Prospective monitoring of BNT162b2 second vaccination booster for prevention of the COVID-19 infection in health care workers (HCW)

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1 Signature Page

Study Title: Prospective monitoring of BNT162b2 second vaccination booster for prevention of the COVID-19 infection in health care workers (HCW)

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Investigator's Responsibility

Prior to participation in the trial, as the site principal investigator I understand that I must obtain written approval from my local Ethics Committee and other regulatory bodies according to the local regulation.

As the site Principal Investigator, I must also:

1. Ensure that the trial is not commenced until approvals have been obtained.
2. Ensure that written informed consent is obtained from each subject prior to any data collection using the most recent EC approved Subject Informed Consent Form.
3. Provide all required data and reports and agree to source document verification of trial data with subject's medical records.
4. Allow EC and/or regulatory bodies representatives, to inspect and copy any documents pertaining to this clinical investigation.

Investigator Signature

I have read and understand the contents of the trial protocol and agree to abide by the requirements set forth in this document.

Investigator's Name	Institution
Investigator's Signature	Date

2 Protocol Summary

OBJECTIVE	**Primary Objective** Examine the protection against any type of COVID-19 infection as confirmed by PCR testing or positive serology for N antigen during the follow up period. **Secondary Objectives** Evaluate the impact of a fourth dose on: 1. Symptomatic COVID 19 infection 2. COVID 19 infection requiring hospitalization 3. Antibody levels including IgG, IgA, IgM, binding and neutralizing activity and the avidity of the antibodies.
STUDY DESIGN	Prospective observational registry
OUTCOMES	**Primary Outcome** COVID-19 infection as confirmed by PCR testing or positive serology for N antigen during the follow up period. We hypothesize that booster of BNT162b2 vaccine in healthcare workers (HCW) will lower the rates of the COVID-19 infection as compared to HCW choosing not to receive the second booster. **Secondary Outcomes** 1. Symptomatic COVID 19 infection 2. COVID 19 infection requiring hospitalization 3. Levels of binding and neutralizing activity and avidity of the antibodies 4. Composite endpoint of serious adverse events during 14 days following the booster vaccination
SAMPLE SIZE CONSIDERATIONS	We aim to enroll up to 1000 subjects. The following assumptions were used to estimate the power of the analysis. • The proportion of the HCW choosing to receive the second booster is 60% • The expected monthly infection rate of 2.5% in the non-vaccinated group • Expected monthly infection rate of 1.0% in the treatment group • Median follow-up until switching to the booster administration in the non-vaccinated group (15%) of 4 months • Type I error (α) = 0.025 (one-sided) • Log-rank test • Enrollment period of 1 month • Lost to follow-up of 10% An evaluable sample size of 1000 subjects provides 80.0% power to reject the null hypothesis, signifying the booster strategy is superior to the comparison group at 6 months post-vaccination.
INCLUSION CRITERIA	1. Able to provide written informed consent. 2. Healthcare worker in one of the Clalit Health Services Medical centers and is at least 18 years of age. 3. Completed three doses of BNT162b2 according to MOH guidelines. 4. Third dose was given at least 4 months prior to enrollment.

EXCLUSION CRITERIA	1. History of COVID-19 infection. 2. History of being treated or is currently being treated for any type of Malignancies or other co-morbid conditions that may result in protocol non-compliance. 3. Currently or in the past three months was treated with any type of Immune suppression medication (including chemotherapy, immunomodulatory drugs, biological agents that affect the immune system, any immunosuppressive drug such as corticosteroids). 4. Received in the past 4 months monoclonal antibodies of any type.
STUDY PROCEDURES	**Screening and Enrollment** The following will take place at this visit: • Confirmation of eligibility and informed consent will be signed. • Demographics and medical history will be recorded. • COVID-19 questionnaire regarding exposure and symptoms. • Blood draw of three 5 ml vials, for antibody testing. • Blood drawn for PBMC and antibody testing: o Will be ONLY at Soroka Medical Center. o 150 subjects will be selected randomly. Subjects participating in this part will have 30 ml (3 vials of 9 ml and 1 vial of 3 ml) of blood drawn for PBMC and antibody testing. Others will have blood draw of three 5 ml vials, for antibody testing. • A nasal and mouth swab will be performed for IgA, IgG and IgM levels testing **Visit 2 –6** • COVID-19 questionnaire regarding exposure and symptoms. Blood draw of 3 vials of 5 ml for antibody testing, • Blood drawn for PBMC and antibody testing: o Will be ONLY at Soroka Medical Center. Subjects who were not part of the PBMC group and are willing to participate, will be asked again to sign inform consent. o Subjects participating in this part will have 30 ml (3 vials of 9 ml and 1 vial of 3 ml) of blood drawn for PBMC and antibody testing. Others will have blood draw of three 5 ml vials, for antibody testing. • A nasal and mouth swab will be performed for IgA, IgG and IgM levels testing • Adverse events following vaccination and changes in medications (chronic and/or Immune suppression medication) will be collected

LABORATORY TESTING	• From each HCW at each visit the following tests will be performed: • The Platelia SARS-CoV-2 Total Ab assay by Bio rad, is a semi-quantitative in vitro diagnostic test, in a one-step antigen capture format, for the detection of IgM/IgA/IgG antibodies to the SARS-CoV-2 in human serum and plasma (EDTA, heparin, ACD or citrate) specimens. This assay will be performed at SMC virology laboratory (head-Prof. Yonat Shemer). • Antibody profiling of blood and nasal samples will be tested using antigen microarrays, this will be performed by using chip antigen microarrays spotted with various coronavirus antigens, as well as antigens of other common respiratory pathogens. This assay will be performed at Prof. Tomer Hertz laboratory at Ben-Gurion University of the Negev. • On select samples from each visit neutralization assays for SARS-CoV-2 virus variants will be performed. Each sample selected will be tested for its ability to inhibit infection of target ACE2-expressing cells by reporter pseudoviruses that express the spike protein of either Delta or Omicron Variant of Concern. This assay will be performed at Dr. Ran Taube laboratory at Ben-Gurion University of the Negev. • Blood samples for PBMC'S will be stored at Prof. Hertz's laboratory at Ben-Gurion University of the Negev for future research.
DATA TO CAPTURE	The following data will be collected: • Demographic data: age, sex, country of birth, healthcare profession, direct contact with covid subjects in working capacity. • Detailed medical history, including but not limited to past illness with COVID, malignancy, diabetes, renal failure, heart disease, hypertension, history of stroke, rheumatological illness. • Current medications used and medications used for more than one week in the past three months. • Any adverse event during the study as a result of vaccination.
DATA MANAGEMENT AND ANALYSIS	The study will be overseen and managed by the Soroka Clinical Research Center.

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3 Abbreviations and Definitions

Abbreviation	Term
AE	Adverse Event
COVID-19	Corona Virus Disease
CRF	Case report form
EC	Ethics Committee
EOS	End of Study
HCW	Health care worker of clalit health care
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MOH	Ministry of Health
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome- corona Virus-2
SMC	Soroka Medical Center
Soroka CRC	Soroka Clinical Research Center

4 Background

The SARS-CoV-2 virus causes severe respiratory illness and is an ongoing global pandemic. On December 12, 2020 the FDA approved Pfizer's BioNTech vaccine BNT162b2 which is a messenger RNA type of vaccine for use. This vaccine has shown in numerus studies the ability to induce a strong immune response and provide both humeral and cellular protection against wild type, alpha and delta variants of SARS-CoV2 virus.
In Israel the national vaccine operation began in mid-December 2020 which included 2 initial doses three weeks apart. In August 2021 a first booster (3rd dose) was provided to enhance protection and due to reports of reduced immune response and clinical protection. Several studies have demonstrated that over time there is a decay in the antibody levels, and with them reduced protection.
Recently a new variant of concern has been identified (Omicron) and is causing a surge of infections worldwide. There is lack of knowledge regarding the effectiveness of the current schedule of vaccine against this new variant and whether a second booster (4th dose) will provide higher levels of clinical protection against this variant, currently the ministry of health is considering recommendations for a fourth dose for HCW.
The purpose of this study is to examine whether a fourth dose of vaccination will provide better protection against infection and clinical disease.

5 Methods

5.1 Working Hypothesis

In HCW, receiving a second booster (fourth dose) of BNT162b2 vaccine will lower the rates of the COVID-19 infection compared to controls.

5.2 Objectives

5.2.1 Primary Objective

Examine the protection against any type of COVID-19 infection as confirmed by PCR testing or positive serology for N antigen during the follow up period.

5.2.2 Secondary Objectives

Evaluate the impact of a fourth dose on:

1. Symptomatic COVID 19 infection
2. COVID 19 infection requiring hospitalization
3. Antibody levels including IgG, IgA, IgM, binding and neutralizing activity and the avidity of the antibodies.

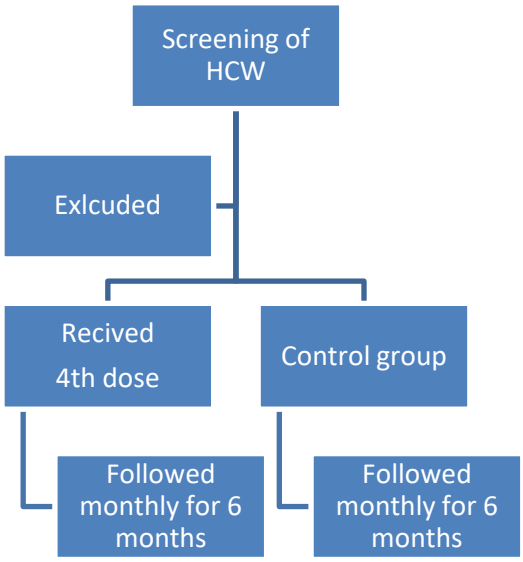
5.3 Schedule of Events

Visit number		**1**	**2**	**3**	**4**	**5**	**6**
Time point (± 2 week)	**Enrollment**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics and Medical History	X						
Blood samples*	X	X	X	X	X	X	X
Nasal and mouth Swab**	X	X	X	X	X	X	X
Questionnaire	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X

* Antibody levels including IgG, IgA, IgM, binding and neutralizing activity and the avidity of the antibodies. Serum separation and freezing at each site. Only at Soroka medical blood will be drawn for PBMC testing in addition to antibodies testing.

** Antibody levels including IgG, IgA, IgM

5.4 Flow chart



Subjects in the control group who are vaccinated after inclusion in the study with a fourth dose will be switched to the vaccinated arm and followed from that point as boosted.

5.5 Study Population

The study population will be screened at each participating medical center

The study population will be enrolled according to the following inclusion and exclusion criteria.

5.5.1 Inclusion Criteria

1. Able to provide written informed consent.
2. Healthcare worker in one of the Clalit Health Services medical centers and is at least 18 years of age.
3. Completed three doses of BNT162b2 according to MOH guidelines.
4. Third dose was given at least 4 months prior to enrollment.

5.5.2 Exclusion Criteria

1. History of COVID-19 infection.
2. History of being treated or is currently being treated for any type of Malignancies or other co-morbid conditions that may result in protocol non-compliance.
3. Currently or in the past three months was treated with any type of Immune suppression medication (including chemotherapy, immunomodulatory drugs, biological agents that affect the immune system, any immunosuppressive drug such as corticosteroids).

4. Received in the past 4 months monoclonal antibodies of any type.

5.5.3 Withdrawal/Discontinuation Criteria

All subjects should be encouraged to remain in the study through the entire follow up. However, if a subject decides to discontinue study participation, the reason for discontinuation must be recorded in the medical record and the Case Report Form (CRF). Subjects who discontinue participation prematurely will be included in the analysis of results (as appropriate) but they will not be replaced in the enrollment of total study subjects.

Every attempt must be made to have all subjects complete the follow up visit schedule. A subject will not be considered lost-to-follow up unless all efforts to obtain compliance are unsuccessful.

Discontinuation in the study will be if the antibody result at enrollment visit shows the participant already had the disease.

5.6 Enrollment

Prior to participation in this study, the Investigator must obtain written approvals from the ethics committee and other local regulatory bodies as appropriate approval for the protocol and the informed consent form. Failure to obtain a signed and hand dated informed consent prior to the procedure constitutes a protocol violation, which is reportable to the EC.

The Investigator will obtain an approval from the ethics committee to recruit and sign participants on informed consent form by study research members (such as study coordinators, research assistant, study investigator and etc.) with GCP certificate.

5.7 Subject Screening

Screening will be performed at each participating medical center; an email or other medical communication will be sent out to the employees of each participating medical center with a short description of the study and invite them to learn more of the study and be screened.

HCW who fulfills all the inclusions and none of the exclusion criteria will be approached and offered participation in the study by a study research member. A study research member will explain the purpose, procedures and intent of the study to each potential

participant. Interested HCW will be asked to provide written consent prior to performing any study procedure.

5.8 Study Procedures

5.8.1 Screening and Enrollment

The following will take place at this visit:

- Confirmation of eligibility and informed consent will be signed.
- Demographics and medical history will be recorded.
- COVID-19 questionnaire regarding exposure and symptoms.
- Blood draw of three 5 ml vials, for antibody testing.
- Blood drawn for PBMC and antibody testing:
    - Will be at ONLY at Soroka Medical Center.
    - 150 subjects will be selected randomly. Subjects participating in this part will have 30 ml (3 vials of 9 ml and 1 vial of 3 ml) of blood drawn for PBMC and antibody testing. Others will have blood draw of three 5 ml vials, for antibody testing.
- A nasal and mouth swab will be performed.

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- Blood drawn for PBMC and antibody testing:
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5.9 Laboratory testing

From each HCW at each visit the following tests will be performed:

- The Platelia SARS-CoV-2 Total Ab assay by Bio rad, is a semi-quantitative in vitro diagnostic test, in a one-step antigen capture format, for the detection of IgM/IgA/IgG antibodies to the SARS-CoV-2 in human serum and plasma (EDTA, heparin, ACD or citrate) specimens. This assay will be performed at SMC virology laboratory (head- Prof. Yonat Shemer).
- Antibody profiling of blood and nasal and mouth samples will be tested using antigen microarrays, this will be performed by using chip antigen microarrays spotted with various coronavirus antigens, as well as antigens of other common respiratory pathogens. This assay will be performed at Prof. Tomer Hertz laboratory at Ben-Gurion University of the Negev.
- On select samples from each visit neutralization assays for SARS-CoV-2 virus variants will be performed. Each sample selected will be tested for its ability to inhibit infection of target ACE2-expressing cells by reporter pseudoviruses that express the spike protein of either Delta or Omicron Variant of Concern. This assay will be performed at Dr. Ran Taube laboratory at Ben-Gurion University of the Negev.
- Blood samples for PBMC'S will be stored at Prof. Hertz's laboratory at Ben-Gurion University of the Negev for future research.

5.10 Study Duration

The subject will participate for 6 months from the initial consent.

We expect that enrollment to the study will be for a period of 1 months.

5.11 Data to Capture

5.11.1 Required Data

All required data for this study will be collected via paper and or electronic case report forms (eCRF).

5.11.2 Data Collection

The final set of CRFs is designed to accommodate the specific features of the trial design. Modification of CRFs will only be made if deemed necessary by Soroka CRC. The CRFs will be filled out manually by the study personal and or will be collected through the computer services database.

The following data will be collected:

- Demographic data: age, sex, country of birth, healthcare profession, direct contact with covid subjects in working capacity.
- Detailed medical history, including but not limited to past illness with COVID, malignancy, diabetes, renal failure, heart disease, hypertension, history of stroke, rheumatological illness.
- Current medications used and medications used for more than one week in the past three months.
- Any adverse event during the study as a result of vaccination.

6 Statistical Considerations and Analysis Plan

In this prospective observational registry we aim to assess the rates of the COVID-19 infection as confirmed by PCR testing or positive serology for N antigen during the follow up period. We hypothesize that booster of BNT162b2 vaccine in healthcare workers (HCW) will lower the rates of the COVID-19 infection as compared to HCW choosing not to receive the second booster.

We aim to enroll up to 1000 subjects. The following assumptions were used to estimate the power of the analysis.

- The proportion of the HCW choosing to receive the second booster is 60%
- The expected monthly infection rate of 2.5% in the non-vaccinated group
- Expected monthly infection rate of 1.0% in the treatment group
- Median follow-up until switching to the booster administration in the non-vaccinated group (15%) of 4 months
- Type I error (α) = 0.025 (one-sided)
- Log-rank test
- Enrollment period of 1 month
- Lost to follow-up of 10%

An evaluable sample size of 1000 subjects provides 80.0% power to reject the null hypothesis, signifying the booster strategy is superior to the comparison group at 6 months post-vaccination.

All statistical analyses will be performed using Statistical Analysis System (SAS) for Windows (version 9.4 or higher, SAS Institute Inc. Cary, NC) or other widely accepted statistical or graphical software. In general, data for all study patients will be combined and presented. Individual data will be presented in patient listings.

Descriptive statistics will be used to present the data and to summarize the results. Categorical variables will be presented using frequency distributions and cross tabulations with numbers and percent per group. Continuous variables will be summarized by presenting the number of observations (N), mean, 95% confidence interval of the mean, standard deviation, median, minimum, and maximum values.

The primary outcome will be analyzed within the paradigm of the survival analysis, i.e. log-rank test for the univariate analysis. Since it is possible that those who chose not to be vaccinated will differ in a number of characteristics, we will utilize Cox regression models with time dependent variable (timing of the booster) with the adjustment for the potential confounders. The confounders will be identified by the DAG approach. In addition different approaches to approximate the counterfactual world assumption such as propensity score adjustment will be considered.

The following subgroup analysis will be performed: stratified by age, gender, immune status, medical center, timing of the booster (before and after the current wave peak).

7 Adverse Events

7.1 Adverse Event Definition

7.1.1 Adverse Event

Any undesirable experience (sign, symptom, illness, abnormal laboratory value, or other medical event) occurring to a subject, that is considered related to the investigational treatment regimen prescribed as part of the clinical protocol, predefined in the clinical protocol and/or Instructions For Use, that is identified or worsens during a clinical study.

7.1.2 Treatment Related Adverse Event

A treatment related adverse event is defined as any adverse event, for which a causal relationship between the treatment and the event is at least a reasonable possibility, i.e., the relationship cannot be excluded.

7.1.3 Serious Adverse Event

A serious adverse event as one when the subject outcome is one of the following: Death, Life-threatening, Hospitalization (initial or prolonged), Disability - significant, persistent, or permanent change, impairment, damage or disruption in the subject's body function/ structure, physical activities or quality of life, Congenital anomaly or requires intervention to prevent permanent impairment or damage.

Since this is an observational registry study, SAEs will not be reported to the EC, but will be included in the statistical analysis.

All side effects as a result of vaccination will be reported to Ministry of Health.

7.2 Anticipated Adverse Event

In this study we do not anticipate adverse event. However, adverse event as a result from blood drawn and nasal and mouth swabbing can occur, such as: pain, hematoma, and discomfort in the sampling area

8 Ethical and Regulatory Considerations

8.1 Subject Confidentiality

Subject confidentiality will be maintained throughout the study in a way that assures that data can always be tracked back to the source data. For this purpose, a unique subject identification code will be used that allows identification of all data reported for each subject.

Data relating to the study might be made available to third parties (for example in case of an audit performed by regulatory authorities) provided the data are treated confidentially and that the subject's privacy is guaranteed.

8.2 Sources of Materials

Research material obtained from study participants includes medical information, blood specimens, and nasal samples obtained for laboratory analysis.

All of the data obtained for this study will be obtained prospectively.

Copies of data obtained as part of the study will be retained by the clinical research center, with appropriate source documentation, on all subjects that sign informed consent. The data utilized in this study are described above and consist of information from medical records, or study-specified measures and interventions.

8.3 Maintaining Records

The principal investigator will maintain copies of all study-related correspondence, regulatory documents, data, shipment of sample logs, adverse events and other records related to the clinical study.

8.4 Site Record Retention Policy

All core laboratories and clinical sites will maintain study records until the principal investigator notifies them and the reviewing regulatory authorities that research is completed or terminated under the clinical investigation in compliance with national law. Record retention dates will be provided to study sites by the principal investigator at the onsite closeout visit.

8.5 Informed Consent and Ethics Committee

All subjects must provide written informed consent in accordance with the local clinical site's EC. A signed Informed Consent must be obtained from each subject prior to

commencing screening/baseline evaluations. One copy of the Informed Consent document will be given to the subject and another retained by the Investigator.

8.6 Protocol Deviation

Any incident in which the investigator or site personnel did not conduct the study according to the clinical protocol or the investigator agreement.
Protocol deviations are classified to three categories:

- Major deviation: Any deviation from subject inclusion and or exclusion criteria or subject informed consent procedures.
- Critical finding: any deviation that can affect the wellbeing of the participant, or the reliability of the data collected is compromised.
- Minor deviation: Deviation from a clinical protocol requirement such as incomplete/inadequate testing procedures, follow-ups performed outside specified time windows, etc.

9 References

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