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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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Manuscripts

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3 1 **TITLE PAGE**
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6 2 **Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in**
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8 3 **the Bruneian population: Protocol for a national longitudinal study**
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24 **ABSTRACT**

25 **Introduction**

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

33 **Methods and analysis**

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

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3 **47 Ethics and dissemination**
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5
6 48 This study has been approved by the Medical and Ethical Research Committee (MHREC) of the
7
8 49 Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each
9
10 50 participant by SMS. The findings from this study will help policymakers in Brunei develop future
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12 51 vaccination strategies and establish regulations across multiple agencies.
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52 STRENGTHS AND LIMITATIONS OF THIS STUDY

53 Strengths

- 54 • Three vaccine platforms used for primary series vaccination can be compared (BBIBP-
55 CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT1626b
56 (mRNA) with and without a third (booster-1) dose.
- 57 • Stratified random sampling recruitment targeting adults across all age groups (18-30; 31-
58 40; 41-50; 51-60; 61 and above) with a target of 3,000 participants across vaccine
59 platforms and controlled sampling time points (2 to 6 weeks post-primary series, then
60 longitudinal follow-up at 1, 3, 6, 9, 12 months \pm 1-week post the booster dose).
- 61 • Data extraction and integration from national electronic health record databases to
62 facilitate participant recruitment and communication.

64 Limitations

- 65 • The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit only measures the ability
66 of participant's antibodies to inhibit the binding of receptor-binding domain (RBD) from the
67 ancestral SARS-CoV-2 strain (Wu01) to its ligand, human angiotensin-converting enzyme
68 2 (hACE2). Further, neutralizing antibodies alone are not absolute correlates of protection
69 against infection, disease severity and death.
- 70 • Breakthrough infections are determined according to either positive polymerase chain
71 reaction (PCR) or Antigen Rapid Test (ART) result and not individually sequenced.

72 INTRODUCTION

73 Background and rationale

74 Vaccination has been instrumental in emerging from the restrictions introduced globally in
75 response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented
76 breakthroughs in vaccine technology¹ and new platforms, e.g., viral vector (AZD1222 developed
77 by Oxford-AstraZeneca²) and mRNA vaccines (mRNA-1273 from Moderna³, and BNT1626b
78 developed by Pfizer-BioNTech⁴) have been approved for human use.⁵ Traditional whole
79 inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by
80 Sinopharm⁶). Immunity develops following exposure to a pathogen, and vaccines aim to mimic
81 this by inducing a primary immune response. This results in a rapid secondary immune response
82 to the wild-type pathogen which prevents the development of infection and/or serious illness.^{7 8}

83
84 It is, therefore, of great interest to monitor immune responses to the various vaccine preparations
85 in populations to evaluate the effectiveness of these vaccination programmes.⁹ There is evidence
86 that antibody measurements can be used as a correlate of protection post-vaccination.¹⁰
87 Antibodies can be isolated from whole blood and subsequently analyzed for specificity and
88 functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2
89 weeks after vaccination and can persist for several months but are likely to wane over time.¹¹⁻¹⁴

90
91 Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein
92 and block viral entry to host cells. High level of NAb have been correlated with survival^{16 17} and
93 appear to be the best measure of vaccine efficacy.¹⁸ Surrogate virus neutralization assays in the
94 form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed and
95 validated to determine specific functional antibodies that can inhibit the interaction of viral

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3 96 receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2)
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5 97 receptors.¹⁵ Commercially available NAb preparations have been authorized for treatment and
6
7 98 are particularly useful in immunocompromised individuals.¹⁹ There is much interest in whether
8
9 99 NAb generated by one variant (or vaccine type) can protect against future variants of concern.²⁰
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15 101 The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct
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17 102 waves. The first wave, in March 2020, was largely import-driven and successfully contained within
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19 103 a month. This was followed by a period of relative stability, with no domestic cases reported for
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21 104 over a year due to strict international travel restrictions.²¹ The second wave commenced in August
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23 105 2021 and was predominantly caused by the Delta variant with more than 15,000 local cases with
24
25 106 only a small proportion of imported cases reported from August until December 2021. Subsequent
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27 107 waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.
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33 109 Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with
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35 110 vaccine administration implemented in three phases. The first phase covered healthcare and
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37 111 frontline workers, and individuals aged 60 years and above; the second phase for individuals with
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39 112 chronic diseases; and the third phase for the general public comprising adults aged 18 years and
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41 113 above. Three different vaccine types were distributed for the primary immunization series: BBIBP-
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43 114 CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to
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45 115 adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT1626b
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47 116 (mRNA) administered at least three months following completion of the primary series.
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49 117 Vaccination for adolescents aged 12 years and above commenced in November 2021 using the
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51 118 BNT162b2 vaccine and they are not included in this study population.
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3 120 The pandemic catalyzed the development of a national mobile health application, BruHealth
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5 121 (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in
6
7 122 May 2020. This provides a virtual health management platform with digital contact tracing, a
8
9 123 premise scan QR code function in public spaces, self-test reporting and vaccination appointment
10
11 124 capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which
12
13 125 links primary and secondary health care data across the entire government health network. Both
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15 126 platforms will be utilized accordingly for the conduct of this study.
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21 128 **Aims and objectives**

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24 129 Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines
25
26 130 in Southeast Asian populations. Thus, our study aims to assess the level of NAb generated by
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28 131 primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated
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30 132 Bruneian population and to compare their immunogenicity. In addition, this study captures
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32 133 participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or
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34 134 BNT1626b. Durability of NAb and rate of breakthrough infections will also be investigated over
35
36 135 a period of one year. Furthermore, our study will allow the evaluation of the effects of a
37
38 136 heterologous booster dose on NAb levels in the study cohort. This will provide new information
39
40 137 on immune responses in a Southeast Asian population and provide data on any differences in
41
42 138 NAb levels across different age groups, gender and vaccine types. This is the first instance where
43
44 139 the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth
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46 140 application for the evaluation of NAb levels across the Bruneian population.
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141 **METHODS**

142 **Study design**

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

154

155 **Study settings**

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

163 **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

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3 165 **Eligibility criteria**

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6 166 ***Inclusion criteria***

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9 167 Participants will be included in the study if they meet the following criteria:

- 10
11 168 1. Aged 18 years and above.
- 12
13 169 2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an
- 14
15 170 additional booster dose (BBIBP-CorV, mRNA-1273, or BNT1626b) for Phase 1. Phase 2
- 16
17 171 will follow up on participants who have received a booster dose over a period of one year.

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23 173 ***Exclusion criteria***

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26 174 Participants will be excluded from the study if they meet any of the following criteria:

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29 175 1. History of travel between the first dose and first blood withdrawal in Phase 1.
- 30
31 176 2. History of COVID-19 infection before the first blood withdrawal in Phase 1.

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36 178 **Outcomes**

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39 179 The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2

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41 180 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's

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43 181 protocol. Presence of NAb can be categorized as either positive (> 30%) or negative (< 30%)

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45 182 based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be

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47 183 analysed across the participant population according to vaccine platform/booster type, time since

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49 184 the last dose and associated with socio-demographic data. This analysis will advise policymakers

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51 185 in Brunei on future vaccination strategies and establishing regulations across multiple agencies.

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187 **Sample size**

188 The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222,
189 and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly
190 selected, stratified by four categories: (1) the three primary vaccination series platform; (2)
191 gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and
192 (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after
193 receiving the primary or booster doses. Considering the outcome variable for NAb measurement
194 as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an
195 expected positive rate of 88%, though the positive rate is expected to be much higher. The sample
196 size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate,
197 a sample size of 100 could detect a difference of 5% between two groups, with a power of 99%
198 for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus
199 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher
200 positive rate e.g., at least higher than 80%, the targeted sample size is considered more than
201 adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine
202 category will achieve a precision of at least 20% of standard deviation (a small effect size) in
203 estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose).
204 In comparing groups of n = 160 for each category, a significant result can be obtained with a mean
205 difference of at least 36% of standard deviation (small to medium effect size), with a power of
206 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software
207 (version 3.1.6).

208

209 **Recruitment and allocation**

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

218

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

227 **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.	

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229 **Blinding mechanism**

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

234

235 **Data collection**

236 ***Study procedures and evaluations***

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

244

245 ***Blood sampling and serum analysis***

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

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3 251 The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2
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5 252 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's
6
7 253 protocol. Briefly, 20 µL of serum sample, positive and negative controls will be diluted in the buffer
8
9 254 (volume ratio of 1:9) and an equal volume of horseradish peroxidase (HRP)-RBD solution will be
10
11 255 added. The mixture will be incubated at 37°C for 30 minutes before being added to a capture
12
13 256 plate which will be further incubated for 15 minutes at 37°C. The well plate will be washed using
14
15 257 a diluted washing buffer solution four times and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB)
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17 258 solution will be added to each well. The well plate will be incubated in the dark at room
18
19 259 temperature for 15 minutes, before the addition of 50 µL of stop solution to each well to stop the
20
21 260 enzymatic reaction. The absorbance value (optical density, OD) of each well will be read using a
22
23 261 microplate reader at 450 nm wavelength. The level of NAb in the serum samples will be measured
24
25 262 by calculating the percentage inhibition as shown below. Serum samples with inhibition of at least
26
27 263 30% will be considered as positive for the presence of NAb,¹⁵ as stated in the kit instructions.
28
29 264 Percentage inhibition will be converted to international units per milliliter (IU/mL) as previously
30
31 265 described.²²

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

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42 268 The results of the NAb measurement as positive or negative and in IU/mL will be disseminated to
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44 269 all participants via a text message. A custom link for additional information on the interpretation
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46 270 of NAb levels will be included. Contact details of a medical doctor will be provided should the
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48 271 participant require further information and to address any enquiry.

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273 **Retention**

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

277

278 **Data management and confidentiality**

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

291

292 **Statistical methods**

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

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6 298 The NAb level elicited by each vaccine platform or subgroup (e.g., by post-vaccination week, by
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8 299 gender, by age group) will be presented with 95% confidence interval (CI) of proportion analysis.
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10 300 Comparison of these positive rates will be analyzed by binomial regression using binreg R
11
12 301 package (RStudio) and presented with positive rate ratios, their 95% CIs and P values. P value
13
14 302 of less than 0.05 will be considered statistically significant.
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304 **Patient and Public Involvement**

305 No patient involved.
306

307 **ETHICS AND DISSEMINATION**

308 **Research ethics approval**

309 This study is approved by the Medical and Ethical Research Committee (MHREC) of the MOH,
310 Brunei Darussalam (Reference No: MHREC/MOH/2021/14(1) dated 18th November 2021). The
311 study will be conducted in conformance with the Good Clinical and Laboratory Practice.
312

313 **Informed consent process**

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

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3 319 understand the PIS and are free to withdraw from the study at any time. They will agree to their
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5 320 medical records being accessed and their blood being withdrawn for the purpose of this study. In
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7 321 addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for
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9 322 better antibody measurement techniques or against future emerging SARS-CoV-2 variants.
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11 323 Participants may agree to the first (current study) but not the second (future relevant studies), if
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13 324 they wish. A physical, hard copy signed consent form will be collected from each participant prior
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15 325 to blood withdrawal. No remuneration will be provided in exchange for their participation.
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21 327 **Declaration of interests**

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23
24 328 CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization
25
26 329 Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD
27
28 330 Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other
29
30 331 authors declare no conflict of interests.
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33 334 34 335 35 336 36 337 **Access to data**

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39 338 Collected data will be secured against unauthorized access and will be stored securely on the
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41 339 online platform.
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46 337 **Dissemination**

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49 338 Study findings will be disseminated to all participants. The results of the study will be submitted
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51 339 for publications in peer-reviewed journals as they become available. The data will also be
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53 340 presented internally to MOH, Brunei Darussalam, universities and in national and international
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55 341 scientific conferences and seminars.
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3 342 **STUDY ADMINISTRATION**
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6 343 **Funders**
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10
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12
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18 348 **Roles and responsibilities**
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20
21 349 ***Protocol contributors***
22

23
24 350 All authors have contributed to the study design and conceptualization, writing and critical revision
25
26 351 of the manuscript. HG, LA, HS, MFA, SB, ST, CYS, YW, SYC, YW, JW, FI, LN, and ACC will
27
28 352 manage the recruitment of study participants, data coordination and blood withdrawal. CWT,
29
30 353 XMO, FZ, and LFW will manage NAb measurement. All authors read and approved the final
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32 354 manuscript.
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BMJ Open

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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Manuscripts

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3 1 **TITLE PAGE**
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6 2 **Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in**
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8 3 **the Bruneian population: Protocol for a national longitudinal study**
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10 4 Ghani H.^{1*}, Ahmad L.¹, Sharif H.¹, Wong J.^{1,2}, Bagol S.², Alikhan M.F.^{2,4}, Taib S.², Tan C.W.³, Zhu
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24 **ABSTRACT**

25 **Introduction**

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

33 **Methods and analysis**

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

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3 **47 Ethics and dissemination**
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6 48 This study has been approved by the Medical and Ethical Research Committee (MHREC) of the
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8 49 Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each
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10 50 participant by SMS. The findings from this study will help policymakers in Brunei develop future
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12 51 vaccination strategies and establish regulations across multiple agencies.
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52 STRENGTHS AND LIMITATIONS OF THIS STUDY

53 Strengths

- 54 • Three vaccine platforms used for primary series vaccination can be compared (BBIBP-
55 CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT162b2
56 (mRNA) with and without a third (booster-1) dose.
- 57 • Stratified random sampling recruitment targeting adults across all age groups (18-30; 31-
58 40; 41-50; 51-60; 61 and above) with a target of 3,000 participants across vaccine
59 platforms and controlled sampling time points (2 to 6 weeks post-primary series, then
60 longitudinal follow-up at 1, 3, 6, 9, 12 months \pm 1-week post the booster dose).
- 61 • Data extraction and integration from national electronic health record databases to
62 facilitate participant recruitment and communication.

64 Limitations

- 65 • The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit only measures the ability
66 of participant's antibodies to inhibit the binding of receptor-binding domain (RBD) from the
67 ancestral SARS-CoV-2 strain (Wu01) to its ligand, human angiotensin-converting enzyme
68 2 (hACE2). Further, neutralizing antibodies alone are not absolute correlates of protection
69 against infection, disease severity and death.
- 70 • Pre-vaccination blood serum sampling was not performed to indicate potential neutralizing
71 antibodies present in participants. Moreover, comparisons of homologous vaccinations
72 using AZD1222 and BNT162b2 were limited due to the difference in vaccine platforms
73 distributed in the primary series and booster vaccinations.
- 74 • Breakthrough infections are determined according to either positive polymerase chain
75 reaction (PCR) or Antigen Rapid Test (ART) result and not individually sequenced. Hence,

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3 76 there will be limited information on the specific SARS-CoV-2 variants in the breakthrough
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5 77 infection cohort.
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For peer review only

78 INTRODUCTION

79 Background and rationale

80 Vaccination has been instrumental in emerging from the restrictions introduced globally in
81 response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented
82 breakthroughs in vaccine technology [1] and new platforms, e.g., viral vector (AZD1222
83 developed by Oxford-AstraZeneca [2]) and mRNA vaccines (mRNA-1273 from Moderna [3], and
84 BNT162b2 developed by Pfizer-BioNTech [4]) have been approved for human use [5]. Traditional
85 whole inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by
86 Sinopharm [6]). Immunity develops following exposure to a pathogen, and vaccines aim to mimic
87 this by inducing a primary immune response. This results in a rapid secondary immune response
88 to the wild-type pathogen which prevents the development of infection and/or serious illness [7],
89 [8].

90
91 It is, therefore, of great interest to monitor immune responses to the various vaccine preparations
92 in populations to evaluate the effectiveness of these vaccination programmes [9]. There is
93 evidence that antibody measurements can be used as a correlate of protection post-vaccination
94 [10]. Antibodies can be isolated from whole blood and subsequently analyzed for specificity and
95 functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2
96 weeks after vaccination and can persist for several months but are likely to wane over time [11],
97 [12], [13], [14].

98
99 Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein
100 and block viral entry to host cells. High levels of NAb have been correlated with survival [15], [16]
101 and appear to be the best measure of vaccine efficacy [17]. Surrogate virus neutralization assays

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3 102 in the form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed
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5 103 and validated to determine specific functional antibodies that can inhibit the interaction of viral
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7 104 receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2)
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9 105 receptors [18]. Commercially available NAb preparations have been authorized for treatment and
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11 106 are particularly useful in immunocompromised individuals [19]. There is much interest in whether
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13 107 NAb generated by one variant (or vaccine type) can protect against future variants of concern
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21 110 The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct
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23 111 waves. The first wave, in March 2020, was largely import-driven and successfully contained within
24
25 112 a month. This was followed by a period of relative stability, with no domestic cases reported for
26
27 113 over a year due to strict international travel restrictions [21]. The second wave commenced in
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29 114 August 2021 and was predominantly caused by the Delta variant with more than 15,000 local
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31 115 cases with only a small proportion of imported cases reported from August until December 2021.
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33 116 Subsequent waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.
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39 118 Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with
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41 119 vaccine administration implemented in three phases. The first phase covered healthcare and
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43 120 frontline workers, and individuals aged 60 years and above; the second phase for individuals with
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45 121 chronic diseases; and the third phase for the general public comprising adults aged 18 years and
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47 122 above. Three different vaccine types were distributed for the primary immunization series: BBIBP-
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49 123 CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to
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51 124 adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT162b2
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53 125 (mRNA) administered at least three months following completion of the primary series.
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3 126 Vaccination for adolescents aged 12 years and above commenced in November 2021 using the
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5 127 BNT162b2 vaccine and they are not included in this study population.
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11 129 The pandemic catalyzed the development of a national mobile health application, BruHealth
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13 130 (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in
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15 131 May 2020. This provides a virtual health management platform with digital contact tracing, a
16
17 132 premise scan QR code function in public spaces, self-test reporting and vaccination appointment
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19 133 capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which
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21 134 links primary and secondary health care data across the entire government health network. Both
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23 135 platforms will be utilized accordingly for the conduct of this study.
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29 137 **Aims and objectives**

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32 138 Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines
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34 139 in Southeast Asian populations. Thus, our study aims to assess the level of NAb generated by
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36 140 primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated
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38 141 Bruneian population and to compare their immunogenicity. In addition, this study captures
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40 142 participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or
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42 143 BNT162b2. Durability of NAb and rate of breakthrough infections will also be investigated over
43
44 144 a period of one year. Furthermore, our study will allow the evaluation of the effects of a
45
46 145 heterologous booster dose on NAb levels in the study cohort. This will provide new information
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48 146 on immune responses in a Southeast Asian population and provide data on any differences in
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51 147 NAb levels across different age groups, gender and vaccine types. This is the first instance where
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53 148 the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth
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55 149 application for the evaluation of NAb levels across the Bruneian population.
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150 **METHODS**

151 **Study design**

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

164 **Study settings**

165 Vaccinated members of the general adult population will be recruited to this study. Blood sampling
166 and serum isolation will take place in PAPRSB Institute of Health Sciences, Universiti Brunei
167 Darussalam for Phase 1 (baseline) of the study and Phlebotomy Services, Central Specimen
168 Receiving Area (CSRA), Department of Laboratory Services (DLS) at RIPAS Hospital, Brunei
169 Darussalam for Phase 2 (longitudinal follow-up). NAb level measurement will be conducted in
170 Duke-NUS Medical School, Singapore for Phase 1, and DLS, RIPAS Hospital, Brunei Darussalam
171 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

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3 **174 Eligibility criteria**
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6 **175 Inclusion criteria**
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8
9 176 Participants will be included in the study if they meet the following criteria:

- 10
11 177 1. Aged 18 years and above.
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13 178 2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an
14
15 179 additional booster dose (BBIBP-CorV, mRNA-1273, or BNT162b2) for Phase 1. Phase 2
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17 180 will follow up on participants who have received a booster dose over a period of one year.
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23 **182 Exclusion criteria**
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26 183 Participants will be excluded from the study if they meet any of the following criteria:

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29 184 1. History of travel between the first dose and first blood withdrawal in Phase 1.
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31 185 2. History of COVID-19 infection before the first blood withdrawal in Phase 1.
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36 **187 Outcomes**
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38
39 188 The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2
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41 189 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's
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43 190 protocol. Presence of NAb can be categorized as either positive (> 30%) or negative (< 30%)
44
45 191 based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be
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47 192 analysed across the participant population according to vaccine platform/booster type, time since
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49 193 the last dose and associated with socio-demographic data. This analysis will advise policymakers
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51 194 in Brunei on future vaccination strategies and establishing regulations across multiple agencies.
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196 **Sample size**

197 The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222,
198 and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly
199 selected, stratified by four categories: (1) the three primary vaccination series platform; (2)
200 gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and
201 (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after
202 receiving the primary or booster doses. Considering the outcome variable for NAb measurement
203 as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an
204 expected positive rate of 88%, though the positive rate is expected to be much higher. The sample
205 size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate,
206 a sample size of 100 could detect a difference of 5% between two groups, with a power of 99%
207 for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus
208 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher
209 positive rate e.g., at least higher than 80%, the targeted sample size is considered more than
210 adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine
211 category will achieve a precision of at least 20% of standard deviation (a small effect size) in
212 estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose).
213 In comparing groups of n = 160 for each category, a significant result can be obtained with a mean
214 difference of at least 36% of standard deviation (small to medium effect size), with a power of
215 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software
216 (version 3.1.6).

217

218 **Recruitment and allocation**

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

227
228 Potential participants will be sent three text messages for recruitment into the study in both English
229 (Table 2) and native Malay languages. The first text message will be an invitation to participate in
230 the study. The second text message acts as a reminder and will be sent the following day to
231 prompt response to the invitation. Both text messages will contain a blood withdrawal appointment
232 date and time with a link to an online form (Qualtrics XM, Qualtrics International Inc., USA),
233 participant information sheet (PIS) and an option to agree or disagree to join the study. Those
234 who agree will be sent a third text message 24 hours before their allocated appointment date.
235 Individuals who do not agree will not be included in the study.

236 **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.	

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238 **Blinding mechanism**

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

243

244 **Data collection**

245 ***Study procedures and evaluations***

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

256

257 ***Blood sampling and serum analysis***

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

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

262
263 The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2
264 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's
265 protocol. Briefly, 20 µL of serum sample, positive and negative controls will be diluted in the buffer
266 (volume ratio of 1:9) and an equal volume of horseradish peroxidase (HRP)-RBD solution will be
267 added. The mixture will be incubated at 37°C for 30 minutes before being added to a capture
268 plate which will be further incubated for 15 minutes at 37°C. The well plate will be washed using
269 a diluted washing buffer solution four times and 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB)
270 solution will be added to each well. The well plate will be incubated in the dark at room
271 temperature for 15 minutes, before the addition of 50 µL of stop solution to each well to stop the
272 enzymatic reaction. The absorbance value (optical density, OD) of each well will be read using a
273 microplate reader at 450 nm wavelength. The level of NAb in the serum samples will be measured
274 by calculating the percentage signal inhibition as shown below. Serum samples with inhibition of
275 at least 30% will be considered as positive for the presence of NAb [18], as stated in the kit
276 instructions. Percentage inhibition will be converted to antibody titers expressed in international
277 units per milliliter (IU/mL) using a WHO International Standard conversion tool [22]. Antibody titers
278 will be log-transformed before statistical analyses and presented as geometric mean titers (GMT).

$$279 \quad \% \text{ Signal inhibition} = \left(1 - \frac{\text{OD value of serum sample}}{\text{OD value of negative control}} \right) \times 100\%$$

280
281 The results of the NAb measurement as positive or negative and in IU/mL will be disseminated to
282 all participants via a text message. A custom link for additional information on the interpretation

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3 283 of NAb levels will be included. Contact details of a medical doctor will be provided should the
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5 284 participant require further information and to address any enquiry.
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286 **Retention**

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

291 **Data management and confidentiality**

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

305 **Statistical methods**

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

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6 311 The NAb level, based on percentage inhibition and GMT, elicited by each vaccine platform or
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8 312 subgroup (e.g., by post-vaccination week, by gender, by age group) will be analyzed using the
9
10 313 Mann-Whitney U and Kruskal-Wallis tests with 95% confidence interval (CI). Comparison of
11
12 314 positive rates will be analyzed by binomial regression using binreg R package (RStudio) and
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14 315 presented with positive rate ratios, their 95% CIs and P values. P value of less than 0.05 will be
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16 316 considered statistically significant.

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20 21 22 318 **Patient and Public Involvement**

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25 319 No patient involved.

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29 30 321 **ETHICS AND DISSEMINATION**

31 32 33 322 **Research ethics approval**

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36 323 This study is approved by the Medical and Ethical Research Committee (MHREC) of the MOH,
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38 324 Brunei Darussalam (Reference No: MHREC/MOH/2021/14(1) dated 18th November 2021). The
39
40 325 study will be conducted in conformance with the Good Clinical and Laboratory Practice.

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44 45 46 327 **Informed consent process**

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48 328 All participants will give written informed consent to participate in this study. PIS will be provided
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50 329 to all participants in their preferred language (English, Malay or Chinese). The PIS will contain all
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52 330 relevant information relating to the study, potential risks and benefits, confidentiality and contact
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54 331 details should further information be required. The PIS will also be explained verbally on-site by

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3 332 research assistants and queries will be addressed. Participants will acknowledge that they
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5 333 understand the PIS and are free to withdraw from the study at any time. They will agree to their
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7 334 medical records being accessed and their blood being withdrawn for the purpose of this study. In
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9 335 addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for
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11 336 better antibody measurement techniques or against future emerging SARS-CoV-2 variants.
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13 337 Participants may agree to the first (current study) but not the second (future relevant studies), if
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15 338 they wish. A physical, hard copy signed consent form will be collected from each participant prior
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17 339 to blood withdrawal. No remuneration will be provided in exchange for their participation.
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22 23 341 **Access to data**

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26 342 Collected data will be secured against unauthorized access and will be stored securely on the
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28 343 online platform.
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32 33 34 345 **Dissemination**

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36 346 Study findings will be disseminated to all participants. The results of the study will be submitted
37
38 347 for publications in peer-reviewed journals as they become available. The data will also be
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40 348 presented internally to MOH, Brunei Darussalam, universities and in national and international
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42 349 scientific conferences and seminars.
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350 STATEMENTS

351 Contributorship

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

357

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362

363 Competing of Interests

364 CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization
365 Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD
366 Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other
367 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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Secondary Subject Heading:	Research methods
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Manuscripts

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3 1 **TITLE PAGE**
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6 2 **Immunogenicity of COVID-19 vaccines and levels of SARS-CoV-2 neutralizing antibody in**
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8 3 **the Bruneian population: Protocol for a national longitudinal study**
9

10 4 Ghani H.^{1*}, Ahmad L.¹, Sharif H.¹, Wong J.^{1,2}, Bagol S.², Alikhan M.F.^{2,4}, Taib S.², Tan C.W.³, Zhu
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24 **ABSTRACT**

25 **Introduction**

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

33 **Methods and analysis**

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

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3 **47 Ethics and dissemination**
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6 48 This study has been approved by the Medical and Ethical Research Committee (MHREC) of the
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8 49 Ministry of Health, Brunei Darussalam. Individual NAb test results will be shared with each
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10 50 participant by SMS. The findings from this study will help policymakers in Brunei develop future
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12 51 vaccination strategies and establish regulations across multiple agencies.
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52 STRENGTHS AND LIMITATIONS OF THIS STUDY

53 Strengths

- 54 • Three vaccine platforms used for primary series vaccination can be compared (BBIBP-CorV
55 (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 or BNT162b2 (mRNA)
56 with and without a third (booster-1) dose.
- 57 • Stratified random sampling and recruitment of adults across five age groups with a target of
58 3,000 participants across vaccine platforms and controlled sampling time points.

60 Limitations

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

70 INTRODUCTION

71 Background and rationale

72 Vaccination has been instrumental in emerging from the restrictions introduced globally in
73 response to the COVID-19 pandemic. The first year of the pandemic led to unprecedented
74 breakthroughs in vaccine technology [1] and new platforms, e.g., viral vector (AZD1222
75 developed by Oxford-AstraZeneca [2]) and mRNA vaccines (mRNA-1273 from Moderna [3], and
76 BNT162b2 developed by Pfizer-BioNTech [4]) have been approved for human use [5]. Traditional
77 whole inactivated virus vaccines have also been developed (e.g., BBIBP-CorV manufactured by
78 Sinopharm [6]). Immunity develops following exposure to a pathogen, and vaccines aim to mimic
79 this by inducing a primary immune response. This results in a rapid secondary immune response
80 to the wild-type pathogen which prevents the development of infection and/or serious illness [7],
81 [8].

82
83 It is, therefore, of great interest to monitor immune responses to the various vaccine preparations
84 in populations to evaluate the effectiveness of these vaccination programmes [9]. There is
85 evidence that antibody measurements can be used as a correlate of protection post-vaccination
86 [10]. Antibodies can be isolated from whole blood and subsequently analyzed for specificity and
87 functional properties. Total antibodies specific to SARS-CoV-2 are typically detectable within 2
88 weeks after vaccination and can persist for several months but are likely to wane over time [11],
89 [12], [13], [14].

90
91 Neutralizing antibodies (NAbs) are functionally anti-viral in that they bind to the virus spike protein
92 and block viral entry to host cells. High levels of NAb have been correlated with survival [15], [16]
93 and appear to be the best measure of vaccine efficacy [17]. Surrogate virus neutralization assays

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3 94 in the form of competitive enzyme-linked immunosorbent assay (ELISA) have been developed
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5 95 and validated to determine specific functional antibodies that can inhibit the interaction of viral
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7 96 receptor-binding domain (RBD) proteins with human angiotensin-converting enzyme 2 (hACE2)
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9 97 receptors [18]. Commercially available NAb preparations have been authorized for treatment and
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11 98 are particularly useful in immunocompromised individuals [19]. There is much interest in whether
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13 99 NAb generated by one variant (or vaccine type) can protect against future variants of concern
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16 100 [20].
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21 102 The COVID-19 pandemic in Brunei Darussalam (pop. 430,000) has been characterized by distinct
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23 103 waves. The first wave, in March 2020, was largely import-driven and successfully contained within
24
25 104 a month. This was followed by a period of relative stability, with no domestic cases reported for
26
27 105 over a year due to strict international travel restrictions [21]. The second wave commenced in
28
29 106 August 2021 and was predominantly caused by the Delta variant with more than 15,000 local
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31 107 cases with only a small proportion of imported cases reported from August until December 2021.
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34 108 Subsequent waves in 2022 have been driven by the Omicron sub-variants BA.2 and BA.5.
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38
39 110 Brunei Darussalam introduced the National Vaccination Programme on 3rd April 2021 with
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41 111 vaccine administration implemented in three phases. The first phase covered healthcare and
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43 112 frontline workers, and individuals aged 60 years and above; the second phase for individuals with
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45 113 chronic diseases; and the third phase for the general public comprising adults aged 18 years and
46
47 114 above. Three different vaccine types were distributed for the primary immunization series: BBIBP-
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49 115 CorV (whole inactivated virus), AZD1222 (viral vector), and mRNA-1273 (mRNA) were offered to
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51 116 adults. This was followed by a booster dose of either BBIBP-CorV, mRNA-1273, or BNT162b2
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54 117 (mRNA) administered at least three months following completion of the primary series.
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3 118 Vaccination for adolescents aged 12 years and above commenced in November 2021 using the
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5 119 BNT162b2 vaccine and they are not included in this study population.
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11 121 The pandemic catalyzed the development of a national mobile health application, BruHealth
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13 122 (EVYD Technology Limited), launched by the Ministry of Health (MOH), Brunei Darussalam, in
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15 123 May 2020. This provides a virtual health management platform with digital contact tracing, a
16
17 124 premise scan QR code function in public spaces, self-test reporting and vaccination appointment
18
19 125 capabilities. BruHealth integrates with the Brunei Health Information System (Bru-HIMS), which
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21 126 links primary and secondary health care data across the entire government health network. Both
22
23 127 platforms will be utilized accordingly for the conduct of this study.
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29 129 **Aims and objectives**

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32 130 Currently, limited data exist on the comparative immunogenicity of different COVID-19 vaccines
33
34 131 in Southeast Asian populations. Thus, our study aims to assess the level of NAb generated by
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36 132 primary (two) doses of the BBIBP-CorV, AZD1222, and mRNA-1273 vaccines in the vaccinated
37
38 133 Bruneian population and to compare their immunogenicity. In addition, this study captures
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40 134 participants who have received a third (booster-1) dose of either BBIBP-CorV, mRNA-1273, or
41
42 135 BNT162b2. Durability of NAb and rate of breakthrough infections will also be investigated over
43
44 136 a period of one year. Furthermore, our study will allow the evaluation of the effects of a
45
46 137 heterologous booster dose on NAb levels in the study cohort. This will provide new information
47
48 138 on immune responses in a Southeast Asian population and provide data on any differences in
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51 139 NAb levels across different age groups, gender and vaccine types. This is the first instance where
52
53 140 the national electronic health record system (Bru-HIMS) is integrated with the local BruHealth
54
55 141 application for the evaluation of NAb levels across the Bruneian population.
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142 **METHODS**

143 **Study design**

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

156 **Study settings**

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

164 **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

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3 166 **Eligibility criteria**

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6 167 ***Inclusion criteria***

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9 168 Participants will be included in the study if they meet the following criteria:

- 10
11 169 1. Aged 18 years and above.
- 12
13 170 2. Vaccinated with either the BBIBP-CorV, AZD1222, or mRNA-1273, with or without an
- 14
15 171 additional booster dose (BBIBP-CorV, mRNA-1273, or BNT162b2) for Phase 1. Phase 2
- 16
17 172 will follow up on participants who have received a booster dose over a period of one year.
- 18
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23 174 ***Exclusion criteria***

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26 175 Participants will be excluded from the study if they meet any of the following criteria:

- 27
28
29 176 1. History of travel between the first dose and first blood withdrawal in Phase 1.
- 30
31 177 2. History of COVID-19 infection before the first blood withdrawal in Phase 1.
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36 179 **Outcomes**

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39 180 The level of NAb in the serum samples will be assessed using the cPass™ SARS-CoV-2

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41 181 Neutralization Antibody Detection Kit (GenScript Biotech, China) according to the manufacturer's

42
43 182 protocol. Presence of NAb can be categorized as either positive (> 30%) or negative (< 30%)

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45 183 based on percentage inhibition of viral RBD binding to hACE2 receptor. NAb levels will be

46
47 184 analysed across the participant population according to vaccine platform/booster type, time since

48
49 185 the last dose and associated with socio-demographic data. This analysis will advise policymakers

50
51 186 in Brunei on future vaccination strategies and establishing regulations across multiple agencies.

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188 **Sample size**

189 The sample size determined for each primary vaccination platform, i.e., BBIBP-CorV, AZD1222,
190 and mRNA-1273 was 1,000 for the Phase 1 study (total n = 3000). Individuals will be randomly
191 selected, stratified by four categories: (1) the three primary vaccination series platform; (2)
192 gender; (3) age groups, i.e., 18 to 30, 31 to 40, 41 to 50, 51 to 60, and above 60 years old; and
193 (4) duration post-vaccination (primary or booster immunization), i.e., 2, 3, 4, 5, and 6 weeks after
194 receiving the primary or booster doses. Considering the outcome variable for NAb measurement
195 as positive or negative, the targeted sample size of 1,000 would give a precision of 2% for an
196 expected positive rate of 88%, though the positive rate is expected to be much higher. The sample
197 size could give a precision of 3% if the positive rate is 63%. In a comparison of the positive rate,
198 a sample size of 100 could detect a difference of 5% between two groups, with a power of 99%
199 for two positive rates of 90% versus 95%; a power of 84% for two positive rates of 80% versus
200 85%, and a power of 71% for two positive rates of 70% versus 75%. As we anticipate a higher
201 positive rate e.g., at least higher than 80%, the targeted sample size is considered more than
202 adequate. In terms of the Phase 2 study, a sample size of 160 participants for each vaccine
203 category will achieve a precision of at least 20% of standard deviation (a small effect size) in
204 estimating the mean values for each time point after the baseline (2 to 6 weeks post the last dose).
205 In comparing groups of n = 160 for each category, a significant result can be obtained with a mean
206 difference of at least 36% of standard deviation (small to medium effect size), with a power of
207 80%. Sample size calculation was done using PS: Power and Sample Size Calculation software
208 (version 3.1.6).

209

210 **Recruitment and allocation**

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

219
220 Potential participants will be sent three text messages for recruitment into the study in both English
221 (Table 2) and native Malay languages. The first text message will be an invitation to participate in
222 the study. The second text message acts as a reminder and will be sent the following day to
223 prompt response to the invitation. Both text messages will contain a blood withdrawal appointment
224 date and time with a link to an online form (Qualtrics XM, Qualtrics International Inc., USA),
225 participant information sheet (PIS) and an option to agree or disagree to join the study. Those
226 who agree will be sent a third text message 24 hours before their allocated appointment date.
227 Individuals who do not agree will not be included in the study.

228 **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.	

229

230 **Blinding mechanism**

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

235

236 **Data collection**

237 ***Study procedures and evaluations***

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

248

249 ***Blood sampling and serum analysis***

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

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

254

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

$$271 \quad \% \text{ Signal inhibition} = \left(1 - \frac{\text{OD value of serum sample}}{\text{OD value of negative control}} \right) \times 100\%$$

272

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

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3 275 of NAb levels will be included. Contact details of a medical doctor will be provided should the
4
5 276 participant require further information and to address any enquiry.
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For peer review only

278 **Retention**

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

283 **Data management and confidentiality**

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

297 **Statistical methods**

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

302

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.

309

Patient and Public Involvement

No patient involved.

312

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.

318

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

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2
3 324 research assistants and queries will be addressed. Participants will acknowledge that they
4
5 325 understand the PIS and are free to withdraw from the study at any time. They will agree to their
6
7 326 medical records being accessed and their blood being withdrawn for the purpose of this study. In
8
9 327 addition, consent will be obtained for the use of serum sample for other similar purposes e.g., for
10
11 328 better antibody measurement techniques or against future emerging SARS-CoV-2 variants.
12
13 329 Participants may agree to the first (current study) but not the second (future relevant studies), if
14
15 330 they wish. A physical, hard copy signed consent form will be collected from each participant prior
16
17 331 to blood withdrawal. No remuneration will be provided in exchange for their participation.
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23 333 **Access to data**

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26 334 Collected data will be secured against unauthorized access and will be stored securely on the
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28 335 online platform.
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32 33 34 337 **Dissemination**

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37 338 Study findings will be disseminated to all participants. The results of the study will be submitted
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39 339 for publications in peer-reviewed journals as they become available. The data will also be
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41 340 presented internally to MOH, Brunei Darussalam, universities and in national and international
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43 341 scientific conferences and seminars.
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3 342 **STATEMENTS**
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6 343 **Contributorship**
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8
9 344 All authors have contributed to the study design and conceptualization, writing and critical revision
10
11 345 of the manuscript. HG, LA, HS, MFA, SB, ST, CYS, YW, SYC, YW, JW, FI, LN, and ACC will
12
13 346 manage the recruitment of study participants, data coordination and blood withdrawal. CWT,
14
15 347 XMO, FZ, and LFW will manage NAb measurement. All authors read and approved the final
16
17 348 manuscript.
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24

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28
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35 355 **Competing of Interests**
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37
38 356 CWT and LFW received a patent for the development of the cPass™ SARS-CoV-2 Neutralization
39
40 357 Antibody Detection Kit used in the study. CYS, YW, SYC, and YW are employed by EVYD
41
42 358 Technology Ltd, who is the provider for the BruHealth and EVYDResearch platforms. All the other
43
44 359 authors declare no conflict of interests.
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