RESEARCH PROTOCOL

'Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2'
(PIENTER Corona)

Including Amendment 3

NL73474.100.20/ Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2 acronym: PIENTER Corona)

PROTOCOL TITLE 'Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2'

Protocol ID	IIV-450, NL73474.100.20 NTR8473
Short title	Immunity against SARS-CoV-2 in Dutch population (acronym: PIENTER Corona)
EudraCT number	Not applicable
Version	5.0
Date	October, 21st 2020
Coordinating investigator/project leader and Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	5.1.2e PhD Centre of Infectious Diseases control - Immunology of Infectious Diseases and Vaccines – Immunosurveillance (IIV/IMS) National Institute of Public Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven Tel. 030- 5.1.2e E-mail: 5.1.2e @rivm.nl
Author	5.1.2e 5.1.2e, PhD National Institute of Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Centre for Immunology of Infectious Diseases and Vaccines Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven
Sponsor (in Dutch: verrichter/opdrachtgever)	National Institute of Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Centre for Immunology of Infectious Diseases and Vaccines Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven
Independent expert	5.1.2e Infectious Diseases and Vaccinology Centre –Epidemiology and Surveillance of Infectious diseases (I&V/EPI/RVP) National Institute of Public Health and the Environment (RIVM)

	Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven
Laboratory sites	Laboratories of Immunosurveillance (IIV/IMS) and Immune mechanisms (IIV/IMM) Laboratory of Infectious Diseases and Screening (IDS) National Institute of Public Health and the Environment (RIVM) Antonie van Leeuwenhoeklaan 9 P.O. Box 1, 3720 BA Bilthoven

NL73474.100.20/ Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2 acronym: PIENTER Corona)

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of the Department 5.1.2e 5.1.2e, PhD Head of the Centre of Immunology of Infectious Diseases and Vaccines, RIVM		October 21st 2020
Coordinating Investigator/Project leader/Principal Investigator 5.1.2e PhD Head of Department Immunosurveillance (IIV/IMS) of Centre of Immunology of Infectious Diseases and Vaccines, RIVM		October 21st2020

TABLE OF CONTENTS

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)
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
EU	European Union
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
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.
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

NL73474.100.20/ Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2 acronym: PIENTER Corona)

SUMMARY

Title: Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2

Rationale: The first person infected with the novel coronavirus, SARS-CoV-2, that presented with COVID-19 disease, emerged on November 2019 in Wuhan, China. Since then, the virus has spread worldwide, with new cases emerging every day. The first COVID-19 case in the Netherlands was confirmed on February 27, 2020. At this moment, the scope of undetected spread of the virus, the fraction of immune persons due to recent infection, and the course of further spread within the Netherlands is largely unknown. Since, the spreads rapidly, laboratory testing of all suspected cases is not feasible anymore. The sera, collected in the previous PIENTER 3 study, provides an unique opportunity to function as baseline for antibody levels against SARS-CoV-2 of the Dutch population prior to the COVID-19 pandemic. In the present study proposal, PIENTER 3 participants, that had previously indicated that they could be approached for a follow-up study as well as invited subjects from a random age-stratified sample of the Netherlands, will be asked to donate a finger prick blood sample by self-sampling and fill in a questionnaire at different time points during and after the coronavirus pandemic in the Netherlands. This follow-up sampling will obtain insight in the obtained humoral immunity against SARS-CoV-2 during this first pandemic wave in the Netherlands. This is important to monitor the status of the generated immunity against SARS-CoV-2 as well as to identify possible gaps among different age groups in The Netherlands, to identify risk groups that are not immune. Furthermore, data from this study can contribute to the evaluation of recently implemented intervention measurements by policy makers and to take decisions for new measurements needed. In addition, it may provide clues how the pandemic will evolve; can we get the pandemic under control, can we stabilize it, or can we expect a new pandemic period?

Objective: To assess achieved immunity against COVID-19 across the different age groups in The Netherlands by testing a representative part of the Dutch population for the presence of SARS-Cov-2 specific antibodies in serum

Study design: An observational, longitudinal prospective study

Study population: Participants reflect a representative part of the Dutch population aged 1-93 years)

Main study parameters/endpoints: Detection of SARS-CoV-2-specific antibody concentrations, and qualitative analysis of antibody functionality (neutralization and avidity), which will be measured in serum obtained from fingerprick blood at 3-6 different time points over a period of 18 months.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The risk of blood collection is considered minimal. There are no

personal benefits for the participants of the study. By joining this study the participants contribute to the public health related to the current coronavirus pandemic.

1. INTRODUCTION AND RATIONALE

The first person infected with the novel coronavirus, SARS-CoV-2, that presented with COVID-19 disease, emerged on November 2019 in Wuhan, China. Since then, the virus has spread worldwide, with new cases emerging every day. The first COVID-19 case in the Netherlands was confirmed on February 27, 2020. On March,19 2020 a total of 2994 persons tested positive for SARS-CoV-2 and 30 patients with COVID-19 disease died. have been reported in the Netherlands. On March 11, 2020, the WHO declared the rapidly spreading novel coronavirus outbreak a pandemic. At this moment, the scope of undetected spread of the virus, the fraction of immune persons due to recent infection, and the course of further spread within the Netherlands is largely unknown. Most ongoing research on SARS-CoV-2 infection is focusing on COVID-19 patients, their contacts and risk groups, such as medical personnel. However, since the virus is currently spreading rapidly, laboratory testing of all suspected cases is not feasible.

Spreading of the virus will inevitably result in herd immunity, a form of indirect protection from infectious disease that occurs when a large percentage of a population has become immune to an infection, thereby providing protection for individuals who are not immune. Herd immunity leads to disruption of viral transmission, and as a consequence the spread of disease will be stopped or delayed. At present, the exact percentage of a population that should be immune to achieve herd immunity against COVID-19 is not known. To get better insight into the current levels of immunity that have been achieved in the Dutch population, the change over time and thereby in the spread of the virus among all age groups, scientific research and immunosurveillance is indispensable. It is expected that persons that experienced an infection with SARS-CoV-2, will develop specific antibodies against the virus (1-4). These antibodies will likely contribute to immune protection, though there is currently no information about the antibody levels which confer this protection. A seropositive result can be considered as first indicator for immune protection, until more sophisticated and functional assays become available. Immunoassays to detect SARS-Cov-2 specific antibodies have currently passed the first provisional stages at RIVM, and patients that have recovered from COVID-19 tested positive with these assays. These immunoassays are essential to analyse population immunity and to gain insight into the spread of the virus.

In 2016/2017, a third large-scale seroprevalence study was initiated among a representative part of the Dutch population (age 0-89 years; PIENTER 3) (5). Within this study, questionnaires, blood, saliva, peripheral blood mononuclear cells, feces, oral and nasal swabs were collected. The primary aim of that study was to obtain insight in the protection against infectious diseases for which vaccines are offered by the national immunization programme (RVP) in the Netherlands (5). However, the PIENTER studies offered also an unique opportunity as baseline sample of the Dutch population (6, 7). For instance, serological data from the PIENTER studies have provided essential baseline seroprevalence data for Q-fever, before the Q-fever outbreak in 2007 took place in the Netherlands (8).

NL73474.100.20/ Third population-based immune surveillance study used for the evaluation of immunity against SARS-CoV-2 acronym: PIENTER Corona)

Antibodies against SARS-CoV-2 will be measured in the serum samples from the PIENTER 3 that had been collected prior to the COVID-19 pandemic and will be used as baseline. In the present study proposal, PIENTER 3 participants, that had previously indicated that they could be approached for a follow-up study, as well as invited subjects from a random age-stratified sample of the Netherlands will be asked to donate a finger prick blood sample by self-sampling and fill in a questionnaire at different time points during and after the coronavirus pandemic in the Netherlands. This follow-up sampling will obtain insight in the obtained humoral immunity against SARS-CoV-2 during this first pandemic wave in the Netherlands. This is important to monitor the status of the generated immunity against SARS-CoV-2 as well as to identify possible gaps among different age groups in The Netherlands, to identify risk groups that are not immune, and to be able to take appropriate intervention measurements by policy makers. In addition, it may provide clues how the pandemic will evolve; can we get the pandemic under control, can we stabilize it, or can we expect a new pandemic period? The completed questionnaires will provide insight in the symptoms that are characteristic for infection with SARS-CoV-2, but also which persons had been at higher risk for severe COVID-19 according to demographic characteristics and medical background.

2. OBJECTIVES

	Primary objective
1	To assess achieved immunity against COVID-19 over time across the different age groups in The Netherlands by testing a representative part of the Dutch population for the presence of SARS-Cov-2 specific antibodies in serum

	Secondary objectives
2	To assess the quantity and quality (i.e. antibody functionality and avidity) of the antibodies raised against SARS-CoV-2 in a representative part of the Dutch population, and to identify subgroups (including risk groups) with different levels of immunity against COVID-19
3	To assess the development of immunity during and after the first pandemic wave and the period thereafter
4	To assess the existence of cross-reactive antibodies against established coronavirus infections in the past

3. STUDY DESIGN

The present study is an observational, longitudinal prospective study in a representative part of the Dutch population (age 1-93 years). A total of approximately 6000 persons will be approached, that previously participated in the PIENTER 3 study (2016/17) and had indicated that they could be approached for a follow-up study. In addition, approximately 27,200 randomly selected subjects spread over the Netherlands representing all age groups will be approached. Participants will be asked to donate a finger prick blood sample by self-sampling and fill in a questionnaire at different time points during and after the coronavirus pandemic in the Netherlands. The intention is to collect samples over a time period of 18 months, with a maximum of 6 different sampling time points, guided by the epidemiology of the pandemic (reporting rates). The first timepoint for sampling will be as soon as possible, thereafter sampling moments will be chosen based on epidemiological information.

In contrast to the previous PIENTER 3 study, in which participants visited the clinic at location, in this study all participants are asked to donate finger prick blood sample at their home. Within RIVM there is good experience with this way of blood collection. In several studies, the number of participants that joined these studies and succeeded to collect this type of self-samples was high. In addition, the quality of the returned fingerprick blood samples was sufficient for reliable antibody testing.

Prior to invitation, it will be screened whether the participants that previously participated in the PIENTER 3 study and had indicated that they could be approached, can be reached at the known addresses (via Basisregistratie Personen (BRP)), and to avoid sending invitation to deceased former PIENTER 3 participants. In addition, an age-stratified sample of 27,200 individuals is drawn from the population register (BRP). The population register contains all individuals with a home or postal address. All screened potential participants will be invited by mail to participate in the study and will receive subject information. Participants can show their interest to participate the study via the website (by filling in their contact details that will be automatically forwarded to the study team). The participants will be given the opportunity to indicate whether they want to receive a questionnaire on paper instead of a digital questionnaire. Subsequently, the potential participants will receive an instruction letter, a self-sampling set with detailed instructions, a login code for access to the digital questionnaire (or a questionnaire on paper) and an informed consent form (pre-signed by investigator). The questionnaire contains several questions about health status, living situation, profession and contact information and travel history. In addition, questions on recent (infectious) diseases, clinical symptoms (intensity and duration) will be included. For the participants younger than

12 years of age, parents/legal representatives are requested to fill in the questionnaire. If the questionnaire is not completed by the participant, a reminder will be send by e-mail, if an e-mail address is available.

Participants are requested to take a finger prick blood sample (maximum of 0.5 ml) by the provided self-sampling set according to the detailed instruction. If needed, a family member or other close contact can assist with the sampling. Subsequently, participants are asked to return the sample(s) in the stamped, addressed safety-envelope provided and signed informed consent. For participants, who have requested for a paper questionnaire, we will provide a stamped envelope. Upon arrival; samples will immediately be aliquoted and stored at -80 °C.

4. STUDY POPULATION

4.1 Population (base)

A cross section of the Dutch population (aged 1-93 years)

4.2 Inclusion criteria

Invited subjects:

- Subject previously participated in the PIENTER 3 study (2016/17), and had indicated that they could be approached for a follow-up study, or

  Subjects from a random age-stratified sample from the Netherlands

4.3 Exclusion criteria

There are no exclusion criteria.

4.4 Sample size calculation

All participants of previous PIENTER 3 who have given permission to be approached again for this follow-up study are invited. In total approximately 6,000 persons will be invited, which is expected to result in approximately 4,000 of participants, taking a response rate of about 70% into account.

In the previous PIENTER 3 study (2016/2017) the Netherlands was divided into five regions and per region eight municipalities have been drawn. We distinguished 18 age-groups. The age strata at that time were 0, 1-4, thereafter intervals of five years, 5-9, ..., until 75-79, and the last age strata is 80-89 years. In addition an extra sample was taken of non-Western migrants from a number of the above 40 municipalities as the number of non-Western migrants in the NS would be too small to determine the seroprevalence in this group with sufficient precision. Four age strata (0-9 years, 10-34 years, 35-59 years and 60-89 years) were defined.

Furthermore, individuals were selected from eight additional municipalities with low vaccination coverage to assess the seroprevalence in four age groups (0-9, 10-34, 35-59, 60-89 years of age) in socio-geographically clustered orthodox Protestant groups who refuse vaccination for religious reasons (5).

The number of clusters (municipalities) and individuals per cluster were chosen such that the expected accuracy of the overall seroprevalence estimates was optimal within financial and logistic constraints. An assumed seroprevalence of 50% was taken since for this value the inaccuracy is likely to be greatest. The accuracy is determined mainly by the total number of clusters and to a lesser extent by the number of individuals per cluster, as the expected variance between clusters is greater than within clusters (9). Taking the above into account

and the results of PIENTER1 and PIENTER2, a total of 40 municipalities were drawn, resulting in eight municipalities per region.
The number of individuals will be sufficient to estimate age- and/or gender specific seroprevalences with a confidence interval with a half-width of about 10% to 15%. For example with 10% precision, $\alpha = 0,05$ and a prevalence of 50% the number of respondents should be about 100. Building on previous experience, at an estimated total sample size of 4,000, it is expected that fairly small changes in prevalence (5%, say), can be detected with high probability.

In addition to the previous PIENTER 3 participants, another approximately 13,600 subjects will be invited to obtain seroprevalence data from a larger sample with more municipalities included. This will provide a more complete picture of seroprevalence of COVID-19, especially considering the geographic clustering of COVID-19 a wider geographic spread is desirable. Furthermore, this allows us to detect minor changes in antibody levels among particular subgroups (1-3%), such as age groups, but also minor changes in time when antibody levels from samples taken at different time points are compared. Furthermore, it enables us to have information from the whole country, including geographically areas that were not sampled in the previous sampling frame. For this purpose, an estimated extra sample size of 3,400 is needed (i.e. 200 per age group; 17 different age-groups of 1-4, thereafter intervals of five years, 5-9, ..., until 75-79, and the last age strata is 80-89 years). It is expected that fairly small changes in seroprevalence per age group (i.e. 3%), can be detected with 95% confidence interval and power of 80%. To achieve an extra inclusion of 3,400 participants, taken into account the response rate of 55% for previous PIENTER 3 participants that will probably be higher than the response rate of newly invited subjects, we estimate that it will be needed to approach approximately 13,600 new subjects taken into account a response rate of about 25%. For these extra inclusions, a random age-stratified sample will be taken from five different regions of the Netherlands with approximately equal number of habitants (similar five regions as used for the precious PIENTER 3 study sampling strategy). Per region and per age group 160 individuals will be randomly sampled according to the total number of individuals in each age class per region (5 regions x 160 individuals x 17 age groups = total of 13.600 subjects to be invited). We now also invite 1-2 year-olds, as they were not included in the PIENTER 3 sample.
The response of the 13,600 invited subjects started low with inclusion rate of 11% in the first week. To achieve the required extra inclusion of 3,400 participants by the end of the registration period an additional 13,600 subjects were invited. The second group of 13,600 subjects were also randomly selected subjects spread over the Netherlands representing all

age groups. Of the 27,200 invited subjects, 5,050 subjects registered at the end of the registration period resulting in a response rate of 18.6%.

5. TREATMENT OF SUBJECTS

Not applicable.

6. INVESTIGATIONAL PRODUCT

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary study parameters of this study are SARS-CoV-2-specific antibody concentrations, and qualitative analysis of antibody functionality (neutralization and avidity), which will be measured in serum obtained from fingerprick blood at 3-6 different time points over a period of 18 months.

8.1.2 Secondary study parameters/endpoints (if applicable)

Other study parameters that are investigated:

- cross-reactive antibodies against established coronavirus infections in the past
- Results of the questionnaires

8.1.3 Other study parameters (if applicable)

Other result include the study parameters from the questionnaire, including health status, living situation, contacts, occurrence of recent (infectious) diseases and corresponding clinical symptoms (intensity and duration).

8.2 Randomisation, blinding and treatment allocation

Not applicable

8.3 Study procedures

Potential participants that received subject information and show interest in the study will receive an instruction letter, a self-sampling set, login code for access to the digital questionnaire and an informed consent form (pre-signed by investigator). The informed consent form (ICF) is signed by the subject (described in section 11.2).
The participants are requested to fill out the digital questionnaire (or questionnaire on paper, if they indicated they preferred that). In addition, the participants will be asked to donate finger prick blood sample (maximum of 0.5 ml) by the provided self-sampling set according to the detailed instruction, and are requested to return the sample(s) in the stamped, addressed safety-envelope provided.
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 presence of SARS-Cov-2 specific antibodies by quantitative multiplex serology (Microarray and/or Luminex or other (commercial) immune-assays), against appropriate controls (SARS/MERS and human coronaviruses). Selected SARS-CoV-2 positive serum samples will be tested for

antibody binding strength (avidity) and virus neutralization, providing a quantitative measure of immune protection
The self-sampling and questionnaire is repeated 3-6 times during the study. Participants are also asked to take part in the online citizen science questionnaire study Infectieradar.nl (considered as nWMO study by METC) . Already 20,000 people have created an account on this website to report to the RIVM health problems and symptoms possibly related to respiratory infectious disease and that could be caused by SARS-CoV-2. Unfortunately, currently the website is overloaded (due to many visitors) and is therefore under maintenance, but is expected to be soon available. If the website is not available during inclusion, an email reminder will be send with the request to join Infectieradar.nl as soon as possible. Participants' permission will be asked explicitly in the ICF for the possibility to link their data obtained from the PIENTER Corona study to data obtained from Infectieradar.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Once personal identifiers are deleted from the database (before statistical analyses), it will no longer be possible to withdraw individual subjects.

8.5 Replacement of individual subjects after withdrawal

Subjects will not be replaced after withdrawal.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable.

8.7 Premature termination of the study

There are no criteria for terminating the study prematurely. If the study should be terminated, this will be done in consultation with the Principal Investigator and the METC will be notified.

9. SAFETY REPORTING

Not applicable.

10. STATISTICAL ANALYSIS

10.1 Descriptive statistics

A detailed description of the number of participants will be given; i.e. by age, gender, municipality, and of all the variables in the questionnaires. 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. To obtain insight into the characteristics of the non-respondents data available from the non-response survey will be analysed and compared with the responders and absolute non responders. 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.

10.2 Statistical analyses

Overall geometric mean titers/concentrations (GMTs/GMCs) for antibodies against SARS-CoV-2, stratified by age, will be compared to baseline concentrations, using serum samples from the same individuals that were obtained prior to introduction of SARS-Cov-2 in The Netherlands (2016/2017). Percentages of participants with SARS-Cov-2-specific antibodies above baseline levels will be calculated.

Data will be weighted for age, gender and ethnicity according to the Dutch population, and all analyses will take account of the survey design. Because of the longitudinal nature of the study, we will analyze the data with a random effects binary mixture model in which the first sample (taken in 2016/2017) represents a baseline measure that will help informing the uninfected component. In this context, the time-dependent prevalence will be estimated using penalized splines. Expertise for the analyses is present within RIVM, and code for analyses is operational. Building on previous experience, at an estimated total sample size of 4,000, it is expected that fairly small changes in prevalence (5%, say), can be detected with high probability. In first instance, univariable analyses will be performed, focusing on age. In a second step, potential risk factors for infection, such as gender, comorbidities (e.g., hypertension, diabetes, cancer), medication use (especially immunosuppressants), and profession (e.g., healthcare workers, teachers) will be taken into account. Finally, we will estimate the sensitivity and specificity of COVID-19-like symptoms for infection, as defined by serology, using receiver operating characteristics.

Longitudinal study

Longitudinal data will be analysed with appropriate statistical tests taking into account that samples are paired.

10.3 Interim analysis (if applicable)

As soon as all samples from the first sending (first timepoint) have arrived at RIVM, laboratory testing will be performed and thereafter the laboratory data will be analysed. This will be done this way for every next timepoint.

ETHICAL CONSIDERATIONS

10.4 Regulation statement

The study will be conducted according to the principles of the World Medical Association Declaration of Helsinki and its amendments effective since 1964 and in accordance with the Medical Research Involving Human Subjects Act (WMO). The guideline ICH Good Clinical Practice (GCP) will be followed, however it is foreseen that some items cannot be adhered to, mainly because of logistical reasons. The ICF, pre-signed by the investigator, will be countersigned at home by the subject and self-sampling by fingerpick and completion of the questionnaire will be taken place at home.

10.5 Recruitment and consent

Recruitment will be done by inviting the subjects by post and simultaneously send the subject information. Participants can show their interest to participate the study via the website (by filling in their contact details that will be automatically forwarded to the study team). The participants will be given the opportunity to indicate whether they want to receive a questionnaire on paper instead of a digital questionnaire. Subsequently, the potential participants will receive an instruction letter, a self-sampling set with detailed instructions, a login code for access to the digital questionnaire (or a questionnaire on paper) and an informed consent form (pre-signed by investigator). The ICF, pre-signed by the investigator, will be countersigned at home by the subject and together with the blood sample and completed questionnaire will be returned to the investigator in the stamped, addressed safety-envelope.

For subjects under 16, both parents/legal representatives have to sign the ICF. In addition, if the subject is over the age of 12, he/she will co-sign the ICF as well.

10.6 Objection by minors or incapacitated subjects (if applicable)

Minor subjects: The Code of Conduct for minors will be respected.

Incapacitated subjects: As the study is designed as a representative sample of the population there is a possibility that incapacitated persons will be invited. Nevertheless in 2016/2017, the study coordinator already judged in consultation with the accompanying person or legal representative whether it was acceptable to include that particular subject for the PIENTER 3 study. In all cases the Code of Conduct for mentally disabled or incapacitated elderly will be respected.

10.7 Benefits and risks assessment, group relatedness

The study is designed to include a representative sample of the Dutch population aged 1-93 years. The main objective can only be investigated in case all age groups are invited. Blood collection by fingerpick is a standard procedure which is generally accepted. The sensation of a fingerprick can be discomfortable for some participants. The risk of blood collection is considered minimal. There are no personal benefits for the participants of the study. By joining this study the participants contribute to the public health related to the current coronavirus pandemic.

10.8 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.

10.9 Incentives (if applicable)

No financial incentives are given to the participants. Hopefully, participants are willing to participate the study in order to be able to collect data that may help to control the current coronavirus pandemic.

Amendment 3:

Children: We plan to send each child up to 13 years of age a certificate that they participate in the PIENTER Corona study. Children like to tell to others that they participate and are proud that they help in this clinical study, showing a certificate can help them. This certificate is our way to say; Thank you.

Gift: Optionally, we consider to send participants a small gift (like a chocolate bar, tea bags or something in this range).

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

The data with identifiers of all invited subject will be stored in the corresponding study database. The electronic clinical data management system used is GLEAN from the company Sidekick-it https://sidekickit.nl/cases/glean-maatwerk-met-veilige-dataverwerking-voor-rivm/

This database is only accessible for the project team of the RIVM.

Subjects who participate are given a unique number (barcode) which is attached to the case report form (CRF), questionnaire and the sample tubes.

All data which can identify the subject will be stored temporarily until 6 months after the last sample is expected. RIVM will respect the Dutch law upon using personal data (Wet Bescherming Persoonsgegevens) at all times. Unless subjects give permission on the ICF to store their personal data, all personal identifiers will be destroyed. All data will be available by their unique code.

The RIVM will store the documents for at least 15 years according to the GCP guidelines. Source documents like ICF, Questionnaire, diaries (if applicable) and CRF will be stored in a safe. Only project team members have access to these documents.

The collected blood will be stored at the RIVM under appropriate conditions. They will be kept for at least 15 years according to GCP guidelines.

Collection of personal identifiers

After the data collection is completed (six months after the last sample is expected), personal identifiers will be removed so the data will only be available by their unique code. At the start of the laboratory analyses no personal identifiers are present. Personal identifiers for individuals who give permission to be approached again in the future for additional research will be stored separately from the data. Participants will receive their personal test result after round 3 and thereafter each consecutive round.

11.2 Monitoring and Quality Assurance

A representative of the sponsor may monitor this study throughout various stages of the study. The frequency of monitoring is determined by the sponsor.

The monitor will verify the following:

- Presence of written approval by METC
- Presence of an up-to-date trial master file
- Proper training and authorisation of study personnel
- Adherence to protocol and GCP
- ICF completion

- Laboratory procedures

11.3 Amendments

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

Amendment 1&2:

Goal of amendment 1&2

Extension of number of participants.

Apart from the approximately 6,000 persons, that previously participated in the PIENTER 3 study and were approached for this study, another approximately 27,200 persons will be approached to be able to get an even better picture of the SARS-CoV-2 antibody seroprevalence in the Dutch population around the whole country.

Justification of amendment 1&2

At present (1 month) after the recruitment of the previous PIENTER 3 participants, approximately 3,200 participants are included (response rate approximately 55%). These previous PIENTER 3 participants give a good overview across the different age groups in The Netherlands. This allows us to test for the presence of SARS-CoV-2 specific serum antibodies in the Dutch population. However, to be able to get a more complete picture of seroprevalence of COVID-19 and considering the geographical clustering of COVID-19, a larger sample including individuals from more municipalities to obtain a geographically broader spread of subjects is desirable. This further allows us to detect smaller changes in antibody levels among particular subgroups (1-3%), such as age groups, but also minor changes in time when antibody levels from samples taken at different time points are compared. Furthermore, it enables us to have information from the whole country, including geographically areas that were not sampled in the previous sampling frame.
For this purpose, an estimated extra sample size of 3,400 is needed (i.e. 200 per age group; 17 different age-groups of 1-4, thereafter intervals of five years, 5-9, ..., until 75-79, and the last age strata is 80-89 years). It is expected that fairly small changes in seroprevalence per age group (i.e. 3%), can be detected with 95% confidence interval and power of 80%. To achieve an extra inclusion of 3,400 participants, taken into account the response rate of 55% for previous PIENTER 3 participants that will probably be higher than the response rate of newly invited subjects, we estimate that it will be needed to

approach approximately 13,600 new subjects taken into account a response rate of about 25%. After an initial slow inclusion rate we invited a second group of 13,600 new subjects to ensure inclusion of the needed number of at least 3,400 participants. In total this extra round of invitations resulted in 5,050 new participants. For these extra inclusions, a random age-stratified sample will be taken from five different regions of the Netherlands with approximately equal number of habitants (similar five regions as used for the precious PIENTER 3 study sampling strategy). Per region and per age group 160 individuals will be randomly sampled according to the total number of individuals in each age class per region (5 regions x 160 individuals x 17 age groups = total of 13.600 subjects to be invited). We now also invite 1-2 year-olds, as they were not included in the PIENTER 3 sample. Seroprevalence data of this age group may be of value for decision makers to take adequate intervention measurements related to opening or closing of nurseries. This sampling method will lead to a more geographic spread, which is important as COVID-19 seem to cluster. For PIENTER 3 sampling, the approach was slightly different. For practical reasons the Netherlands was for purpose of that study divided in the same five regions, and per region a sample from a fixed number of municipalities were drawn. This approach was chosen since participants were requested to visit a study location in their own municipality. Now we have chosen for a sampling method leading to a more geographic spread of the participants based without the risk of missing some clusters of COVID-19 cases.

Similar to the already included participants, the new invited subjects will be personally invited to participate in the study, and participants are asked to donate finger prick blood sample at their home and fill in a questionnaire. As we do not have any background information of the newly invited 27,200 subjects, the first questionnaire will be adapted to include additional questions to be able to collect sufficient demographic data, including data on ethnicity, religion and relevant vaccination history, from these subjects that was already collected from previous PIENTER 3 participants during the PIENTER 3 study. In addition few questions relevant for COVID-19 surveillance have been added to the questionnaires.

Amendment 3:

Goal of amendment 3

- To give all participants their personal test result.
- Incentives; to give all children (<13 years of age) a certificate of participation in a clinical trial, and possibly participants a small gift.
- Update website with information for participants.

Justification of amendment 3

Personal test results

Participants ask for their personal test results. We currently estimate that the test results have a 1% wrong positive rate. With this accurate testing we are confident that we can inform the participants of their personal outcome. We will inform each participant that the test results will be send. They can inform us if they do not want to know the personal outcome. Thereafter we will send each participant a letter with the personal test results of round 1-3, and thereafter after each consecutive round.

Incentives

- **Children:** We plan to send each child up to 13 years of age a certificate that they participate in the PIENTER Corona study. Children like to tell to others that they participate and are proud that they help in this clinical study, showing a certificate can help them. This certificate is our way to say; Thank you.
- **Gift:** Optionally, we consider to send participants a small gift (like a chocolate bar, tea bags or something in this range).

Update website

- To increase the information that participants receive, by updating the website, with a frequently asked questions section (FAQ) en general study outcomes.

All documents will be updated accordingly. The protocol has changes in paragraph 10.1, 11.1, 11.3 and 11.6.

11.4 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.

11.5 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 we receive the last sample from the last participant.
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 preliminary study report with the results of the establishment of the serumbank with data on inclusion numbers and study population profile to the accredited METC.
Due to the number of analyses to be performed a final report containing all the results of laboratory and epidemiology analyses might be not feasible. The results will be made public in peer reviewed journals.

11.6 Public disclosure and publication policy

Summaries of the study results will be placed on https://www.rivm.nl/pienter-corona-studie, with links to publications.
The study results will be submitted for publication in peer reviewed journals.
The study will be registered in a public clinical trial registry.

12. STRUCTURED RISK ANALYSIS

Not applicable.

13. REFERENCES

1. Hsueh PR, Huang LM, Chen PJ, Kao CL, Yang PC. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. Clin Microbiol Infect. 2004;10(12):1062-6.
2. Nie Y, Wang G, Shi X, Zhang H, Qiu Y, He Z, et al. Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. J Infect Dis. 2004;190(6):1119-26.
3. Subbarao K, McAuliffe J, Vogel L, Fahle G, Fischer S, Tatti K, et al. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. J Virol. 2004;78(7):3572-7.
4. Liu W, Fontanet A, Zhang PH, Zhan L, Xin ZT, Baril L, et al. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J Infect Dis. 2006;193(6):792-5.
5. Verberk JDM, Vos RA, Mollema L, van Vliet J, van Weert JWM, de Melker HE, et al. Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis. 2019;19(1):470.
6. van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med. 2009;67(7):301-8.
7. van der Maas NA, Mollema L, Berbers GA, van Rooijen DM, van der Avoort HG, Conyn-Van Spaendonck MA, et al. Immunity against poliomyelitis in the Netherlands, assessed in 2006 to 2007: the importance of completing a vaccination series. Euro Surveill. 2014;19(7):20705.
8. Schimmer B, Notermans DW, Harms MG, Reimerink JH, [redacted], Schneeberger P, et al. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2012;140(1):27-35.
9. Cochran W.G. Sampling techiques. New York: John Wiley & Sons; 1977.