Immune Responses Induced by Vaccination Against COVID-19 in Dutch healthy subjects

Acronym:

IIVAC

Immune responses Induced by Vaccination Against COVID-19 in Dutch healthy subjects

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Coordinating investigator/project leader	5.1.2e PhD 5.1.2e Infectious Diseases and Vaccinology Centre (CIb)-Immunology of Infectious Diseases and Vaccines (IIV) National Institute of Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9, PO box 1, 3720 BA Bilthoven, The Netherlands T: 5.1.2e E: 5.1.2e @rivm.nl
Principal investigator(s):	5.1.2e 5.1.2e Center of Infectious Diseases Control (CIb), Immunology of Infectious Diseases and Vaccines (IIV), National Institute of Health and the Environment (RIVM) PO box 1, 3720 BA Bilthoven, The Netherlands T: 5.1.2e E: 5.1.2e @rivm.nl 5.1.2e Infectious Diseases and Vaccinology Centre (CIb)-Immunology of Infectious Diseases and Vaccines (IIV) National Institute of Health and the Environment

(RIVM)
Antonie van Leeuwenhoeklaan 9, PO box 1, 3720
BA Bilthoven, The Netherlands
T: 5.1.2e
E: 5.1.2e @rivm.nl

5.1.2e 5.1.2e
5.1.2e
Infectious Diseases and Vaccinology Centre (CIb)-
Immunology of Infectious Diseases and Vaccines
(IIV)
National Institute of Health and the Environment
(RIVM)
Antonie van Leeuwenhoeklaan 9, PO box 1, 3720
BA Bilthoven, The Netherlands
T: 5.1.2e
E: 5.1.2e @rivm.nl

5.1.2e
5.1.2e
Infectious Diseases and Vaccinology Centre (CIb)-
Immunology of Infectious Diseases and Vaccines
(IIV)
National Institute of Health and the Environment
(RIVM)
Antonie van Leeuwenhoeklaan 9, PO box 1, 3720
BA Bilthoven, The Netherlands
T: 5.1.2e
E: 5.1.2e @rivm.nl

5.1.2e
5.1.2e
Infectious Diseases and Vaccinology Centre (CIb)-
Immunology of Infectious Diseases and Vaccines
(IIV)
National Institute of Health and the Environment

	(RIVM) Antonie van Leeuwenhoeklaan 9, PO box 1, 3720 BA Bilthoven, The Netherlands T: 5.1.2e E: 5.1.2e @rivm.nl 5.1.2e 5.1.2e 5.1.2e Infectious Diseases and Vaccinology Centre (CIB)-Immunology of Infectious Diseases and Vaccines (IIV) National Institute of Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9, PO box 1, 3720 BA Bilthoven, The Netherlands T: 5.1.2e E: 5.1.2e @rivm.nl
Sponsor (in Dutch: verrichter/opdrachtgever)	National Institute of Public Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven T: 5.1.2e
Subsidising party	Ministry of Health Welfare and Sport Parnassusplein 5 P.O. Box 20350, 2511 EJ, The Hague
Independent expert (s)	5.1.2e 5.1.2e National Institute of Public Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven
Laboratory sites	National Institute of Public Health and the Environment (RIVM); Centre of Infectious Diseases Control, Centre for Immunology of Infectious Diseases and Vaccines (IIV)

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: 5.1.2e	5.1.2e	**30th of April 2021**
Principal Investigator: 5.1.2e	5.1.2e	**30th of April 2021**
Coordinating Investigator/ Principal Investigator: 5.1.2e		**30th of April 2021**

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
ATAC-seq	**Assay for Transposase-Accessible Chromatin Sequencing**
BRP	**Basisregistratie Personen**
CA	**Competent Authority**
CBG	**College ter Beoordeling van Geneesmiddelen**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CD	**Cluster of Differentiation**
COVID-19	**Coronavirus disease 2019**
CV	**Curriculum Vitae**
DCS	**Doetinchem Cohort Study**
DSMB	**Data Safety Monitoring Board**
EMA	**European Medicines Agency**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FACS	**Fluorescence-Activated Cell Sorting**
FFX	**First Few X (*hundred*)**
FPFV	**First Subject, First Visit**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
GMC	**Geometric Mean Concentration**
GMT	**Geometric Mean Titer**
GP	**General Practitioner**
IB	**Investigator's Brochure**
IC	**Informed Consent**
Ig	**Immunoglobulin (antibody)**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
LSFV	**Last Subject, First Visit**

LSLV	**Last Subject, Last Visit**
METC	**Medical research ethics committee (MREC);** **in Dutch: Medisch-ethische toetsingscommissie (METC)**
MLF	**Mucosal Lining Fluid**
NA	**Not Applicable**
MIA	**Multiplex Immunoassay**
PBMC	**Peripheral Blood Mononuclear Cells**
PIF	**Participants Information Leaflet**
(S)AE	**(Serious) Adverse Event**
SAM	**Synthetic Absorptive Matrix**
SARS-CoV-2	**Severe acute respiratory syndrome coronavirus-2**
SmPC	**Summary of medicinal Product Characteristics;** **in Dutch: Officiële productinformatie IB1-tekst**
SOP	**Standard Operating Procedure**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation;** **in Dutch: Uitvoeringswet AVG**
VASCO	**Vaccine Study COvid-19**
VIDO	**Vaccinaties tegen het coronavirus en Immuniteit binnen het Doetinchem Onderzoek**
VIVO	**Vaccinaties, Immuniteit en Verouderings Onderzoek**
VITAL	**Vaccines and InfecTious diseases in the Ageing PopuLation**
VNT	**Virus Neutralization Titer**
VOCAAL	**Vaccinatie van Ouderen tegen SARS-CoV-2 en Cellulaire Afweer voor de Lange termijn bescherming binnen de Doetinchem Cohort Studie**
WMO	**Medical Research Involving Human Subjects Act;**

in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Vaccination against SARS-CoV-2 is the most effective way to end the current pandemic. However, it is currently unknown which level and type of immune responses will be induced by COVID-19 vaccination in the Dutch general population. This study aims to evaluate humoral, cellular and innate immune responses induced by COVID-19 vaccines in generally healthy subjects in the Netherlands. This includes neutralizing antibodies, associated with prevention of infection by SARS-CoV-2, as well as other immune parameters that may govern vaccine-induced protection against disease and transmission. Such knowledge forms a crucial basis for a future estimation of the proportion of Dutch healthy subjects that is immune protected by vaccination, once correlates of protection are identified; and will provide a comparison of immune responses in these healthy subjects with responses in risk groups that are being assessed in other trials. Ultimately, the study supports evidence-based vaccination strategies to reach and sustain optimal population immunity.

Objective: Monitoring & evaluation of immune responses induced by COVID-19 vaccines in the general population in the Netherlands.

Study design: Longitudinal observational study.

Study population: Healthy human volunteers 18-60 years old, who receive COVID-19 vaccination as part of the national program, will be included in this study. Immune responses in subjects >60 yrs old are being assessed in other studies (VIDO study NL76551.041.21, VOCAAL study NL76719.041.21, VIVO study amendment NL74843.041.20 and VITAL study amendment NL69701.041.19) that have synchronized sampling timepoints and assays. Participants (N=450 per vaccine) will be requested to provide capillary blood by finger prick self-sampling at home at various timepoints following vaccination. A subset of subjects (90-150 per vaccine) will be invited to donate blood by venapuncture and nasal mucosal lining fluid, at various timepoints, as well as a pre vaccination fecal sample, for additional analyses. Total follow-up duration is 12 months following vaccination.

Intervention (if applicable): N.A.

Main study parameters/endpoints: The **primary parameter** of the study is COVID-19 vaccine (e.g. Spike protein)-specific serum IgG (GMC) in Dutch healthy subjects, at day 28 after completion of COVID-19 vaccination, by bead-based multiplex immune assay (MIA).

The **secondary endpoints** are, summarized:

a. Humoral, cellular and innate COVID-19 vaccine-induced immune responses
b. Virus neutralizing capacity of antibodies induced by COVID-19 vaccination
c. Fc functionality of antibodies (e.g. complement deposition) and antibody glycosylation status induced by COVID-19 vaccination
d. COVID-19 vaccine-induced antibodies in nasal mucosal lining fluid
e. Reactogenicity self-reported in questionnaires shortly after vaccination

Exploratory endpoints consist of

a. COVID-19 vaccine-induced epigenetic reprogramming of innate immune cells
b. Intestinal microbiome composition that may be related to vaccine response
c. Diversity and functional differentiation of the adaptive immune receptor repertoire induced by COVID-19 vaccination
d. Anti-vector antibodies that might interfere with viral vector 2nd dose response

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden associated with participation involves collection of finger prick blood samples by self-sampling at home for all participants (5 times max. 800 ul per finger prick). In a subset of participants, 3 of 5 finger pricks are replaced by venapuncture blood collections (4 times; 32-89 mL per visit with a max. total volume of 269 mL over 1 year), faeces collection (once) and nasal mucosal lining fluid collection by nasosorption strips (3 times) during the follow up of 1 year. In addition, all subjects will be asked to fill in questionnaires at almost all timepoints. All participants will receive COVID-19 vaccination as part of the national immunization program provided by the Dutch government, that they would have received regardless of this study. The potential risks of sampling performed within the proposed study are considered minimal. The benefit for the individual subjects in this trial is low. Results of the study will support evidence-based optimisation of vaccination strategies within the Dutch population.

Planning:

- First Subject, First Visit (FSFV): May 2021
- Last Subject, First Visit (LSFV): Depending on national program and vaccine availability, most likely Dec 2021
- Last Subject, Last Visit (LSLV): Depending on national program and vaccine availability, most likely Dec 2022
- End of Project (including follow-up and data analysis): Depending on national program and vaccine availability; most likely Dec 2023.

1. INTRODUCTION AND RATIONALE

Vaccination against SARS-CoV-2 is the most effective way to end the current pandemic. Vaccination in the Netherlands has been targeted at those with highest burden of disease and health care workers according to national policies [1, 2]. In time, the vast majority of the population will need to be vaccinated for individual and herd protection. Various vaccines are being implemented, including vaccines that are novel in architecture, such as nucleotide-based, vaccine platforms [3,4]. A spectrum of immune data from clinical trials is provided by developers and manufacturers before registration [5-8], but this is dominated by short-term antibody response data and it is to be established which level and type of immune responses will be induced by real world implementation of COVID-19 vaccination in the Dutch general population. Amplitude, longevity and quality of vaccine-induced immune responses need to be monitored in various (age) segments of healthy subjects, to be able to evaluate the proportion of individuals in the Dutch population that is protected by vaccination against COVID-19 once correlates of protection become known. This study aims to measure these immune responses induced by COVID-19 vaccines in generally healthy subjects in the Netherlands. In addition, this study provides a comparison of vaccine-induced immune responses in healthy subjects with those in risk groups that are examined in other, ZonMw-funded trials, for which major timepoints and sampling have been synchronized [9,10,11]. This study also provides a comparison of vaccine-induced immune responses to responses induced by natural SARS-CoV-2 infection (FFX study NL13529.041.06).

Prevention of infection (and thus prevention of transmission) is associated with virus neutralization, therefore current COVID-19 vaccines are focused on inducing antibodies directed against the viral (S1) Spike protein mediating host cell binding. Binding of the virus to the respiratory tract epithelium has been shown to be inhibited by neutralizing antibodies, provided these are present or are rapidly increasing in levels at mucosal surfaces at the time of infection. Neutralizing antibodies may be of the IgA, IgM and IgG isotype. IgG levels predominantly determine systemic neutralizing capacity, whereas at mucosal surfaces the contribution of IgA is prevailing. In this study IgG and IgA will be sampled systemically and from the respiratory mucosal surface (by nasal mucosal lining fluid, MLF) to determine kinetics and longevity of antibody subclasses and to measure avidity for the Spike protein domain involved in (ACE2) receptor binding. Importantly, the coronavirus is rapidly evolving away from the strain that has been used as a blue print for existing vaccines. Current SARS-CoV-2 variants express enhanced binding and infectivity patterns, most of which were indicated to have indeed evolved with a structural change in the S1 protein. It is uncertain whether current COVID-19 vaccines will induce antibody responses not only neutralizing the original SARS-CoV-2 strain but also sufficiently neutralizing these major variants. For this

reason, in this study serum IgG from a subset of participants will be tested in (pseudo)neutralization assays against original and dominant virus variants. In addition to neutralization, antibodies are capable of modulating the immune response by interacting with various (innate) immune cells and complement via their Fc-tails. These Fc-mediated effector functions likely contribute to protection [12], though it has been suggested that in severe COVID-19 cases they potentially also contribute to the development of severe disease [13,14]. This study intends to obtain a broader understanding of the profile of antibody Fc-mediated effector functions elicited by novel vaccines and vaccine platforms. Levels of antibody responses, Fc functionality and neutralizing potential will be assessed both in SARS-CoV-2-naive participants following vaccination as well as in participants with (serological) proof of infection prior to, during and after vaccination.

Importantly, though efficacy and short-term immunogenicity has been proven by pharmaceutical companies, data on cellular immunity of B- and T-cell responses, driving forces behind long-term immunity following vaccination, is limited. Induction of memory B-cells is vital as these will proliferate and differentiate into plasma cells, which will quickly reproduce antibodies upon renewed contact with the virus. Induction of CD8+ T-cell responses is pivotal for the ability to kill virus-infected cells and induction of CD4+ helper T-cell responses is needed for optimal maturation of B- and CD8+ T-cell responses. Data on induction of T-cell responses by COVID-19 vaccines are often limited to frequencies of responding cells, without specifying T-cell type (effector, memory, regulatory) or kinetics and longevity of T-cell responses for the various vaccines. In addition, underlying the induction and steering of adaptive immunity is the activation of innate immunity [15]. The current phase in this pandemic in which most people are still vaccine- and SARS-CoV-2-naïve necessitates to assess in the very near future the quality and magnitude of immune responses induced by different vaccine formulations, and to study how innate immune signatures shape the quality, type and longevity of adaptive serological and cellular immune responses. Thus, in this study innate and cellular adaptive immune responses induced by various COVID-19 vaccines will be studied in depth in a subset of participants; also these data will be compared to cellular immunity data from infected cases (RIVM/FFX study NL13529.041.06). More generally, this will contribute to the understanding of vaccine-induced biomarkers and mechanisms governing protection against disease.

In conclusion, a spectrum of immune responses induced by COVID-19 vaccines in generally healthy subjects will be studied. This analysis will contribute to understanding of vaccine-induced biomarkers of protection against disease and transmission and thereby support evidence-based vaccination strategies to reach and sustain optimal population immunity.

2. OBJECTIVES

The overall objective of the study is to monitor and evaluate immune responses induced by COVID-19 vaccines in the general, healthy population of the Netherlands; which will support future evaluations of vaccine-induced protection in the population. Immune responses will be linked with those in the older Dutch population of 60-90 years of age that will be studied under separate protocols (VIDO study NL76551.041.21, VOCAAL study NL76719.041.21, VIVO study NL74843.041.20 and Vital study NL69701.041.19).

2.1 Primary objective

- Measuring vaccine (e.g. Spike protein)-specific serum IgG GMC in Dutch healthy subjects at day 28 after completion of COVID-19 vaccination by bead-based multiplex immune assay (MIA).
-

2.2 Secondary objectives

The secondary objectives of the study are to monitor important biomarkers of innate, humoral and cellular immune responses induced by COVID-19 vaccines in the general, healthy population of the Netherlands, that are thought to be mechanistically relevant to immune protection against COVID-19. These secondary endpoints not only can and will be linked with those in the older Dutch population of 60-90 years of age that will be studied under separate protocols (VIDO study NL76551.041.21, VOCAAL study NL76719.041.21, VIVO study NL74843.041.20 and Vital study NL69701.041.19), but also provide a comparison to immune response data in ZonMw-funded risk group vaccination studies, as listed below, that have been harmonized for study objectives and endpoints.

Secondary objectives are:

- Measuring the dynamics and longevity of antibody subclasses induced by various COVID-19 vaccines;
    a. including measuring and comparing the IgG response after the 1st dose with that after completion of the 2nd dose;
    b. including to provide a comparison of immune response data in these healthy subjects with those in COVID-19 risk groups examined in ZonMw-funded risk group vaccination studies (details provided in ref. [9,10,11]):
        - Primary immune deficiency patients (VACOPID study)
        - Patients with kidney failure, dialysis treatment and/or kidney transplant (RECOVAC study)

- Patients with pulmonary disease who will/have receive(d) a lung transplant (COVALENT study)
- Autoimmune disease patients (Target to B! (sub)study)
- People with Down syndrome (PRIDE study)
- People living with HIV (COVIH study)
- Cancer patients and patients on cancer treatment (VOICE study)

Haematological patients (COBRA-KAI study);c. including to compare vaccine-induced immune responses to responses to natural SARS-CoV-2 infection (FFX study NL13529.041.06).

- Measuring vaccine-induced antibody binding and capacity for (pseudo)neutralization of circulating SARS-CoV-2 variants;
    a. in addition, serum from day 28 after completion of vaccination from a subset of participants will be stored for analysis of neutralizing capacity of future SARS-CoV-2 variants.
- Measuring the impact of pre-existing immunity to SARS-CoV-2 (seropositivity at baseline) on the serum antibody response after 1 and after 2 doses of vaccination.
- Assessing the magnitude, characteristics and longevity of the adaptive cellular response induced by various COVID-19 vaccines, in a subset of participants;
    a. including to provide a comparison of cellular responses in healthy subjects in this trial for responses in risk groups that are examined in other trials [9,10,11];
    b. including to compare vaccine-induced cellular immune responses to responses to natural SARS-CoV-2 infection (FFX/RIVM study NL13529.041.06).
- Measuring the (functional) characteristics of the innate immune response that is activated by various COVID-19 vaccines, in a subset of participants.
- Measuring (subclasses of) antibodies in nasal mucosal lining fluid (MLF), induced by various COVID-19 vaccines, in a subset of participants.
- Measuring functional Fc capacity and glycosylation status of antibody responses induced by various COVID-19 vaccines, in a subset of participants.
- Measuring (local and systemic) reactogenicity by self-reporting in a digital questionnaire in a subset of participants, to assess the relation between reactogenicity and ensuing vaccine response.
- Comparison of functional characteristics of humoral, cellular and innate responses (in a subset of participants) induced by different COVID-19 vaccines.
-

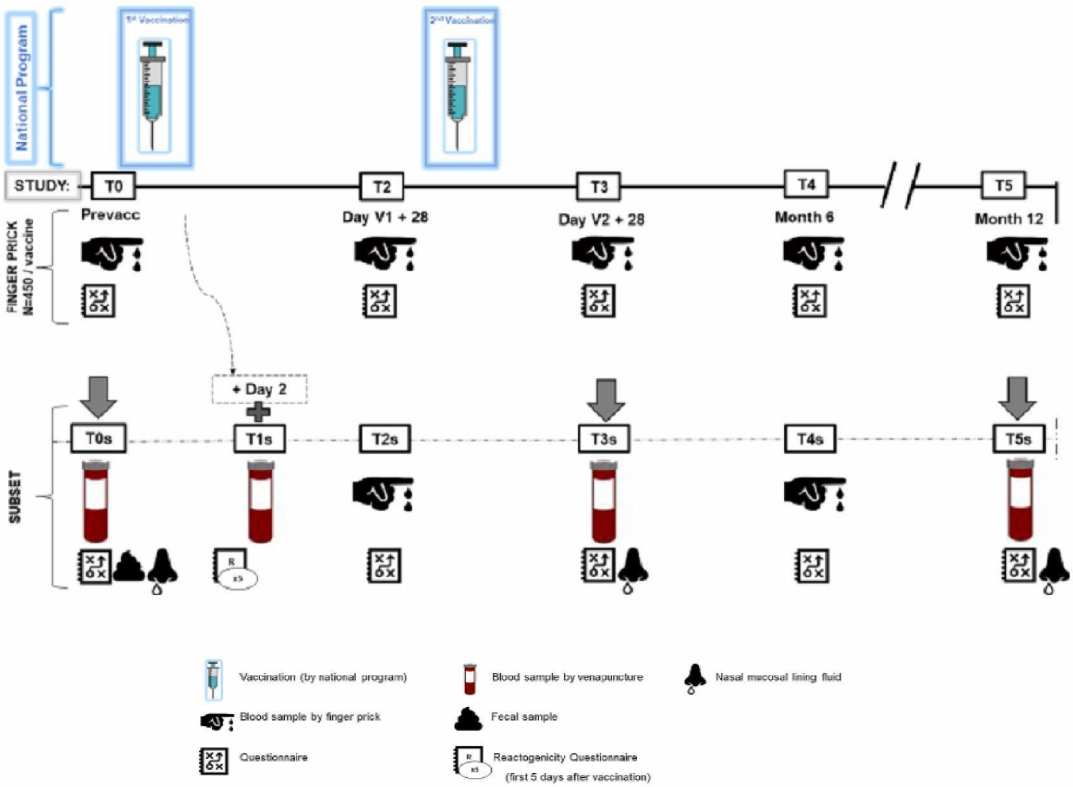
2.3 Exploratory objectives

- Assessing training of immunity of innate cells (myeloid progenitors [16]) in a subset of participants, to explore epigenetic reprogramming by various COVID-19 vaccines.
- Assessing intestinal microbiome by fecal sampling shortly pre vaccination in a subset of participants, to explore immune regulation of vaccine responses by the intestinal microbiome.
- Assessing diversity and functional differentiation status of vaccine-induced CD4+ and CD8+ T-cells at the single cell level in a subset of participants, to explore the cellular immune receptor repertoire induced by various COVID-19 vaccines.
- Assessing anti-vector antibodies in a subset of participants, to explore possible interference with COVID-19 viral vector-based second vaccine dose response.

3. STUDY DESIGN

This is a longitudinal study, wherein vaccine responses will be measured at different time points throughout a 12-month follow up period. Timepoints were selected by aligning with studies that are being prepared for evaluation of immune responses following vaccination of risk groups [9,10,11].

Figure 1 Setup of the study with timeline



All subjects will receive COVID-19 vaccination as provided by the Dutch government. Participants will be vaccinated either by their GP (general practitioner), the GGD or via provisional vaccination sites. Participants will communicate the date of vaccination(s) and vaccine brand to the study investigators.

For the primary and secondary serological aims, participants will be asked to donate a finger prick blood sample by self-sampling at home at 5 timepoints (T0, T2, T3, T4, T5). Participants will receive the necessary materials and a special return envelope.

All participants are requested to complete questionnaires on COVID-19, sampling and general health status at all timepoints. At baseline, participants will be requested to complete questionnaires on quality of life (SF-36 and EQ-5D) for detailed description of baseline characteristics as in linked studies (VIDO study NL76551.041.21, VOCAAL study NL76719.041.21, VIVO study NL74843.041.20 and Vital study NL69701.041.19).

Table 1: Definition of timepoints and sampling for general participants

All subjects	Timepoint	Sampling
T0	Pre vaccination sample (week -2 to 0)	Self-sampling finger prick
T2	Day 28 (± 2 days) after 1st Dose (before 2nd dose) If 2nd Dose of vaccination is before day 28, then T2 is the day (-2-0 days) before 2nd Dose of vaccination	Self-sampling finger prick
T3	Day 28 (± 2 days) after 2nd Dose (or completion) of vaccination T3 = T2 if vaccination series consists of 1 Dose*	Self-sampling finger prick
T4	6 Months (±2 weeks) after completion of vaccination	Self-sampling finger prick
T5	12 Months (±2 weeks) after completion of vaccination	Self-sampling finger prick

**This applies to vaccine posology consisting of one dose as registered. It also applies to participants who were tested COVID-19-positive less than 6 months before vaccination and who may be vaccinated with only one dose, as recently advised by the Gezondheidsraad [17].*

A subset of participants will be asked to donate larger blood samples by venapuncture for additional analyses, during home visits by a research nurse, at 4 timepoints (labeled 's' for subset: T0s, T1s, T3s, T5s). These timepoints replace corresponding self-samplings. Of note: in this subset of participants, timepoints with blood sampling by finger prick and not venapuncture (day 28 after 1st vaccination and 6 months following completion of vaccination, T2s and T4s, resp.) are still labeled 's' for clarity as denoted in figure 1. All participants of the subset have given consent in the ICF to take part in this subgroup.

Pre-vaccination (week -2 to 0; T0s) blood will be sampled for (max.) 71 mL. At day 2±1 day after vaccination (T1s; where vaccination day is counted as day '0'), blood will be sampled for (max.) 32 mL, to measure the innate immune response in this subset of participants. Samples for cellular and (functional) antibody analysis will be collected 28 days and 12 months post-vaccination (T3s, T5s; blood sampled for (max.) 89 mL and 77 mL, resp.).

Also, in this subset of participants, faeces samples will be collected at baseline (T0s) for microbiome analysis (exploratory aim); and nasal mucosal lining fluid (MLF) will be collected by nasosorption devices at baseline (T0s), at day 28 (T3s) and at 12 months (T5s) following completion of vaccination, by the research nurse. This subset of participants will be asked to complete a digital questionnaire on (local) reactogenicity during the first 5 days after the 1st dose of vaccination or until symptoms have subsided.

Table 2: Complete timepoints and sampling in a subset of participants

Subset	Timepoint	Sampling during home visit
T0s*	Pre vaccination sample: week -2 to 0 but as close to vaccination as possible	Blood sample by venapuncture (replacing finger prick by self-sampling) Nasal MLF Faeces sample
T1s	Day 2 ± 1 day after 1st Dose of vaccination	Blood sample by venapuncture
T2s**	Day 28 (± 2 days) after 1st Dose (before 2nd dose) If 2nd Dose of vaccination is before day 28, then T2s is the day (-2-0 days) before 2nd Dose of vaccination	Self-sampling finger prick
T3s	Day 28 (± 2 days) after 2nd Dose of vaccination or completion of vaccination	Blood sample by venapuncture (replacing finger prick by self-sampling) Nasal MLF
T4s	6 Months (± 2 weeks) after completion of vaccination	Self-sampling finger prick
T5s	12 Months (± 2 weeks) after completion of vaccination	Blood sample by venapuncture (replacing finger prick by self-sampling) Nasal MLF

**Participants in this subset of the study will be asked to fill in a digital reactogenicity questionnaire during the first 5 days after the first vaccination, to score local reactogenicity daily.*

***T2s will no longer apply in case of one-dose vaccination and thus sampling will occur as T3s by venapuncture. This is the case when vaccine posology consists of one dose as registered, or when participants who were tested COVID-19-positive less than 6 months before vaccination are vaccinated with only one dose as was recently advised [17].*

In Chapter 8, a detailed overview is given of sampling for all participants and broken down by vaccination series (one or two -dose vaccines).

4. STUDY POPULATION

4.1 Population (base)

Adults (N=450 per vaccine) aged 18-60 years can participate in the study. Subjects can be invited to participate in this study in multiple ways:

1. Via participation in the VASCO Study (Vaccine Study COvid-19; NL76815.056.21), a population-based prospective cohort study on vaccine effectiveness of COVID-19 vaccines in the Netherlands by EPI-RIVM/UMCU/Julius. In the VASCO Study, participants are invited by random sampling from the BasisRegistratie Personen (BRP). Participants who consent to be contacted for additional research will be invited.
2. By random sampling from the BasisRegistratie Personen (BRP; excluding subjects already invited for VASCO).

Of note, elderly > 60 yrs old are not included in this study but are included in separate protocols (VITAL NL69701.041.19, VIDO NL76551.041.21, VOCAAL NL76719.041.21 and VIVO NL74843.041.20). These three latter studies capitalize on the infrastructure and data provided by the ongoing longitudinal Doetinchem Cohort Study (DCS; NL63779.041.17) and aim to follow up vaccine-induced immune responses in elderly persons related to age and frailty; as immune variability was previously demonstrated to increase in this cohort (from 60 yrs old) with age.

Within this study, immune responses to vaccines implemented < 60 yrs old according to national policy, are evaluated. Depending on national policy on vaccine use and vaccine availability, different vaccines will be implemented and implementation may vary by age. Therefore, 3 age segments have been defined between 18 and 60 years: range 18-30 years, range 30-45 years and range 45-60 years. Per vaccinated age segment 150 participants will be included (thus max. 450 participants per vaccine in total if the same vaccine is implemented in 3 age segments). Though currently unsure, it is possible that in addition to the 4 currently licensed COVID vaccines (Pfizer/BioNTech, Moderna, AstraZeneca/Oxford and Janssen; in which case 1800 participants would be included in the study) additional vaccines will be implemented between 18-60 years.

From the 150 participants per vaccinated age segment, between 30 and 50 per vaccinated age segment will be included in the (optional) subset of participants for additional sampling and analyses (this means per vaccine a maximum of 90 - 150 subset participants of the max. total 450 participants per vaccine, divided over 3 age segments). Inclusion will be adjusted if needed (e.g. by sending extra invitations to participate) to ensure a balanced representation of the various age segments. Statistical analyses will, per vaccine, be stratified by these age segments (see Chapter 9).

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Be 18 – 60 years at the time of inclusion
- Be capacitated mentally and physically
- Be willing to receive SARS-CoV-2 vaccine
- Having signed the Informed Consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study (also when arising during the trial):

- Having had a previous SARS-CoV-2 vaccine; or participation in a phase I/II/III vaccination trial where the subject will be vaccinated with a pre-registration (COVID-19) vaccine
- Participation in a phase I/II/III medicine (pre-registration) trial
- Belonging to a risk group for COVID-19 that is studied in one of the ZonMw-funded risk group vaccination studies (details of risk group studies provided in ref. [9,10,11]) that this study provides a comparison for:
    - Primary (inherited) immune deficiency (VACOPID study)
    - Severely decreased kidney function (defined as Chronic Kidney Disease stage 4 or 5 (eGFR<30)), treatment by dialysis or recipient of a kidney transplant (RECOVAC study)
    - Pulmonary disease for which the patient will receive or has received a lung transplant (COVALENT study)
    - Autoimmune disease (e.g. MS, rheumatoid arthritis, IBD, SLE etc) (Target to B! (sub)study)
    - Down syndrome (PRIDE study)
    - (Known) infection with Human Immunodeficiency Virus (HIV) (COVIH studie)
    - Cancer patients and patients with active cancer treatment (including hormone therapy), receipt of chemotherapy in the last 3 years and/or any history of cancer immune therapy (VOICE study)
    - Haematological patients, such as haematological malignancies (leukemia and lymphomas), myelodysplastic and -proliferative syndromes,

hemoglobinopathies (sickle cell disease and thalassemia), receipt of stem cell transplantation or cell therapy such as CAR T-cell therapy (COBRA-KAI study)

- Any other immune deficiency through disease
- Active or past immunosuppressive or immune modulating medication.
- However, for steroid treatment the exclusion criteria are: receipt of any high-dose (≥ 20 mg of prednisone daily or equivalent) steroid treatment; daily corticosteroids (locally, incl. inhaled steroids, are acceptable) within 2 weeks of study entry; or repeated use of any high dose of corticosteroids (a dose of > 30 mg of prednisone or equivalent per day for multiple days) in the recent past.
- Women who are pregnant or breastfeeding
- Having a (functional) asplenia
- Receipt of blood products or immunoglobulin, within 3 months of study entry
- Receipt of an organ transplant not mentioned above
- For the subgroup: Known or suspected coagulation disorder, also by treatment, that would contraindicate undergoing frequent blood sampling

4.4 Temporary exclusion criteria

A potential subject who meets any of the following criteria will be temporary excluded from participation in the study subgroup:

- If a subject has an elevated body temperature fewer than 48 hours before a visit with blood sampling the visit should be postponed until this criterion is no longer met
- Receipt of any other vaccine(s) 2 weeks before blood collection

Pregnancy during the study is not an exclusion criterium, however immune responses in pregnant participants will be analyzed separately (chapter 10).

4.5 Sample size calculation

The primary objective of this study is to measure vaccine (e.g. Spike protein)-specific IgG GMC at day 28 after completion of COVID-19 vaccination (primary parameter) in healthy subjects.

In this study,with 150 participants per vaccinated age group, we are able to estimate the GMC and 95% confidence interval with 0.13 precision on a natural log scale, assuming a standard deviation of the IgG level on a natural log scale of 0.8. The standard deviation of 0.8 was conservatively based on serum IgG GMC data from phase I/II vaccine trials in subjects 18-55 years old, of which publications of the Moderna vaccine [6], Pfizer vaccine [8] and

AstraZeneca vaccine [5] could be analyzed for variability. Here, for example, with 150 participants per vaccine and age group, we would estimate a 95% confidence interval of 8,800-11,400 around a GMC of 10,000. In total, we thus aim to include 450 participants per vaccine equally divided over three age groups (18-30 years, 30-45 years and 45-60 years), depending on national policy on vaccine use and vaccine availability (section 4.1),
This cohort of healthy participants will also serve as a control cohort for other studies performed in the Netherlands investigating immunogenicity of COVID-19 vaccines in specific risk groups. These risk group vaccination trials generally include 100-200 subjects per cohort [9,10,11]. Here, with 150 participants per vaccine and age group, we have 90% power to detect a GMC ratio of 1.35 between healthy controls and a specific risk group, with 5% significance level and an assumed standard deviation on the natural log scale of 0.8 (under the assumption that the standard deviation is equal for these groups).

The sample size of the subset of participants for additional analyses is based on the sample size required for cellular immunity assays. Cellular immunological parameters aimed in this study are secondary and exploratory endpoints, supporting immunogenicity evaluation in the general vaccinated population in a mostly descriptive manner. Cellular endpoints are expected to be rather variable and (donor) dependent and are typically assessed in labour-intensive assays including in vitro culturing and maturation of lymphocytes before specific and functional read-out. No standardized SARS-CoV-2 specific B- and T-cell assays are currently available. Yet across published platforms used in studies assessing B- and T-cellular immunity in COVID-19 case cohorts or vaccine trials [18-20), as well as across our own numerous immunogenicity studies in COVID-19 and other infectious diseases (e.g. [21]), typical trends can be revealed. These include that the two major drivers of variation in specific cellular immunity endpoints are donor age and time elapsed since the immunizing event (infection or vaccination). To take this into account, specific B- and/or T-cell analysis in such studies is typically evaluated per age subgroup and per time point, with group sizes ranging between 15-40 participants per subgroup. Therefore in this study, to capture the variation in B- and T-cell immunogenicity of COVID-19 vaccination in the overall population, we aim to include subgroup sizes of minimally 30 participants per age stratum (minimally n=30 and maximally n=50) for cellular analysis. Although the results will be descriptive, longitudinal and cross-sectional trends in the induction and maintenance of B- and T-cell responses will be assessed and compared at the subgroup level using t-tests, (M)ANOVA and non-hypothesis driven biostatistical analysis.

5. TREATMENT OF SUBJECTS

Not applicable. Vaccination will be provided by the Dutch government. This study only investigates immune responses and reactogenicity. If subjects had not participated in this study, they would have received the same vaccine without additional testing.

5.1 Investigational product/treatment

COVID-19 vaccines are provided by the Dutch government and are administered as part of the national vaccination program. Therefore, this intervention is not part of our study. A flowchart with the current strategy can be found at: *https://www.rijksoverheid.nl/onderwerpen/coronavirus-vaccinatie/documenten/publicaties/2021/01/13/flowchart-vaccins*

All information on COVID-19 vaccines can be found in the SmPC as approved by the European Medicines Agency (EMA) and College ter Beoordeling van Geneesmiddelen (CBG; www.geneesmiddeleninformatiebank.nl).

5.2 Use of co-intervention

-
- Not applicable.
-

5.3 Escape medication

-
- Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Depending on the vaccination strategy of the Dutch Government the subject will receive one of the available COVID-19 vaccines.
SmPCs of the currently available vaccines are provided.

6.2 Summary of findings from non-clinical studies

Not mentioned.

6.3 Summary of findings from clinical studies

Summary of findings from clinical studies can be found in COVID-19 Vaccine Summary of Product Characteristics section 5.

6.4 Summary of known and potential risks and benefits

Summary of known risks can be found in COVID-19 Vaccine Summary of Product Characteristics (SPC) section 4.

6.5 Description and justification of route of administration and dosage

Not applicable, as the vaccine is not administered in the trial.

6.6 Dosages, dosage modifications and method of administration

Not applicable, as the vaccine is not administered in the trial.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable, as the vaccine is not administered in the trial.

6.8 Drug accountability

Not applicable, as the vaccine is not administered in the trial.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

- 1. Primary parameter of the study is vaccine (e.g. Spike protein)-specific IgG GMC at day 28 after completion of COVID-19 vaccination (T3, T3s), using the bead-based multiplex immune assay (MIA; [22]).
-

8.1.2 Secondary study parameters/endpoints (if applicable)

2. IgG/IgA/IgM GMC reactive to vaccine antigen (e.g. Spike protein) in serum at baseline and following vaccination, in all participants, by MIA.

   a. Antibody kinetics will be compared to those in specific risk groups (ref. [9, 10,11] and as specified in Secondary Objectives) and represented correspondingly as GMC and proportion seroconversion (seropositive compared to baseline; proportion responders (seropositive/seronegative)).

3. IgG seropositivity for SARS-CoV-2 (eg. N-protein; including at baseline), in all participants, by MIA.

4. IgG antibody avidity following vaccination, which determines antibody binding strength and maturation, in all participants, by MIA.

5. (Pseudo)neutralization potential (virus neutralization titer (VNT)) of S1-recombinant virus at day 28 following completion of vaccination, in a subset of participants (T3s)

6. Phenotype and function (such as memory, follicular and regulatory) of CD4+ and CD8+ T-cells, by antigen stimulation of PBMC followed by e.g. ELISpot, supernatant analysis for cytokines and Fluorescence-Activated Cell Sorting (FACS), in a subset of participants (T0s, T3s, T5s);

   a. Cellular characteristics will be compared to specific risk groups [9,10,11] and to cellular responses following natural SARS-CoV-2 infection assessed in another trial (FFX/RIVM study, number NL13529.041.06).

7. Phenotype and functional properties of vaccine-acquired memory B-cells, by antigen stimulation of PBMC followed by ELISpot and limiting dilution procedures to establish clonally derived memory B-cells, in a subset of participants (T3s, T5s).
8.
9. Vaccine-activated number and phenotype of innate cells, cytokine/chemokine production by innate cells, serum proteome and gene expression profile of PBMC/monocytes, in a subset of participants, 2 days after vaccination (T1s) compared to baseline (T0s).
10.
11. IgA1/IgA2 and IgG GMC in nasal mucosal lining fluid (MLF) in a subset of participants, at baseline and at 28 days and 12 months following completion of vaccination (T0s, T3s, T5s).
12.
13. Vaccine-specific antibody phagocytic score, antibody-mediated cellular cytotoxicity score and antibody-mediated complement deposition score; and antibody glycosylation status, in a subset of participants (T0s, T3s, T5s).
14.
15. Local reactogenicity of the vaccine injection site and systemic reactogenicity, as reported in digital questionnaires during the first 5 days following the 1st dose of vaccination, in a subset of participants.
16.
17.

8.1.3 Other study parameters (if applicable)

18. Epigenetic changes of isolated monocytes by ATAC-seq (Assay for Transposase-Accessible Chromatin Sequencing) and (long term) ability for cytokine production of isolated monocytes, in a subset of participants (T3s).
19.
20. Microbiome composition of faeces collected at baseline (T0s) by next generation sequencing, in a subset of participants.

21. Diversity, functional differentiation status and maintenance of the adaptive immune receptor repertoire induced on a T-cellular / clonal level by single cell sorting, in a subset of participants (T3s, T5s).
22.
23. Anti-vector antibody GMC and vaccine-antigen (e.g. Spike protein)-reactive IgG 2nd vaccine dose response in serum, in a subset of participants (T0s, T3s)
24.

8.2 Randomisation, blinding and treatment allocation

Not applicable.

8.3 Study procedures

Persons that received study information, show interest in the study (by filling in an application form) and are willing to be vaccinated will receive an instruction email with a login code for access to the pre-study digital questionnaire (verifying in- and exclusion criteria). Thereafter, applicants receive an email where we explain if they fulfil the criteria for participation or not. In case we are not sure if a potential participant fulfils the criteria, we will contact that person. If the persons adhere to the in- and exclusion criteria, they receive the finger prick set, with IC forms. Thesubset participants will be contacted by phone to plan the home visits and - if needed - ask questions. The subset participants will then receive the feces collection set with the IC forms. The informed consent form (ICF) is pre-signed by the investigator and signed by the subject before inclusion in the study (further described in section 11.2). Participants receiving the finger prick set return the signed IC form by mail. The subset participants give the signed IC and feces sample to the study nurse during the home visit.

During office hours the study team is available for questions, concerning participation, IC signing and other questions.

Table 3-6 depict timepoints and sampling for participants including maximum blood volume for finger prick and venapunctures; and structured by vaccination posology (one or two-dose vaccine series).

Table 3 depicts timepoints and sampling for all participants that are vaccinated with a SARS-CoV-2 vaccine posology consisting of 1 Dose:

Vaccination with 1 Dose (vaccine administration is not part of the study)				
Timepoint	T0 Baseline (-2 till 0 weeks)	T2 = T3 Day 28 (± 2 days)	T4 Month 6 (± 2 weeks)	T5 Month 12 (± 2 weeks)
Obtain Informed Consent	x			
Blood collection by finger prick (800 µL)	x	x	x	x
Questionnaires: -Standard sampling	x	x	x	x

questionnaire -Corona questionnaire -General health status				
Questionnaire on quality of life	x			

Table 4 depicts timepoints and sampling for participants that are vaccinated with a SARS-CoV-2 vaccine posology consisting of 2 Doses:

Vaccination with 2 Doses (vaccine administration is not part of the study)	V1		V2		
Timepoint	T0 Baseline (-2 till 0 weeks)	T2 Day 28 (± 2 days)	T3 Day 28 (± 2 days)	T4 Month 6 (± 2 weeks)	T5 Month 12 (± 2 weeks)
Obtain Informed Consent	x				
Blood collection by finger prick (800 µL)	x	x	x	x	x
Questionnaires: -Standard sampling questionnaire -Corona questionnaire -General health status	x	x	x	x	x
Questionnaire on quality of life	x				

Table 5 depicts all timepoints and sampling for the subset of participants that consent to donate additionally by venapuncture and to provide mucosal and faeces samples; and who are vaccinated with a SARS-CoV-2 vaccine posology consisting of 1 Dose:

Vaccination with 1 Dose (vaccine administration is not part of the study)					
Timepoint	T0s* Baseline (-2 till 0 wks)	T1s Day 2 (±1 day)	T3s* Day 28 (± 2 days)	T4s Month 6 (± 2 weeks)	T5s* Month 12 (± 2 weeks)
Obtain Informed Consent	x				
Blood collection by finger prick (800 µL)				x	
Blood collection by venapuncture:	x	x	x		x
-Serum tubes	1x8mL	1x5mL	1x8mL		1x5mL
-Heparin tubes	7x9mL	3x9mL	9x9mL		8x9mL
-Total max blood volume (mL)	71	32	89		77
Nasal mucosal lining fluid	x		x		x
Faeces collection	x				
Questionnaires: -Standard sampling questionnaire -Corona questionnaire -General health status	x		x	x	x
Questionnaire on quality of life	x				
Local Reactogenicity Questionnaire		vaccination day till 5 days after vaccination			

**For this timepoint serum obtained from venapuncture replaces the finger prick in this subset of participants. It was recently demonstrated that SARS-CoV-2 specific IgG can be as accurately detected in capillary blood by bead-based multiplex immune assay (MIA) as in serum obtained by venapuncture [22]. Of note: in this subset of participants, timepoints with blood sampling by finger prick and not venapuncture (day 28 after 1st vaccination and 6 months following completion of vaccination) are still labeled 's' for clarity.*

Table 6 depicts all timepoints and sampling for the subset of participants that consent to donate additionally by venapuncture and consent to provide mucosal and fecal samples; and who are vaccinated with a SARS-CoV-2 vaccine posology consisting of 2 Doses:

Vaccination with 2 Doses (vaccine administration is not part of the study)	V1			V2		
Timepoint	T0s* Baseline (-2 till 0 weeks)	T1s Day 2 (±1 day)	T2s Day 28 (± 2 days)	T3s* Day 28 (± 2 days)	T4s Month 6 (± 2 weeks)	T4s* Month 12 (± 2 weeks)
Obtain Informed Consent	x					
Blood collection by finger prick (800 µL)			x		x	
Blood collection by venapuncture:	x	x		x		x
-Serum tubes	1x8mL	1x5mL		1x8mL		1x5mL
-Heparin tubes	7x9mL	3x9mL		9x9mL		8x9mL
-Total max blood volume (mL)	71	32		89		77
Nasal mucosal lining fluid	x			x		x
Faeces collection	x					
Questionnaires: -Standard sampling questionnaire -Corona questionnaire -General health status	x	x	x	x	x	x
Questionnaire on quality of life	x				x	x
Local Reactogenicity Questionnaire		vaccination day till 5 days after vaccination				

**For this timepoint serum obtained from venapuncture replaces the finger prick in this subset of participants. It was recently demonstrated that SARS-CoV-2 specific IgG can be as accurately detected in capillary blood by bead-based multiplex immune assay (MIA) as in serum obtained by venapuncture [22]. Of note: in this subset of participants, timepoints with blood sampling by finger prick and not venapuncture (day 28 after 1st vaccination and 6 months following completion of vaccination) are still labeled 's' for clarity.*

8.4 Procedures for sample collection, labelling, processing and storage

All collected samples will be labelled with the unique subject number and a bar code that is linked to the subject number, time point of collection or visit number. All collected samples at home visits will be transported to the RIVM on the day of collection. Venapuncture and the collection of the nasal MLF samples will be performed by qualified and authorized staff. In case the blood collection fails a second attempt can be made, but only if the participant agrees. Blood collected via finger prick will be sent in a sealed envelope to the RIVM. After collection, blood samples will be kept at room temperature and will be processed either for direct analyses requiring fresh material or for storage prior to further use and analysis (serum at -80 ^{0}C, peripheral blood mononuclear cells (PBMCs) at -135 ^{0}C, respectively) according to the study's standard operating procedures (SOPs).

8.4.1 Blood

Blood collection by finger prick: the participants will be asked to donate a finger prick blood sample (maximum of 0.8 mL) by the provided self-sampling set according to the detailed instructions, and are requested to return the sample(s) in the stamped, addressed safety-envelope provided. This method has been tested extensively in previous and ongoing large scale serological studies [23,24]. Upon arrival blood samples will be processed for serum separation and subsequently aliquoted and stored at -80 °C. Serum samples will be tested for the quantitative and qualitative presence of SARS-CoV-2 specific antibodies by selected immunoassays.

Venapuncture, serum tubes: Serum is stored in aliquots at -80°C. Serum will be used for all systemic antibody measurements, analysis of antibody functionality and glycosylation, (pseudo)neutralization assays and (innate) serum proteome profiling.

Venapuncture, heparin tubes: Blood collected in heparin tubes will be used for PBMC isolation by Ficoll density gradient using standard techniques. PBMCs will be stored at -135°C. PBMCs are used for analysis of presence and frequencies of innate and SARS-CoV-2-specific B and T cells. As adaptive cellular responses are expected to peak around day 28 following completion of vaccination (T3s), this timepoint has most heparin tubes (9 tubes) sampled of all timepoints.

Antibody-secreting B cells and IFN-gamma producing T cells will be measured semi-quantitatively and semi-high-throughput e.g. by ELISPOT assays. The number of cells required for ELISPOTs is 10-20 million PBMCs per subject per timepoint. Once identified, the innate and adaptive immune responses will be further characterized at the single cell level by flow cytometry (FACS) and *in vitro* stimulation assays. Sorting

and analysis of SARS-CoV-2 specific T-cells will be guided by activation-induced marker expression or by binding to HLA-epitope tetramers. In-depth receptor repertoire or transcriptome analysis will only be applicable for those participants having given consent for genetic analysis, including HLA typing.

Participants from whom, at any given timepoint, the preferred number of tubes cannot be sampled thus yielding a smaller blood volume by venapuncture, will explicitly not be withdrawn from the study. In these participants, cellular assays will be prioritized using the objectives and methods described.

Plasma will be stored in aliquots at -80°C for plasma-specific markers (e.g. CRP) and as a potential back up for other serological assays.

8.4.2 Nasal mucosal lining fluid (MLF)

Mucosal lining fluid (MLF) will be collected using Synthetic Absorptive Matrix (SAM) strips, as these were demonstrated to lead to the recovery of a more concentrated sample of material [25]. Following transportation to the laboratory, freshly collected MLF samples will be stored in freezers at -80ºC, until further testing.

8.4.3 Faeces

Fecal samples will be collected as close as possible to the start of the vaccination series. A sampling kit and detailed instructions will be sent to subgroup participants: participants will be requested to put a small amount of faeces in a container with DNA/RNA shield buffer and preserve the sample at room temperature until the research nurse' visit. Following transportation to the RIVM, samples will be stored at -80°C on arrival, for future microbiome characterization by next generation sequencing. If the fecal sample could not be collected during the visit the participant can send the sample to the RIVM later in a return envelope.

8.4.4 Genetic analyses

Genetic analysis will only be performed in samples of participants who have given informed consent explicitly for genetic analysis. In this study, COVID-19 vaccine-induced epigenetic reprogramming of innate immune cells (exploratory objective) will be assessed in isolated monocytes by ATAC-seq (Assay for Transposase-Accessible Chromatin Sequencing). Furthermore, in the subgroup of participants, genetic analyses include in-depth receptor repertoire and transcriptome analysis of adaptive cells, as well as HLA-typing. The latter also assists in isolation of SARS-CoV-2 specific T-cells by binding to HLA-epitope tetramers.

8.4.5 Questionnaires

Health assessment questionnaires on quality of life (T0, T0s; SF36 and EQ-5D) will be completed digitally by the subject at baseline. At every timepoint, a standard sampling questionnaire, general health status questionnaire and a corona questionnaire (containing questions on COVID-19 clinical symptoms and/or positive tests) will be included.

In the subgroup, a digital questionnaire on reactogenicity (redness, swelling, pain of vaccine injection site, fever, etc) will be completed digitally every day during the first 5 days after 1st vaccination or until symptoms subside.

8.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. If a subject decides to withdraw from the study, the samples and data collected up to that point will be used in the analysis. In case the subject doesn't approve storage of the collected samples after the study is finished, samples will be destroyed after the study analysis is considered complete.

The investigator can decide to withdraw a subject from the study for urgent medical reasons or health concerns. Depending on the event, follow up may require referral to the general physician.

8.5.1 Specific criteria for withdrawal (if applicable)

Not applicable.

8.6 Replacement of individual subjects after withdrawal

Subjects will not be replaced after withdrawal.

8.7 Follow-up of subjects withdrawn from treatment

Not applicable.

8.8 Premature termination of the study

The sponsor may end the study prematurely if this is beneficial to the health or welfare of the subjects, if continuation no longer serves a scientific purpose, and if this is confirmed by the METC. The METC will be informed about the decision. Premature

termination has no effect on the subjects. In case the study is terminated prematurely, subjects will be notified about this. All study materials should be returned to the sponsor as soon as possible.

9. SAFETY REPORTING

SARS-CoV-2 vaccines have been granted a conditional marketing authorization. These vaccines are given by the participants' own GP or the GGD as part of the immunization program provided by the Dutch government and are not part of this study.

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or trial procedure.

The study staff will only record any adverse event spontaneously reported by the subject related to and occurring within one week after nasal fluid collection or blood sampling.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Serious adverse event related to blood collection or nasal mucosal fluid collection occurring within a week after blood sampling will be recorded, provided they take place during the home visit, or if reported by the subject. The sponsor will report these SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported yearly in a line listing to the METC.

If a subject reports in the month after vaccination an SAE, possibly related to vaccination, we will take the following steps; ask the subject if the SAE has been reported to Lareb (by the subject/GP/GGD); if not ask the subject to report to Lareb; if the subject is not willing/able to report to Lareb, ask the subject if we can report to Lareb.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions (AR) are all unfavorable and unintended responses to an investigational product related to any dose administered.
Unexpected adverse reactions are SUSARs if the following three conditions are met:

- the event must be serious (see chapter 9.2.2);
- there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
- the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

These vaccines are provided by the Dutch government and are not part of this study. The vaccines are given by the GP or GGD. They are responsible for the correct handling of any side effects regarding vaccination. We also have no access to the medical records related to a SUSAR.

If a subject reports a SUSAR in the month after vaccination, we will take the following step; call the METC to discuss if reporting through ToetsingOnline is needed.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

Geometric mean concentrations (GMCs) and 95% confidence intervals (CIs) will be calculated for vaccine-specific IgG in serum. Overall GMC for vaccine-specific antibodies, stratified by age, will be compared to baseline concentrations per vaccine. Longitudinal data will be analyzed with appropriate statistical tests taking into account that samples are paired (analysis further described in 10.2).

10.2 Secondary study parameter(s)

A detailed description of the number of participants will be given; i.e. by age, gender, vaccine, and of all the variables in the questionnaires. Appropriate descriptive statistics will be presented. Categorical variables will be summarized by means of frequency counts and percentages, whereas for continuous variables mean, standard deviation, minimum, maximum, median, where appropriate, will be presented.

GMC of antibodies of various subclasses will be determined at all time-points per vaccine and per age group. Kinetics of antibody responses, including longevity of responses in vaccinated age groups, will be evaluated. Immune responses in subjects who report pregnancy in questionnaires during the study will be analyzed separately for possible differences in immune response. Summary statistics for concentrations and titers of neutralizing antibodies will be reported and possible correlations between virus neutralizing titers (VNT) and the primary endpoint IgG GMC will be analyzed, including the neutralizing capacity of antibodies towards new variants in neutralization assays. Longitudinal and cross-sectional trends in the induction and maintenance of B- and T-cell responses will be assessed and compared between vaccinated age groups at the subgroup level; and possible correlations with the primary endpoint IgG GMC will be evaluated. To test whether there are statistically significant differences, t-tests (or a non-parametric test if appropriate) will be performed for continuous variables and chi-square tests (or Fisher's Exact test if appropriate) will be performed for categorical variables. Differences will if necessary be adjusted by means of regression analysis or stratification.

For the further comparison of antibody responses to other linked protocols (VIDO, VIVO, VOCAAL from the Doetinchem Cohort Studies (>60 yrs) and the VITAL study), all data will be used as continuous variables. Antibody levels and age will be considered as continuous 'response' variables. Multiple covariates and their interactions will be explored by linear regression models. If needed the Benjamini-Hochberg method will be used to take into account multiple testing.

Expertise for the analyses is present within RIVM, and code for analyses is operational.

10.3 Other study parameters

The results of the health questionnaire will be presented as descriptive statistics.

10.4 Interim analysis (if applicable)

Interim analysis is expected to take place once for a COVID-19 vaccine a primary endpoint has been met by the majority of participants receiving that vaccine. Interim analysis may further depend on public health signals and/or signals from risk group vaccination studies.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This clinical study will be conducted conform the current rules for Good Clinical Practice (GCP), as described by the Committee for Proprietary Medical Products (CPMP) of the European Union and the International Committee on Harmonisation (ICH) in "Note for Guidance on Good Clinical Practice, document CPMP/ICH/135/95", effective since January 17th 1997,Clinical Trial Directive 2001/20/EC, the principles of the Declaration of Helsinki (The World Medical Declaration of Helsinki adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and last amended by the 649th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

The EU General Data Protection Regulation (GDPR) of the European Parliament and of the Council (2018) will be followed.

11.2 Recruitment and consent

In the VASCO Study, participants are invited by random sampling from the Basisregistratie Personen (BRP). Participants who consent to be contacted for additional research and meet the inclusion criteria can be invited in the current study. Also persons via random sampling from the Basisregistratie Personen (BRP) can be invited to participate in the current study (excluding those already invited for the VASCO study).

Participants will have at least 1 week between receiving Participant Information Form (PIF) on the study and receiving the first fingerprick set / faeces collection set. This PIF will include information and objectives of the study and an explanation of why certain participant characteristics such as age, (lack of) comorbidities and general health status are required to reach the objectives of the study. An Informed Consent (IC) form will be included for viewing.

A telephone number and an email address will be provided for questions. If the subject decides to participate, he/she will be asked to respond via the application form on the website, www.rivm.nl/IIVAC. Participants will thereafter receive a login code and will be asked to fill in an inclusion/exclusion criteria questionnaire in the secured participant Portal. Thereafter, applicants receive an email where we explain if they fulfil the criteria for participation or not. In case we are not sure if a potential participant fulfils the criteria, we will contact that person. Subsequently, if the participant meets the in-/exclusion criteria, the potential participants will receive an instruction letter, an informed consent form (pre-signed by investigator), a self-sampling set with detailed instructions (and reference to the website with a video instruction) and fills in the digital questionnaire (or a questionnaire on

paper). The ICF, pre-signed by the investigator, will be countersigned at home by the subject and returned to the investigator together with the blood collected by finger prick and the potential hard-copy completed questionnaire in the stamped, addressed safety-envelope.

Participants that register to be willing to participate in the subgroup will be contacted by a study team member to schedule the home visits and will receive the faeces self-sampling kit, with the IC form in advance. Faeces sample and signed IC form will be handled over to the study nurse during the home visit.

The participant will also be asked to inform the study team of the vaccination dates and (vaccine details) preferably via the participant portal or by email, an extra form or by telephone. The participants will receive a self-sampling set at all following timepoints with detailed instructions.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

All subjects will receive an offer for a COVID-19 vaccine, depending on availability and national vaccine implementation policy. The vaccines will be provided by the Dutch government and are not part of this study.

The risk of sampling of blood via finger pricks and venapuncture, faeces and nasal mucosal fluid is considered low. Blood collection could result in a small bruise at the site of blood withdrawal, which will disappear within a few days. The amount of blood drawn within the study is maximum 269 mL and is well within the standard that is maintained by Sanquin Bloodbank,

There are no personal benefits for the participants of the study. By joining this study the participants contribute to knowledge on COVID-19 vaccination and to improving the strategies to fight the current coronavirus pandemic.

11.5 Incidental findings

Incidental findings are unexpected observations or test results that have a potential clinical significance for the subject's health or the health of his relatives. The study includes analyses of different components of the immune system). Incidental findings that

might indicate potential health problems will be communicated with the subject's general practitioner, who will be asked to discuss the finding with his patient.

11.6 Compensation for injury

The METC has decided that participating in the study is without risks, and has granted the RIVM dispensation from the compulsory participants' indemnification as laid down in article 7, paragraph 5 of the WMO. According to a Ministerial Order, RIVM is excluded from compulsory liability insurance for clinical research as determined by the Dutch law on Medical Investigations (WMO, section 7, paragraph 10). Any liability claims should be directed to the RIVM.

11.7 Incentives (if applicable)

After T5 of this study, the participants will receive a gift with a value of about €15. Participants that also participate in the subgroup will receive instead a gift card of 25 euros.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The Good Clinical Data Management Practices (GCDMP) guideline, a document that provides guidance on the accepted practices in Clinical Data Management (CDM) that are consistent with regulatory practices will be followed. A Data Management Plan (DMP) will be designed to ensure optimal collection, processing, storage, and dissemination of data. The Guidelines on FAIR (findable, accessible, interoperable and reusable) Data Management in Horizon 2020 as well as the General Data Protection Regulation (GDPR) the Declaration of Helsinki (and the clinical trial regulation/GCP if appropriate) will be followed. Direct identifiable information, like name and contact information, of study subjects will only be accessible by selected, authorized members of the project team, by monitors and auditors, by members of the METC and the Dutch competent authorities. To secure sensitive, private information from study subjects, all subjects participating in the study will receive a unique subject number. All study documents, data (both retrospective and newly generated) and materials will be recorded/collected using this subject number. The direct identifiable information will be stored separate from all study data, which is used for scientific purposes, including human samples like blood and saliva. The identification key will be stored at the participating sites. The investigator of the trial site or a designated representative will be responsible for the key to the code. Pseudonymization will be applied to minimize the risk of tracing data back to individual subjects and prevents the leaking of private, sensitive information. Data processing of pseudonymized data will be performed in compliance with the GDPR. Data will be accessible to authorised, trained persons only.

Clinical Data Management is a crucial part of a clinical trial and includes, electronic Case Record Forms (eCRF), database design and validation, query handling, medical history and medication coding, database locking, data extraction and assessment of data quality and completeness and integrity at regular intervals during the conduct of the trial.
A remote data capture system will be used for data collection. The electronic CRFs are created and completed in a validated system that complies with the Code of Federal Regulations (CFR), 21 CFR Part 11, that ensures accurate, reliable data by using a secure computer-generated, date and time-stamped audit trail of operator entries modifications and/or deletion of data or electronic records. Procedures are in place to guarantee data integrity and confidentiality.

All protocol required information collected during the study visits will be recorded directly on electronic Case Record forms, these entries will be considered source data, as these recordings will be made during the visits. Personal identifiers will not be recorded on the CRF. The questionnaires will be, as much as possible, completed by the subject directly in the Data Management System. Data from the data management system will be transferred to a central database over the internet using secured data communication protocols (with specific consent of the data subject). Pseudonymized data, and collected samples, of individual subjects are only accessible in EU countries. All data will be stored automatically and regular back-ups will make sure that data will not be lost. Databases and web servers of data management systems will be hosted in data centres that meet the highest possible security requirements. Procedures will be implemented to guarantee that study subjects can exercise their rights as required by the GDPR and GCP.

Specific categories will be created to indicate the level of security/safety involved, and for each respective category appropriate guidelines will be designed. The data categories will be as follows: 1) anonymous, non-sensitive data will be marked as "public data"; 2) data regarding cohort information and statistics, as well as low-risk aggregated data, which have been adequately anonymized, will be marked as "low-risk". Access to this data will be possible for the RIVM and the research community; 3) data which is high risk, sensitive, or other info which can be led back to an individual will be marked as "classified". The latter category contains all individual and pseudonymized data of study subjects as well as all genetic data and human samples. Access to this data will only be possible with specific authorization, which can be acquired only after accepting the Data Access Agreements and notification to the study lead investigators.

After project closure, all data will be exported out of the data management systems (taking into account the requirements for data minimization) and stored on a secured network for at least 25 years as required by the clinical trial regulation.

12.2 Handling and storage of samples

All collected samples will be coded as described in section 8 and stored at the RIVM for 20 years after the end of study. The RIVM has an infrastructure for collecting, storing and managing biomaterial and associated clinical data in a standardized manner, sample processing SOPs and standardized sample storage conditions.
Samples will be used for the study purposes and objectives of the clinical study. The types of analyses that will be done on the samples during the project are described in the

protocol. Samples that are left over after all analysis are done will only be used to answer other research questions as mentioned in the protocol/PIF. Samples that are left over after the study will only be stored and used if the subject had given consent to use his/her materials for further research as described in the PIF. The lead investigators of the study decide on the use of the samples after all research questions have been answered. They can also ask for advice of the Clinical Expertise Centre and the privacy coordinator of the RIVM.

12.3 Monitoring and Quality Assurance

A detailed risk based monitoring plan will be written by the sponsor detailing the monitoring activities and frequencies. The first monitoring visit will be scheduled after the first subjects have been enrolled in the study to assess informed consent procedure and protocol adherence. Informed consent forms of subjects will be reviewed for completeness as described in the monitoring plan. During the study, 1-2 monitoring visits per year will be performed, the number of monitoring visits per year depends on the number of study visits in a year. During these monitoring visits the following items will be verified: adherence to protocol, presence of personally signed and dated Informed Consent forms, accuracy and completeness of study data recorded on the CRFs, consistency between source data and data recorded in the CRF/database, reporting of (serious) adverse events incl. investigators response, presence of an up-to-date study site file, sample labelling, storage and transport. Source data verification will be performed (actual numbers will be determined in the risk-based monitoring plan).

For the purpose of compliance with Good Clinical Practice it may be necessary to conduct a site audit performed by authorized representatives of the sponsor and/or a Regulatory Authority and/or the Ethics Committee. This may occur at any time from start to after conclusion of the study.

12.4 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.5 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the

first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.6 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.7 Public disclosure and publication policy

The study results will be reported in progress reports and submitted for publication in peer-reviewed journals. The study will be registered in a public clinical trial registry.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable.

13.2 Synthesis

All subjects will receive COVID-19 vaccination. This is not part of the study, and therefore no risk.

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