion of the primary series.

Vaccination for adolescents aged 12 years and above commenced in November 2021 using the BNT162b2 vaccine and they are not included in this study population.

The pandemic catalyzed the development of a national mobile health application, BruHealth (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in May 2020. This provides a virtual health management platform with digital contact tracing, a premise scan QR code function in public spaces, self-test reporting and vaccination appointment capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which links primary and secondary health care data across the entire government health network. Both platforms will be utilized accordingly for the conduct of this study.

Aims and objectives

Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines in Southeast Asian populations. Thus, our study aims to assess the level of NAbs generated by primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated Bruneian population and to compare their immunogenicity. In addition, this study captures participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or BNT162b2. Durability of NAbs and rate of breakthrough infections will also be investigated over a period of one year. Furthermore, our study will allow the evaluation of the effects of a heterologous booster dose on NAb levels in the study cohort. This will provide new information on immune responses in a Southeast Asian population and provide data on any differences in NAb levels across different age groups, gender and vaccine types. This is the first instance where the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth application for the evaluation of NAb levels across the Bruneian population.

METHODS

Study design

This study is designed to assess the immune response generated in adult Bruneian population who have been vaccinated with BBIBP-CorV, AZD1222, or mRNA-1273 (primary series), and BBIBP-CorV, mRNA-1273, or BNT162b2 (booster-1). NAb levels will be analyzed across the participant population according to vaccine platforms and associated with socio-demographic data. There are two phases to this study: Phase 1 is a baseline study to compare the levels of NAb induced by each primary series vaccine platform with and without a booster dose across the Bruneian population; Phase 2 is a longitudinal study to evaluate the potential effects of waning and breakthrough infection following primary series plus one booster dose on NAb levels over a period of a year. Individuals who had a confirmed COVID-19 diagnosis prior to the first blood withdrawal will be excluded from the study. The overview of the participant events and timeline is summarized in Table 1.

Study settings

Vaccinated members of the general adult population will be recruited to this study. Blood sampling and serum isolation will take place in PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam for Phase 1 (baseline) of the study and Phlebotomy Services, Central Specimen Receiving Area (CSRA), Department of Laboratory Services (DLS) at RIPAS Hospital, Brunei Darussalam for Phase 2 (longitudinal follow-up). NAb level measurement will be conducted in Duke-NUS Medical School, Singapore for Phase 1, and DLS, RIPAS Hospital, Brunei Darussalam for Phase 2.

172 **Table 1.** Participant event and timeline.

Timepoint (post-last dose)	Phase 1	Phase 2				
	2 to 6 weeks	1 month	3 months	6 months	9 months	12 months
Eligibility screening	x	x	x	x	x	x
Allocation	x	x	x	x	x	x
Informed consent	x	x	x	x	x	x
Sociodemographic data	x					
COVID-19 vaccination history	x	x	x	x	x	x
Medical history	x					
Drug history	x					
Blood samples	x	x	x	x	x	x
COVID-19 breakthrough infection history	x	x	x	x	x	x

Eligibility criteria

Inclusion criteria

Participants will be included in the study if they meet the following criteria:

1. Aged 18 years and above.
2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an additional booster dose (BBIBP-CorV, mRNA-1273, or BNT162b2) for Phase 1. Phase 2 will follow up on participants who have received a booster dose over a period of one year.

Exclusion criteria

Participants will be excluded from the study if they meet any of the following criteria:

1. History of travel between the first dose and first blood withdrawal in Phase 1.
2. History of COVID-19 infection before the first blood withdrawal in Phase 1.

Outcomes

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Presence of NAbs can be categorized as either positive (> 30%) or negative (< 30%) based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be analysed across the participant population according to vaccine platform/booster type, time since the last dose and associated with socio-demographic data. This analysis will advise policymakers in Brunei on future vaccination strategies and establishing regulations across multiple agencies.

Sample size

The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222, and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly selected, stratified by four categories: (1) the three primary vaccination series platform; (2) gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after receiving the primary or booster doses. Considering the outcome variable for NAb measurement as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an expected positive rate of 88%, though the positive rate is expected to be much higher. The sample size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate, a sample size of 100 could detect a difference of 5% between two groups, with a power of 99% for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher positive rate e.g., at least higher than 80%, the targeted sample size is considered more than adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine category will achieve a precision of at least 20% of standard deviation (a small effect size) in estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose). In comparing groups of n = 160 for each category, a significant result can be obtained with a mean difference of at least 36% of standard deviation (small to medium effect size), with a power of 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software (version 3.1.6).

Recruitment and allocation

Eligible participants who are within 2 to 6 weeks after their last vaccination will be identified from the BruHealth database and invited on a weekly basis in the Phase 1 study. The Phase 2 study will follow up on participants who had received the third booster dose and sampled at 1, 3, 6, 9, and up to 12 months following the booster dose. The number of participants invited for both study phases is dependent on the target recruitment according to the maximum capacity of the blood sampling center, with an assumed response rate evaluated on a weekly basis. A balance in sample size for each stratum by age group, gender and vaccine platform will be included in the weekly target.

Potential participants will be sent three text messages for recruitment into the study in both English (Table 2) and native Malay languages. The first text message will be an invitation to participate in the study. The second text message acts as a reminder and will be sent the following day to prompt response to the invitation. Both text messages will contain a blood withdrawal appointment date and time with a link to an online form (Qualtrics XM, Qualtrics International Inc., USA), participant information sheet (PIS) and an option to agree or disagree to join the study. Those who agree will be sent a third text message 24 hours before their allocated appointment date. Individuals who do not agree will not be included in the study.

Table 2. Text message template for recruitment of study participants.

Text Message Type	Text content
First (Invitation)	[MOH] IC:01234567. You are invited to the next phase of the vaccine research study on dd/mm/yy at hh:mm - hh:mm. This will measure the status of your antibody level after vaccination. Please respond at bit.ly/abcdefg by Wednesday, 5:00 PM. Thank you.
Second (Invitation Reminder)	[MOH] IC:01234567. dd/mm/yy hh:mm - hh:mm. An antibody test is reserved for you next week. Please confirm your attendance at bit.ly/abcdefg by Wednesday, 05:00 PM. Thank you for your support. Please disregard this message if you have already responded.
Third (Appointment Reminder)	[MOH] IC:01234567. Reminder for your appointment tomorrow at Central Receiving Area (CSRA), RIPAS Hospital on dd/mm/yy at hh:mm - hh:mm. Location: bit.ly/tuvwxyz.

MOH, Ministry of Health; IC, Identity Card number; dd/mm/yy, allocated date; hh:mm, allocated time; bit.ly/abcdefg, custom link to online form; bit.ly/tuvwxyz, custom link for directions to blood sampling center.

Blinding mechanism

All data will be anonymized and each participant will be given a unique identifier following agreement to participate and recruitment. Identifying information is required to verify participant details at the time of blood sampling only. Otherwise, this information will not be included in any sample labels or data sharing.

Data collection

Study procedures and evaluations

Participant identities will be verified on-site at the blood sampling center. The PIS containing the study design and purpose will be explained to all participants. Written consent will be subsequently obtained prior to sampling. Socio-demographic data including age, gender, ethnicity, co-morbidities (i.e., diabetes mellitus, chronic kidney disease, hypertension, ischemic heart disease and cancer) and immunosuppressive drug use will be enquired and recorded in a secured online platform (EVYDResearch, EVYD Technology Limited, Singapore). For Phase 2, participants with history of COVID-19 breakthrough infection (determined via either positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) results), if any, will be documented. The symptoms and severity of breakthrough infections in these participants may be analyzed and compared to those without breakthrough infections.

Blood sampling and serum analysis

A whole blood sample of 5 mL will be drawn from each participant by venipuncture and allowed to incubate at room temperature for 15 to 30 minutes to allow clotting. Serum will be separated

by centrifugation at 1,000 – 2,000 x g for 10 minutes. Serum samples will be isolated and stored at -20°C.

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Briefly, 20 µL of serum sample, positive and negative controls will be diluted in the buffer (volume ratio of 1:9) and an equal volume of horseradish peroxidase (HRP)-RBD solution will be added. The mixture will be incubated at 37°C for 30 minutes before being added to a capture plate which will be further incubated for 15 minutes at 37°C. The well plate will be washed using a diluted washing buffer solution four times and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) solution will be added to each well. The well plate will be incubated in the dark at room temperature for 15 minutes, before the addition of 50 µL of stop solution to each well to stop the enzymatic reaction. The absorbance value (optical density, OD) of each well will be read using a microplate reader at 450 nm wavelength. The level of NAb in the serum samples will be measured by calculating the percentage signal inhibition as shown below. Serum samples with inhibition of at least 30% will be considered as positive for the presence of NAbs [18], as stated in the kit instructions. Percentage inhibition will be converted to antibody titers expressed in international units per milliliter (IU/mL) using a WHO International Standard conversion tool [22]. Antibody titers will be log-transformed before statistical analyses and presented as geometric mean titers (GMT).

$$\% \text{ Signal inhibition } = \left(1 - \frac{\text{OD value of serum sample}}{\text{OD value of negative control}}\right) \text{x } 100\%$$

The results of the NAb measurement as positive or negative and in IU/mL will be disseminated to all participants via a text message. A custom link for additional information on the interpretation

of NAb levels will be included. Contact details of a medical doctor will be provided should the participant require further information and to address any enquiry.

Retention

All data recorded and stored on the online platform (EVYDResearch) will only be stored for the duration of the study. Following cessation of the study, data will be securely deleted and disposed of in accordance to EVYD Technology Limited's Data Retention Framework.

Data management and confidentiality

Data confidentiality will be maintained through the online platform and only the research team will have access to full participant information. MOH, Brunei Darussalam will retain ownership of the participant data on Bru-HIMS and BruHealth and hold the rights to grant access to authorized personnel for the purposes of conducting the research. EVYDResearch platform follows the data security and privacy requirement as set forth by the MOH, Brunei Darussalam. There is a management system for the platform administrator to distribute or withdraw permission for authorized users. The platform administrator will grant access to the relevant fields as permitted by MOH, Brunei Darussalam. Authorized users will be required to sign the Officials Secret Act and to maintain participant confidentiality at all times. The platform strictly abides by the Data Privacy Policy in the collection, usage and disclosure of participant data. In accordance with Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, all identifiers of the individual will be removed using the Safe Harbor method.

Statistical methods

Socio-demographic variables, medical and drug history will be used to produce visualization plots and descriptive statistics and simple univariate analyses will be performed. Statistical software including RStudio (RStudio, MA, USA) and GraphPad Prism (GraphPad, CA, USA) may be used when necessary for advanced statistical analyses and data visualization.

The NAb level, based on percentage inhibition and GMT, elicited by each vaccine platform or subgroup (e.g., by post-vaccination week, by gender, by age group) will be analyzed using the Mann-Whitney U and Kruskal-Wallis tests with 95% confidence interval (CI). Comparison of positive rates will be analyzed by binomial regression using binreg R package (RStudio) and presented with positive rate ratios, their 95% CIs and P values. P value of less than 0.05 will be considered statistically significant.

Patient and Public Involvement

No patient involved.

ETHICS AND DISSEMINATION

Research ethics approval

This study is approved by the Medical and Ethical Research Committee (MHREC) of the MOH, Brunei Darussalam (Reference No: MHREC/MOH/2021/14(1) dated 18th November 2021). The study will be conducted in conformance with the Good Clinical and Laboratory Practice.

Informed consent process

All participants will give written informed consent to participate in this study. PIS will be provided to all participants in their preferred language (English, Malay or Chinese). The PIS will contain all relevant information relating to the study, potential risks and benefits, confidentiality and contact details should further information be required. The PIS will also be explained verbally on-site by

research assistants and queries will be addressed. Participants will acknowledge that they understand the PIS and are free to withdraw from the study at any time. They will agree to their medical records being accessed and their blood being withdrawn for the purpose of this study. In addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for better antibody measurement techniques or against future emerging SARS-CoV-2 variants. Participants may agree to the first (current study) but not the second (future relevant studies), if they wish. A physical, hard copy signed consent form will be collected from each participant prior to blood withdrawal. No remuneration will be provided in exchange for their participation.

Access to data

Collected data will be secured against unauthorized access and will be stored securely on the online platform.

Dissemination

Study findings will be disseminated to all participants. The results of the study will be submitted for publications in peer-reviewed journals as they become available. The data will also be presented internally to MOH, Brunei Darussalam, universities and in national and international scientific conferences and seminars.

STATEMENTS

Contributorship

All authors have contributed to the study design and conceptualization, writing and critical revision of the manuscript. HG, LA, HS, MFA, SB, ST, CYS, YW, SYC, YW, JW, FI, LN, and ACC will manage the recruitment of study participants, data coordination and blood withdrawal. CWT, XMO, FZ, and LFW will manage NAb measurement. All authors read and approved the final manuscript.

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Competing of Interests

CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other authors declare no conflict of interests.

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Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study

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TITLE PAGE

Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in the Bruneian population: Protocol for a national longitudinal study

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ABSTRACT

Introduction

Neutralizing antibodies (NAbs) have been shown to be correlative of immune protection against SARS-CoV-2. We report the protocol for a national longitudinal study to assess and compare the level of NAb generated in response to COVID-19 vaccines in Brunei Darussalam in adults 2 to 6 weeks post-primary series (BBIBP-CorV, AZD1222, or mRNA-1273 vaccines) and their subsequent follow-up after administration of a third (booster-1) dose (BBIBP-CorV, mRNA-1273, or BNT162b2).

Methods and analysis

Participant data will be extracted and processed from the national electronic health record system ('Bru-HIMS') and the national mobile health application ('BruHealth') into a research data platform. Eligible adults who have received their primary or booster vaccine will be invited using a stratified random sampling strategy based on age, gender and vaccine type (baseline target population, n=3,000; 2 to 6 weeks post last dose). Blood serum will be isolated, and NAb level assessed using the cPass™ surrogate virus neutralization test. Baseline participants will then be screened for eligibility for subsequent longitudinal analysis. Those who have received a third dose will be followed up at 1, 3, 6, 9, and up to 12 months. NAb levels will be evaluated across the participant population according to vaccine platform/booster type, time since the last dose and correlated with demographic data. The study period is from December 2021 to January 2023 and aims to evaluate how NAb levels wane following a third vaccine dose across different vaccine platforms and determine the impact and rate of breakthrough infections.

Ethics and dissemination

This study has been approved by the Medical and Ethical Research Committee (MHREC) of the Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each participant by SMS. The findings from this study will help policymakers in Brunei develop future vaccination strategies and establish regulations across multiple agencies.

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- Three vaccine platforms used for primary series vaccination can be compared (BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT1626b2 (mRNA) with and without a third (booster-1) dose.
- Stratified random sampling and recruitment of adults across five age groups with a target of 3,000 participants across vaccine platforms and controlled sampling time points.

Limitations

- Neutralizing antibodies alone are not absolute correlates of protection against infection, disease severity and death, and the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit only measures antibodies against the ancestral SARS-CoV-2 strain (Wu01).
- Limited information on pre-vaccination neutralizing antibody levels and immunogenicity generated from homologous vaccinations using AZD1222 and BNT162b2.
- Breakthrough infections are determined based on positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) results and are not individually sequenced, which restricts data on the causative SARS-CoV-2 variant.

INTRODUCTION

Background and rationale

Vaccination has been instrumental in emerging from the restrictions introduced globally in response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented breakthroughs in vaccine technology [1] and new platforms, e.g., viral vector (AZD1222 developed by Oxford-AstraZeneca [2]) and mRNA vaccines (mRNA-1273 from Moderna [3], and BNT162b2 developed by Pfizer-BioNTech [4]) have been approved for human use [5]. Traditional whole inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by Sinopharm [6]). Immunity develops following exposure to a pathogen, and vaccines aim to mimic this by inducing a primary immune response. This results in a rapid secondary immune response to the wild-type pathogen which prevents the development of infection and/or serious illness [7], [8].

It is, therefore, of great interest to monitor immune responses to the various vaccine preparations in populations to evaluate the effectiveness of these vaccination programmes [9]. There is evidence that antibody measurements can be used as a correlate of protection post-vaccination [10]. Antibodies can be isolated from whole blood and subsequently analyzed for specificity and functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2 weeks after vaccination and can persist for several months but are likely to wane over time [11], [12], [13], [14].

Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein and block viral entry to host cells. High levels of NAb have been correlated with survival [15], [16] and appear to be the best measure of vaccine efficacy [17]. Surrogate virus neutralization assays

in the form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed and validated to determine specific functional antibodies that can inhibit the interaction of viral receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2) receptors [18]. Commercially available NAb preparations have been authorized for treatment and are particularly useful in immunocompromised individuals [19]. There is much interest in whether NAb generated by one variant (or vaccine type) can protect against future variants of concern [20].

The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct waves. The first wave, in March 2020, was largely import-driven and successfully contained within a month. This was followed by a period of relative stability, with no domestic cases reported for over a year due to strict international travel restrictions [21]. The second wave commenced in August 2021 and was predominantly caused by the Delta variant with more than 15,000 local cases with only a small proportion of imported cases reported from August until December 2021. Subsequent waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.

Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with vaccine administration implemented in three phases. The first phase covered healthcare and frontline workers, and individuals aged 60 years and above; the second phase for individuals with chronic diseases; and the third phase for the general public comprising adults aged 18 years and above. Three different vaccine types were distributed for the primary immunization series: BBIBP-CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT162b2 (mRNA) administered at least three months following completion of the primary series.

Vaccination for adolescents aged 12 years and above commenced in November 2021 using the BNT162b2 vaccine and they are not included in this study population.

The pandemic catalyzed the development of a national mobile health application, BruHealth (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in May 2020. This provides a virtual health management platform with digital contact tracing, a premise scan QR code function in public spaces, self-test reporting and vaccination appointment capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which links primary and secondary health care data across the entire government health network. Both platforms will be utilized accordingly for the conduct of this study.

Aims and objectives

Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines in Southeast Asian populations. Thus, our study aims to assess the level of NAbs generated by primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated Bruneian population and to compare their immunogenicity. In addition, this study captures participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or BNT162b2. Durability of NAbs and rate of breakthrough infections will also be investigated over a period of one year. Furthermore, our study will allow the evaluation of the effects of a heterologous booster dose on NAb levels in the study cohort. This will provide new information on immune responses in a Southeast Asian population and provide data on any differences in NAb levels across different age groups, gender and vaccine types. This is the first instance where the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth application for the evaluation of NAb levels across the Bruneian population.

METHODS

Study design

This study is designed to assess the immune response generated in adult Bruneian population who have been vaccinated with BBIBP-CorV, AZD1222, or mRNA-1273 (primary series), and BBIBP-CorV, mRNA-1273, or BNT162b2 (booster-1). NAb levels will be analyzed across the participant population according to vaccine platforms and associated with socio-demographic data. There are two phases to this study: Phase 1 is a baseline study to compare the levels of NAb induced by each primary series vaccine platform with and without a booster dose across the Bruneian population; Phase 2 is a longitudinal study to evaluate the potential effects of waning and breakthrough infection following primary series plus one booster dose on NAb levels over a period of a year. Individuals who had a confirmed COVID-19 diagnosis prior to the first blood withdrawal will be excluded from the study. The overview of the participant events and timeline is summarized in Table 1.

Study settings

Vaccinated members of the general adult population will be recruited to this study. Blood sampling and serum isolation will take place in PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam for Phase 1 (baseline) of the study and Phlebotomy Services, Central Specimen Receiving Area (CSRA), Department of Laboratory Services (DLS) at RIPAS Hospital, Brunei Darussalam for Phase 2 (longitudinal follow-up). NAb level measurement will be conducted in Duke-NUS Medical School, Singapore for Phase 1, and DLS, RIPAS Hospital, Brunei Darussalam for Phase 2.

Table 1. Participant event and timeline.

Timepoint (post-last dose)	Phase 1	Phase 2				
	2 to 6 weeks	1 month	3 months	6 months	9 months	12 months
Eligibility screening	x	x	x	x	x	x
Allocation	x	x	x	x	x	x
Informed consent	x	x	x	x	x	x
Sociodemographic data	x					
COVID-19 vaccination history	x	x	x	x	x	x
Medical history	x					
Drug history	x					
Blood samples	x	x	x	x	x	x
COVID-19 breakthrough infection history	x	x	x	x	x	x

Eligibility criteria

Inclusion criteria

Participants will be included in the study if they meet the following criteria:

1. Aged 18 years and above.
2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an additional booster dose (BBIBP-CorV, mRNA-1273, or BNT162b2) for Phase 1. Phase 2 will follow up on participants who have received a booster dose over a period of one year.

Exclusion criteria

Participants will be excluded from the study if they meet any of the following criteria:

1. History of travel between the first dose and first blood withdrawal in Phase 1.
2. History of COVID-19 infection before the first blood withdrawal in Phase 1.

Outcomes

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Presence of NAbs can be categorized as either positive (> 30%) or negative (< 30%) based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be analysed across the participant population according to vaccine platform/booster type, time since the last dose and associated with socio-demographic data. This analysis will advise policymakers in Brunei on future vaccination strategies and establishing regulations across multiple agencies.

Sample size

The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222, and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly selected, stratified by four categories: (1) the three primary vaccination series platform; (2) gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after receiving the primary or booster doses. Considering the outcome variable for NAb measurement as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an expected positive rate of 88%, though the positive rate is expected to be much higher. The sample size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate, a sample size of 100 could detect a difference of 5% between two groups, with a power of 99% for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher positive rate e.g., at least higher than 80%, the targeted sample size is considered more than adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine category will achieve a precision of at least 20% of standard deviation (a small effect size) in estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose). In comparing groups of n = 160 for each category, a significant result can be obtained with a mean difference of at least 36% of standard deviation (small to medium effect size), with a power of 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software (version 3.1.6).

Recruitment and allocation

Eligible participants who are within 2 to 6 weeks after their last vaccination will be identified from the BruHealth database and invited on a weekly basis in the Phase 1 study. The Phase 2 study will follow up on participants who had received the third booster dose and sampled at 1, 3, 6, 9, and up to 12 months following the booster dose. The number of participants invited for both study phases is dependent on the target recruitment according to the maximum capacity of the blood sampling center, with an assumed response rate evaluated on a weekly basis. A balance in sample size for each stratum by age group, gender and vaccine platform will be included in the weekly target.

Potential participants will be sent three text messages for recruitment into the study in both English (Table 2) and native Malay languages. The first text message will be an invitation to participate in the study. The second text message acts as a reminder and will be sent the following day to prompt response to the invitation. Both text messages will contain a blood withdrawal appointment date and time with a link to an online form (Qualtrics XM, Qualtrics International Inc., USA), participant information sheet (PIS) and an option to agree or disagree to join the study. Those who agree will be sent a third text message 24 hours before their allocated appointment date. Individuals who do not agree will not be included in the study.

Table 2. Text message template for recruitment of study participants.

Text Message Type	Text content
First (Invitation)	[MOH] IC:01234567. You are invited to the next phase of the vaccine research study on dd/mm/yy at hh:mm - hh:mm. This will measure the status of your antibody level after vaccination. Please respond at bit.ly/abcdefg by Wednesday, 5:00 PM. Thank you.
Second (Invitation Reminder)	[MOH] IC:01234567. dd/mm/yy hh:mm - hh:mm. An antibody test is reserved for you next week. Please confirm your attendance at bit.ly/abcdefg by Wednesday, 05:00 PM. Thank you for your support. Please disregard this message if you have already responded.
Third (Appointment Reminder)	[MOH] IC:01234567. Reminder for your appointment tomorrow at Central Receiving Area (CSRA), RIPAS Hospital on dd/mm/yy at hh:mm - hh:mm. Location: bit.ly/tuvwxyz.

MOH, Ministry of Health; IC, Identity Card number; dd/mm/yy, allocated date; hh:mm, allocated time; bit.ly/abcdefg, custom link to online form; bit.ly/tuvwxyz, custom link for directions to blood sampling center.

Blinding mechanism

All data will be anonymized and each participant will be given a unique identifier following agreement to participate and recruitment. Identifying information is required to verify participant details at the time of blood sampling only. Otherwise, this information will not be included in any sample labels or data sharing.

Data collection

Study procedures and evaluations

Participant identities will be verified on-site at the blood sampling center. The PIS containing the study design and purpose will be explained to all participants. Written consent will be subsequently obtained prior to sampling. Socio-demographic data including age, gender, ethnicity, co-morbidities (i.e., diabetes mellitus, chronic kidney disease, hypertension, ischemic heart disease and cancer) and immunosuppressive drug use will be enquired and recorded in a secured online platform (EVYDResearch, EVYD Technology Limited, Singapore). For Phase 2, participants with history of COVID-19 breakthrough infection (determined via either positive polymerase chain reaction (PCR) or Antigen Rapid Test (ART) results), if any, will be documented. The symptoms and severity of breakthrough infections in these participants may be analyzed and compared to those without breakthrough infections.

Blood sampling and serum analysis

A whole blood sample of 5 mL will be drawn from each participant by venipuncture and allowed to incubate at room temperature for 15 to 30 minutes to allow clotting. Serum will be separated

by centrifugation at 1,000 – 2,000 x g for 10 minutes. Serum samples will be isolated and stored at -20°C.

The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's protocol. Briefly, 20 µL of serum sample, positive and negative controls will be diluted in the buffer (volume ratio of 1:9) and an equal volume of horseradish peroxidase (HRP)-RBD solution will be added. The mixture will be incubated at 37°C for 30 minutes before being added to a capture plate which will be further incubated for 15 minutes at 37°C. The well plate will be washed using a diluted washing buffer solution four times and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) solution will be added to each well. The well plate will be incubated in the dark at room temperature for 15 minutes, before the addition of 50 µL of stop solution to each well to stop the enzymatic reaction. The absorbance value (optical density, OD) of each well will be read using a microplate reader at 450 nm wavelength. The level of NAb in the serum samples will be measured by calculating the percentage signal inhibition as shown below. Serum samples with inhibition of at least 30% will be considered as positive for the presence of NAbs [18], as stated in the kit instructions. Percentage inhibition will be converted to antibody titers expressed in international units per milliliter (IU/mL) using a WHO International Standard conversion tool [22]. Antibody titers will be log-transformed before statistical analyses and presented as geometric mean titers (GMT).

$$\% \text{ Signal inhibition } = \left(1 - \frac{\text{OD value of serum sample}}{\text{OD value of negative control}}\right) \text{x } 100\%$$

The results of the NAb measurement as positive or negative and in IU/mL will be disseminated to all participants via a text message. A custom link for additional information on the interpretation

of NAb levels will be included. Contact details of a medical doctor will be provided should the participant require further information and to address any enquiry.

Retention

All data recorded and stored on the online platform (EVYDResearch) will only be stored for the duration of the study. Following cessation of the study, data will be securely deleted and disposed of in accordance to EVYD Technology Limited's Data Retention Framework.

Data management and confidentiality

Data confidentiality will be maintained through the online platform and only the research team will have access to full participant information. MOH, Brunei Darussalam will retain ownership of the participant data on Bru-HIMS and BruHealth and hold the rights to grant access to authorized personnel for the purposes of conducting the research. EVYDResearch platform follows the data security and privacy requirement as set forth by the MOH, Brunei Darussalam. There is a management system for the platform administrator to distribute or withdraw permission for authorized users. The platform administrator will grant access to the relevant fields as permitted by MOH, Brunei Darussalam. Authorized users will be required to sign the Officials Secret Act and to maintain participant confidentiality at all times. The platform strictly abides by the Data Privacy Policy in the collection, usage and disclosure of participant data. In accordance with Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, all identifiers of the individual will be removed using the Safe Harbor method.

Statistical methods

Socio-demographic variables, medical and drug history will be used to produce visualization plots and descriptive statistics and simple univariate analyses will be performed. Statistical software including RStudio (RStudio, MA, USA) and GraphPad Prism (GraphPad, CA, USA) may be used when necessary for advanced statistical analyses and data visualization.

The NAb level, based on percentage inhibition and GMT, elicited by each vaccine platform or subgroup (e.g., by post-vaccination week, by gender, by age group) will be analyzed using the Mann-Whitney U and Kruskal-Wallis tests with 95% confidence interval (CI). Comparison of positive rates will be analyzed by binomial regression using binreg R package (RStudio) and presented with positive rate ratios, their 95% CIs and P values. P value of less than 0.05 will be considered statistically significant.

Patient and Public Involvement

No patient involved.

ETHICS AND DISSEMINATION

Research ethics approval

This study is approved by the Medical and Ethical Research Committee (MHREC) of the MOH, Brunei Darussalam (Reference No: MHREC/MOH/2021/14(1) dated 18th November 2021). The study will be conducted in conformance with the Good Clinical and Laboratory Practice.

Informed consent process

All participants will give written informed consent to participate in this study. PIS will be provided to all participants in their preferred language (English, Malay or Chinese). The PIS will contain all relevant information relating to the study, potential risks and benefits, confidentiality and contact details should further information be required. The PIS will also be explained verbally on-site by

research assistants and queries will be addressed. Participants will acknowledge that they understand the PIS and are free to withdraw from the study at any time. They will agree to their medical records being accessed and their blood being withdrawn for the purpose of this study. In addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for better antibody measurement techniques or against future emerging SARS-CoV-2 variants. Participants may agree to the first (current study) but not the second (future relevant studies), if they wish. A physical, hard copy signed consent form will be collected from each participant prior to blood withdrawal. No remuneration will be provided in exchange for their participation.

Access to data

Collected data will be secured against unauthorized access and will be stored securely on the online platform.

Dissemination

Study findings will be disseminated to all participants. The results of the study will be submitted for publications in peer-reviewed journals as they become available. The data will also be presented internally to MOH, Brunei Darussalam, universities and in national and international scientific conferences and seminars.

STATEMENTS

Contributorship

All authors have contributed to the study design and conceptualization, writing and critical revision of the manuscript. HG, LA, HS, MFA, SB, ST, CYS, YW, SYC, YW, JW, FI, LN, and ACC will manage the recruitment of study participants, data coordination and blood withdrawal. CWT, XMO, FZ, and LFW will manage NAb measurement. All authors read and approved the final manuscript.

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Competing of Interests

CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other authors declare no conflict of interests.

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