SARS-COV-2 PREVALENCE, SEROPREVALENCE AND SEROCONVERSION AMONG HEALTHCARE WORKERS IN BELGIUM DURING THE 2020 COVID-19 OUTBREAK: STUDY PROTOCOL

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1. Background

The novel SARS-CoV-2 (Severe Acute Respiratory Syndrome-associated Coronavirus type 2), belongs to the betacoronavirus subfamily and is closely related to SARS-CoV-1 that caused the SARS epidemic in 2002-2003 and more distantly related to various human coronaviruses that circulate in the population since a long time and are associated with rather mild respiratory symptoms. SARS-CoV-2 is rapidly spreading over the world causing a condition called Coronavirus disease 2019 (COVID-19) whose symptoms range from mild respiratory symptoms to a serious, sometimes life-threatening condition requiring in-hospital treatment. As of 26th March 2020, around 415,798 cases have already been reported worldwide since the beginning of the epidemics, among which 18,552 deaths (1). Although transmission occurs via droplet in the first place, touching contaminated surfaces with hands and touching mouth, nose or eyes subsequently, can also transmit the virus. Fecal-oral route of transmission is now also considered (2). Furthermore, there is evidence that infected subjects could remain asymptomatic and yet be infectious to others (3).

Like other European countries, Belgium has mobilized the entire health sector to prepare for the treatment of hundreds or even thousands of seriously ill people in the coming weeks. Hospital health care workers (HCW) therefore represent a highly exposed population and are also in close contacts of vulnerable patients at high risk for COVID-19. HCW have received detailed instructions to protect themselves against this infection, but, in view of the high infectiousness of this virus, it is likely that at least some of them will also get infected either at their workplace or elsewhere. According to the current testing procedures (4), symptomatic HCW are tested with the standard, molecular techniques, based on naso/oro pharyngeal swabbing (NOPS) and RT-qPCR (5). Because of the crucial role of HCW in the chain of transmission, it is of upmost importance to assess the proportion of asymptomatic infections in this population. However the seroconversion rate among asymptomatic persons is not well known until now. Hence the urgency for validating a serological test as soon as possible.

NOPS is the standard material to perform RT-qPCR for diagnostic purposes. It has been shown however, that saliva is also a suitable sample to use, with good (but somewhat lower) sensitivity (6,7). A validation of this technique would become very useful for future screening purposes.

Additionally, there is only limited information about antibody responses towards SARS-CoV-2 in the literature, but it seems to follow the classical course: seroconversion for IgM and later for IgG within 1-2 weeks after the onset of symptoms (8). Several mainly Chinese biotech companies have developed serological tests for the new SARS-CoV-2, based on various techniques (fluorescence, chemiluminescence, colloidal gold) and various antigenic preparations (crude viral lysate, recombinant proteins …) (9). The information on specificity and sensitivity of these assays is limited today, but based on the experience with the closely related SARS-CoV-1, it is likely that there will be issues of cross-reactivity with the more established "common cold" coronaviruses HKU-1 and OC-43 (which belong to the beta subfamily) or even 229-E and NL-63 (alpha CoV), as argued in a review on serology of emerging coronavirus, before the advent of the present SARS-CoV-2 pandemic (10). Clearly, even if pure recombinant proteins are used, cross-reactivity might still occur and it is not excluded that titers of antibodies to "common" CoV increase to a significant extent after seroconversion for SARS-CoV-2. Some preliminary, rather encouraging validation efforts have very recently been performed in Europe (11), but this type of studies clearly needs to be expanded, in view of the limited number of patients tested.

This Belgian multicentric study therefore aims to address the knowledge gap regarding specificity and sensitivity of various SARS-CoV-2 serological assays as well as saliva testing and the likelihood of cross-reactivity. In addition, it aims to investigate seroprevalence and seroconversion among healthcare workers, including asymptomatic ones, in order to generate insights on the clinical presentation of the disease as well as natural immunity, and an evaluation of the effectiveness of the preventive measures in place to protect the healthcare personnel.
In this context, it is urgent to start this study as soon as possible, as it is crucial to obtain "baseline" samples before HCW will be too heavily exposed to the virus.

2. Methods

The aim of this study is to broaden the knowledge on COVID-19 in Belgium and to contribute to scientific research supporting the fight against this epidemics.

2.1. OBJECTIVES

Primary objectives

- Document prevalence and seroprevalence of SARS-CoV-2 among Belgian active hospital healthcare workers at D0 (beginning of the study, planned early April 2020).
- Document number of new cases (incidence) of COVID-19 and SARS-CoV-2 seroconversions among Belgian hospital healthcare workers during a period of five months from D0 (duration of follow-up will depend on the findings and the evolution of the outbreak).

Secondary objectives

- Validate the EUROIMMUN serological tests (subject to change/addition depending on the evolution of scientific research) against the plaque reduction neutralization test (PRNT) (gold standard (11));
- Validate the saliva sample (sampling with Oracol or equivalent) against the standard (NOPS) to perform RT-qPCR for SARS-CoV-2 diagnostic purposes, as well as against the standard serology (serum);

- Validate the nasal swab against the standard (NOPS) to perform RT-qPCR for SARS-CoV-2 for diagnostic purposes
- Investigate potential risk factors for the infection;
- Quantify the proportion of asymptomatic cases among new cases that develop during a period of 5 months;
- Investigate virus shedding in urine, feces and sperm as well as cellular immune response (sub study in the Antwerp region on volunteering SARS-CoV-2 positive HCW**,** currently on hold).

2.2. STUDY POPULATION

Study population include health care workers currently working in Belgian hospitals.
According to current national guidelines, HCW in contact with persons at risk for COVID-19[1] experiencing mild respiratory symptoms without fever are allowed to work if clinical condition permits, while wearing a mask and observing usual hygiene measures. Healthcare workers experiencing respiratory symptoms and fever are systematically tested and if positive, isolated at home, if negative, eventually working wearing a mask if the clinical state allows it. Those who recovered from a documented infection are also allowed to work. HCW who are not in contact with persons at risk for COVID-19 are not tested and systematically isolated in case of any unusual respiratory symptom. This implies that at baseline, the study population will only include asymptomatic or paucisymptomatic HCW applying proper infection prevention measures.

Inclusion Criteria:

- Any permanent medical and paramedical staff working in the hospital who provided a signed informed consent to participate in the study.
- Participants must have a social security insurance (mandatory in Belgium).
- This study only include adults.

Exclusion Criteria:

- Staff hired on a temporary (interim) basis will be excluded as follow-up over time will be compromised.
- Administrative staff or technical staff will also be excluded.
- HCW who were not active during the inclusion period will automatically be excluded.

2.3. STUDY DESIGN

This study will be set up as a prospective cohort study.
Participants will be recruited at D0 in the participating hospitals. Ideally, samples will be taken at the same point in time or at least during the same week for all the participants.
Participants will need to fill in a questionnaire at each of the timepoints providing basic socio-demographic characteristics (at baseline only) and health characteristics including presence of symptoms during the past 30 days (at each of the timepoints). The questionnaire will be completed through a secured online application at the same time as sampling.
Follow-up will last five months (duration of follow-up will depend on the evolution of the outbreak). Data and sample collection will occur at baseline (D0), day 14 (D14), day 28 (D28),

[1] Adult>65 years old
Cardiovascular disease, diabetes or HTA
Severe chronic heart, lung or kidney condition
Immunsuppression, malignant hemopathy or active neoplasia

then monthly (M2, M3, M4, M5; meaning seven contacts in total). Ideally, recruitment and data and sample collection should occur between 1 and 10 April 2020.
A sub-study investigating virus shedding and cellular immune response in PCR-confirmed SARS-CoV-2 infected individuals will additionally be performed by the Institute of Tropical Medicine (ITM). Details are provided in Appendix 1.

2.4. OPERATIONAL DEFINITIONS

- "COVID-19 case": in this study, relates to HCW with or without acute unusual respiratory symptoms, in whom SARS-CoV-2 was detected by RT-qPCR on the nasopharyngeal swab;
- "SARS-CoV-2 seroconversion": Presence of specific SARS-CoV-2 IgG detected in the serum by ELISA during any of the monthly follow-up with a previous sample showing no specific antibody, and confirmed by PRNT.

2.5. SAMPLE SIZE

To estimate a prevalence of 50% with a precision of 5% requires a sample of 385 HCW (see appendix 2). For operational and practical reasons we opted for cluster sampling, choosing 16 clusters from 104 Belgian hospitals. Taken into account their close geographic proximity (104 hospitals on a surface area of 30,689 square kilometers), we assume a design effect of 2 will be largely sufficient to account for variability in SARS-CoV-2 prevalence among HCW between the Belgian hospitals. Thus we required 48.15 subjects per cluster, which we rounded upwards to 50.

2.6. SAMPLING PROCEDURE

The study includes a two stage sampling:

- **1st stage: Selection of hospitals (cluster sampling with probability proportional to size)**

To select the HCW to be included in the sample, we first selected 16 clusters. Hospitals were listed based on their aggregation number (from lowest to highest number) and in a second column we listed the number of beds as a proxy of numbers of HCW, while in a third column we listed the cumulative total of beds. Based on the FOD acute hospital list of December 2019, the total number of hospital beds is 52,651. Divided by 16 (number of clusters) this results in a sampling step of 3,291. We used Stata/SE15.1 to obtain a random starting point between 1 and 3,291 (random sample of one from a list of 3,291 IDs, setting the seed to 25). This starting point was used to identify the location of the first cluster from the cumulative beds column. The next cluster was located from the same column, 3,291 beds further down the list. This procedure was repeated until the total of 16 clusters had been identified. In case one or more selected hospital(s) refuse(s) to participate, this procedure will be repeated, adapting the number of clusters needed and the number of hospital beds accordingly.

- **2nd stage: Selection of HCW in each hospital (simple random sampling in each cluster)**

To select the 50 HCWs in each hospital, the local research coordinator (identified by each hospital participating in the study) will compile a list of HCW in the hospital and assign an ID number to each individual in the list (a sequence of integers starting from 1) . He/she will provide the study coordinator at Sciensano the total number of HCW in the hospital and in return will receive from the Sciensano coordinator a random list of 50 numbers of HCWs to be included. The random list

will be generated in Stata/SE15.1, always creating a sequence of integers starting from 1 until the total number of HCW in the hospital from which 50 will be sampled at random (setting the seed to 25). If the selected HCW is not eligible, the HCW next on the list will be chosen.

2.7. DATA COLLECTION

2.7.1. Information collected

Laboratory data and epidemiological data will be collected simultaneously at baseline (D0), D14, D28 and then every four weeks during a period of minimum five months.
In each hospital, one contact person will be designated to coordinate the study locally (ideally a staff member of the local infection prevention and control team) and communicate with the researchers. This person will be responsible of setting up a small team to:

- Help in the participants selection (see above) and inform the participants,
- Explain, provide and collect the informed consent for each of the participants,
- Collect biological samples, label them appropriately, fill-in the laboratory request form, and send samples to the laboratory for analysis,
- Simultaneously collect epidemiological data via questionnaires and send them to the researchers,
- Ensure any logistical support required for the study.

Laboratory data will be collected via

- A nasopharyngeal swab, in all centers, during each of the timepoints;
- A saliva sample (Oracol or equivalent), in all centers, starting at D0 and ongoing until 50 samples are collected in PCR negative and serology negative participants (this will be managed at D0) and 50 samples are collected in PCR positive as well as serology positive participants (this might need an additional sample collection at D14 and eventually D28);
- A nasal swab, in all centers, starting at D0 and ongoing until 50 samples are collected in PCR negative participants (this will be managed at D0) and 50 samples are collected in PCR positive participants (this might need an additional sample collection at D14 and eventually D28);
- A blood sample: two 10ml serum dry tubes, in all centers, during each of the timepoints;
- (Additionally, urine, semen and feces (rectal swab) samples as well as four heparin blood tubes will be collected only in some PCR positive participants as part of the sub-study performed by ITM. Details are provided in Appendix 1.) On hold.

Epidemiological data will be collected at each study timepoint via a questionnaire presented in Appendix 3. This questionnaire will be developed by the two Sciensano researchers and validated by the other study investigators (academics, virologists), then transcribed in LimeSurvey. The questionnaire will be filled-in online (via tablet/smartphone) by the participant during each contact, and will include his unique identifier code.
Information collected will include basic socio-demographic characteristics (age, gender, hospital number…), professional exposure (specific function, specialty of ward, contact with confirmed case etc.), and health characteristics (co-morbidities, presence of symptoms, use of medications). This information will be needed to assess the association between the presence and clinical presentation of the disease and these potential risk factors.
The timeline for the data collection of the main study is displayed in table 1.

		D0	Day 14	Day 28	Month 2	Month 3	Month 4	Month 5
All participant centers	Informed consent	X						
	Questionaire	X	X	X	X	X	X	X
	Nasopharyngeal swab	X	X	X	X	X	X	X
	Nasal swab	X	X*	(X*)				
	Saliva sample	X	X*	(X*)				
	two 10 ml serum_dry tubes	X	X	X	X	X	X	X

Figure 1: Timeline for data collection.

Note: Sample collection duration of +/- 2 days

X: until 100 samples reached (50 PCR - ; 50 PCR+)*

2.7.2. Laboratory specimen collection, transport and analysis

Kits containing the material for the four samples as well as labelling stickers (see below) will be provided by Sciensano. Samples dispatching and collection will be organized by Sciensano using a chauffeur.

In the laboratories, a standard RT-qPCR will be done on all nasopharyngeal swabs and, for the first 50 RT-qPCR positive and 50 RT-qPCR negative samples, RT-qPCR will also be done on the matching saliva sample and the nasal swab, enabling to validate or not saliva sampling and nasal swab for SARS-CoV-2 detection (calculating specific test properties as sensitivity and specificity).

For serological analyses, the "background" serological positivity of the HCW to various CoV will be tested in order to have a clear view on true seroconversions. Antibody titers against SARS-CoV-2; HKU-1 and OC-43 as well as 229-E and NL-63 will be determined in order to assess specificity and document potential seroconversion. Furthermore, the presently available serological tests need to be validated and confirmed with a plaque reduction neutralization test (PRNT). To that end researchers will use a Vero cell-based in vitro virus neutralization test. These cells are highly susceptible to infection with coronaviruses, including SARS-CoV-2 and show clear cytopathic effects. PRNT is not only a WHO-recognized confirmation test for SARS viruses (12), it obviously also provides information on the potential functionality of the antibodies. As for RT-qPCR, serology will equally be performed on saliva samples for those first 50 samples with a positive serology, and the first 50 samples with a negative serology enabling to validate or not the saliva sampling for antibody detection.

The urine, fecal and semen samples collected as part of the sub-study will be analysed by RT-qPCR by the ITM. The heparin tubes will be sent to Sciensano for PBMC isolation and storage. Details are provided in Appendix 1.

2.8. DATA ANALYSIS

Epidemiological and laboratory data will be linked via a unique code assigned to each participant. This code will start with the aggregation number of each hospital (three specific figures) followed by a chronological two figures number (eg. "143-01" for the first HCW tested in this hospital, "143-02" for the second one etc). At each contact, a sticker labelled with this code will be affixed on each sampling kit (and each tube/swab, lab request form) and the same corresponding code will be entered in each questionnaire, enabling the link for data analysis. This code must stay the same during all the duration of follow-up and a list of each participants and their assigned codes will be kept in a secure and protected way by each local contact person and destroyed upon completion of the study.

Epidemiological data

Analysis will be done by the two Sciensano researchers involved in this study. Questionnaires' responses will be coded. Data will be cleaned and validated, incomplete questionnaires will be manually checked to see if they can be included. Analysis will be mainly descriptive and done on STATA 14.

Among others following indicators will be calculated:

- "Prevalence of COVID-19 cases": number of HCW in whom SARS-CoV-2 was detected by RT-qPCR on the nasopharyngeal swab / Total number of HCW tested.
- "Seroprevalence of SARS-CoV-2": number of HCW in whom specific SARS-CoV-2 IgG were detected in the serum by ELISA and/or PRNT / Total number of HCW tested
- "Incidence of COVID-19": number of confirmed new cases (symptomatic and asymptomatic) of COVID-19 among HCW (SARS-CoV-2 detected by RT-qPCR on the nasopharyngeal swab) / Total number of HCW followed-up with a baseline negative PCR sample, monthly during a period of 5 months.
- "SARS-CoV-2 seroconversion rate": Number of HCW in whom presence of specific IgG was detected by ELISA and PRNT during any of the monthly follow-up with a previous sample showing no specific antibody / Total number of HCW followed-up with a baseline negative serum sample, monthly during a period of 5 months

Laboratory data

The samples will be analyzed in Sciensano and ITM laboratories. The tests used will be identical or have nearly identical properties regarding of the lab doing the analysis.

As stated above, standard RT-qPCR will be performed on NOPS. On serum samples, IgG and IgA will be detected using EUROIMMUN IgG and IgA ELISA respectively. Results will be confirmed by PRNT testing. RT-qPCR will be performed on nasal swabs and both RT-qPCR and serology will be performed on saliva sample in a limited number of participants in order to validate these sampling procedure.

Results will be communicated to participants who desire so by phone, using a line specifically dedicated for this purpose, and using no other identifier than the unique code of the participant. However, as these tests are done for research and not diagnostic purposes, and depending on lab capacity, communication of the results will take longer if compared to diagnostic testing (within one week for RT-qPCR and at least one week more for serology results). If a participant becomes symptomatic (respiratory symptoms + fever) during the study, he will follow the recommended procedure and get tested in parallel (for which results will be provided in a few hours in order to take appropriate measures).

2.9. QUALITY ASSURANCE

As for the questionnaire, this protocol will be reviewed by other researchers and experts in the field. The informed consent and the questionnaire will be available in two languages (French and Dutch). The sample collection will be performed by trained HCW (nurses). Results will be validated by the scientists in charge of the assays. Data analysis will be done by the two Sciensano researchers in collaboration with statisticians, biologists etc.

2.10. BIAS AND LIMITATIONS

- Potential selection bias because of the "late" start of the study: if all the most vulnerable HCW have already been infected at the time of the start of this study, then the incidence among the remaining HCW may be lower (because better immune system, etc…)
- Insufficient sample size: due to the current heavy workload in Belgian hospitals and time constraints, it might be difficult to recruit a team to ensure adequate local recruitment and data collection. However we will aim for a security margin in the number of participants.
- Loss of follow-up or missing data will be possible, eg/ if a HCW becomes sick in between two data collection points without providing immediate samples and is isolated at home, or if participant does not provide data at one point because of heavy workload etc. In these case, the HCW will be invited to come back in the study and participate in the following data collection timepoint. However, in the current outbreak situation HCW are supposedly highly interested in knowing their infection status and therefore in participating in the study. Furthermore, their profession might make them more inclined to contribute to medical research. Finally the duration of follow-up being relatively short, loss of follow-up should be minimized.
- Underestimation of the presence of SARS-CoV-19 among this population due to:
    - imperfect testing methods (imperfect sensitivity)=> however, bias minimized by using best available diagnostic methods (gold standard)
    - errors in the data collection => however, staff in charge of collecting the samples are experienced HCW and questionnaires will be as short and clear as possible to ensure completeness and accuracy of data and minimize reporting bias.

2.11. PROTECTION OF HUMAN SUBJECTS

2.11.1. Risks

The risks for participants are minimal, they include side effects of the sampling procedure: a nasopharyngeal swab is an unpleasant procedure while a blood test can sometimes result in a hematoma, and rarely in vagal discomfort. Participants will be clearly informed about these risks, that are minimized due to the expertise of the persons in charge of collecting the samples.

2.11.2. Benefits

In this challenging COVID-19 situation, asymptomatic HCW would be eager to benefit from a monthly diagnostic testing and follow-up they would otherwise not have as they currently do not meet the criteria for testing. Not only would they be reassured if tested negative, but it would also help them to apply timely appropriate prevention and control measures if tested positive (via NOPS) thus reducing the number of contaminated people. They would also benefit from a type of assay that is not routinely performed in Belgium for asymptomatic individuals during this COVID-19 epidemics.

2.11.3. Confidentiality

Samples results and questionnaires will be pseudonymized via an individual code attributed to each participant Tests results will be communicated by the researcher team to each participant who

demands so via phone, using only the unique code of the participant. None of the researchers who will analyse the data will be involved in data collection, nor in the care of COVID-19 patients.

2.11.4. Biological specimen

As mentioned before, we will collect a nasopharyngeal swab, a nasal swab, a saliva sample, and a blood sample, which will each be stored after entering the biobank for a duration of 10 years after which they will be destroyed. If the participant wishes its biological specimens to be destroyed upon completion of the study, this will need to be mentioned in the informed consent form.

2.11.5. Informed consent

Information on the study will be provided by the local coordinating team and informed consent will be obtained from all participants, in their working language. The informed consent can be found in appendix 4.

2.11.6. Study insurance

We have an insurance for this study with Ethias. Insurance reference is: 45.433.271 – SARS-CoV-2

2.11.7. Ethical committee clearance

The current epidemic context justifies the extremely rapid implementation of this study, with the aim of improving the care of the population and contributing to a better control of the epidemic. **An expedited ethical committee review has been sought for this protocol in University hospital Ghent and the project was approved on the 08/04/2020.**

2.11.8. Data processing and protection (GDPR)

The data protection officer for this study is Melissa Van Bossuyt (melissa.vanbossuyt@sciensano.be).

The General Data Protection Regulation (GDPR, the European legislation governing the protection of personal data, applies to this study. The legal basis on which the data are processed is consent.

- See Article 6 § 1 (a) of the GDPR
- See Article 9 § 2(a) of the GDPR

2.11.9. Practical considerations

2.11.9.1. Timeline

- Study start date: ASAP, ideally during week 14 or 15.
- Estimated study completion date: October 2020

2.11.9.2. Field work

- Purchase, preparation and transport of kits for the seroprevalence study will be done by Sciensano (for the sub-study, kits will be prepared and dispatched by ITM)
- Lab analyses will be done in both Sciensano and ITM
- Epidemiological and laboratory data will be analysed by the Sciensano researchers
- A budget of 300.000€ was estimated and approved by the management board on the 30th of March 2020

2.12. EXPECTED OUTCOME

The benefits to get out of the achievement of this study would be numerous:

- Validating saliva tests and nasal swabs could lead to the use of a less invasive, easier, cheaper testing procedure. Equally, it is crucial to gather knowledge on serological tests, hoping to quickly ensure availability of rapid, point-of care COVID-19 tests.
- Data on the current burden of COVID-19 in asymptomatic people is currently missing worldwide and would help to understand better the dynamics of the disease. This would help inform scientist and policy makers thus optimizing infection prevention and control practices.
- Furthermore, HCW represent an important element in the infection transmission chain, as they are both highly exposed to infectious cases and to vulnerable population. This is why getting a better picture of the proportion of infected HCW could help to review the measures to protect hospital personnel from infectious threats, thereby protecting the general population.
- Understanding the natural immunity regarding SARS-CoV-19 is a crucial element in the control of the epidemics and at present, evidence is dramatically lacking in this regard.

3. Appendices

3.1. APPENDIX 1: SUB-STUDY ON SARS-COV-2 SHEDDING IN FECES, URINE AND SEMEN. (ON HOLD)

As part of the study on SARS-CoV-2 seroprevalence and seroconversion among healthcare workers in Belgium, a sub-study will be performed by the Institute of Tropical medicine in Antwerp, in collaboration with Sciensano.

3.1.1. Specific objective

- Investigate virus shedding and persistence in urine, feces and semen;
- Investigate cellular immune response to the virus;
- Guide future research.

3.1.2. Study population

Study population will be the RT-qPCR positive healthcare workers tested for the purpose of the main study among the subset of selected hospitals.

Inclusion Criteria:

- Any of these RT-qPCR positive HCW who provided a signed informed consent to participate in this sub-study (not the same as for the main study).

3.1.3. Selection procedure

A sub-set of hospitals will be selected among those selected for the seroprevalence study, by convenience (geographical proximity in the neighbourhood of Antwerp). For this reason, the Antwerp University Hospital (UZA) will be added to the primary selection.
Recruitment in the study will start once the results of the D0 samples are known, and will stop once 50 RT-qPCR positive HCW are included, among which at least 20 men.

3.1.4. Study design

Prospective cohort study. Participants will be followed-up every week to investigate virus shedding and persistence, from the moment they are diagnosed positive and for a duration of minimum 5 months (subject to change, eg/ follow-up will stop for participants whose samples are tested negative before the end of the study.)
Participants will continue participating in the seroprevalence study and samples will be collected jointly when timepoints coincide (once a month). Additionnally, during these monthly timepoints, heparin tubes will be collected.

3.1.5. Data collection and analysis

Only biological specimens will be collected. No additional questionnaire will be needed.
The following samples will be collected once a week:

- A urine sample;
- A semen sample (in males);
- A rectal swab.

The following samples will be collected during the regular (monthly) timepoints, only in the participants of this sub-study:

- Four blood heparin tubes.

The kits will be dispatched by ITM to the selected hospitals and the local team coordinator will provide them to each participant. Sampling can be made at home, after what samples must be returned to the ITM laboratory via the local coordinator.
RT-qPCR will be performed on these three samples to quantify the presence of the virus and its evolution through time.
No pseudonymisation will be done at this stage so that results can be communicated to the participants by the clinical biologist if they wish so. This will be done by e-mail in an encrypted way (a valid personal e-mail address will be asked in the lab request form).
In a second stage, results will then be coded and analysed by an independant local researcher in collaboration with UZA.

3.1.6. limitations

This will be a research project done on a small, non-representative sample. However the aim is to generate new insights in this completely unknown area to guide future research.

3.1.7. Risks

The risks for the participants include those linked with the sampling procedures. A rectal swab is quite unpleasant, semen sampling is quite sensitive while a blood test can sometimes result in a hematoma, and rarely in vagal discomfort.

3.1.8. Benefits

The benefits for the participants include knowledge of their results and satisfaction obtained from having contributed to the advancement of medical research.
In these pandemic times, improving knowledge of SARS-CoV-2 shedding in human biological specimen is crucial to guide infection prevention and control measures in the short and long term.

3.1.9. Informed consent and ethics

A specific paragraph about this research project will be included in the informed consent of the seroprevalence study. Additionally, a specific informed consent form will be obtained upon participants recruitment in this study.
This study was approved by the ethical committee of University Hospital Ghent as part of the seroprevalence study.

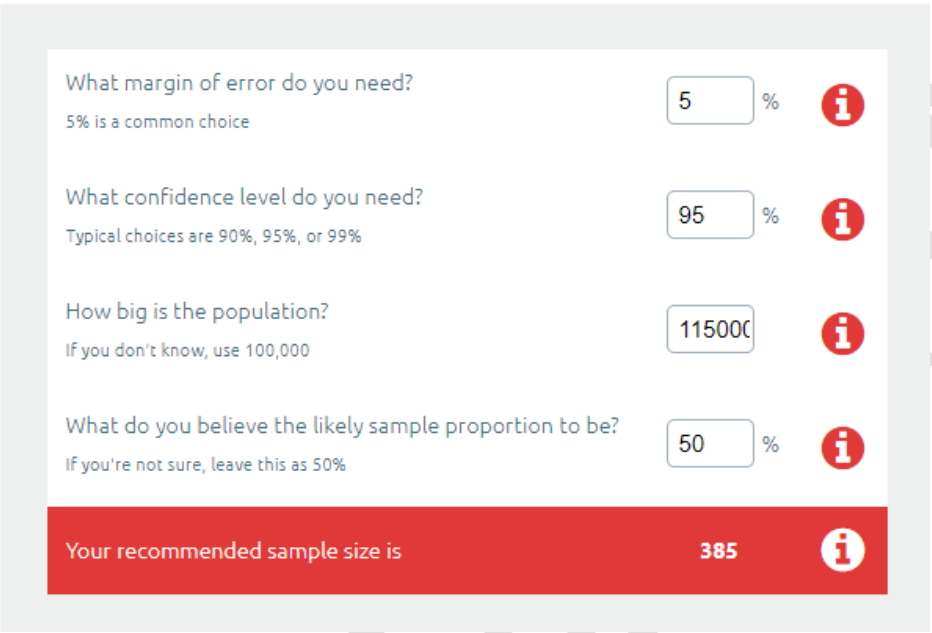
3.2. APPENDIX 2: SAMPLE SIZE CALCULATION TO ESTIMATE A PROPORTION[2]

n = N*X / (X + N – 1),

where,

X = Zα/22 *p*(1-p) / MOE2,
and Zα/2 is the critical value of the Normal distribution at α/2 (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), MOE is the margin of error, p is the sample proportion, and N is the population size.

Calculator



We would therefore need around **385** participants to estimate a prevalence of 50% of asymptomatic COVID-19 infection with a precision of 5%.

[2] https://select-statistics.co.uk/calculators/sample-size-calculator-population-proportion/

3.3. APPENDIX 3: QUESTIONNAIRE (FRENCH VERSION)

Prévalence, séroprévalence et séroconversion du SARS-CoV-2 chez les professionnels de la santé en Belgique lors de l'épidémie de COVID-19 en 2020

Sciensano (Institut belge de santé publique) a une mission statutaire de recherche dans le domaine de la santé publique. Dans le contexte épidémique actuel, Sciensano réalise entre autres une étude portant sur la prévalence et séroprévalence des infections dues au SARS-CoV-2 au sein du personnel soignant travaillant dans les hôpitaux belges. Dans ce cadre, Sciensano collecte et traite des données personnelles des participants de façon anonyme. Les questionnaires remplis seront traités par des chercheurs autorisés de Sciensano dans le but d'investiguer la prévalence de l'infection chez le personnel soignant asymptomatique, l'incidence de l'infection et la séroconversion sur une durée de 5 mois, ainsi que de tester de nouvelles méthodes d'échantillonnage et de diagnostic du COVID-19. Merci de contribuer grâce à votre participation à l'avancée de la recherche et à la lutte contre l'épidémie !

3.4. QUESTIONNAIRE AU J0 (RÉFÉRENCE)

Section 1: Informations de base

Code patient (numéro d'agrément de l'hôpital suivi de 2 chiffres attribués chronologiquement aux participants) : _ _ _/_ _
Date du jour : (jour/mois/année): __/__/____
Date de naissance: (jour/mois/année): __/__/____
Genre: □ Homme □ Femme □ Autre

Section 2 : Informations professionnelles

Occupation professionnelle dans l'hôpital (plusieurs réponses possibles)

- □ Médicale
  - □ Chef de service
  - □ Médecin
  - □ Assistant en médecine
  - □ Stagiaire
  - □ Autre : _____
- □ Paramédicale
  - □ Infirmier-ère
  - □ Kinésithérapeute
  - □ Aide-soignant
  - □ Ergothérapeute
  - □ Technicien
  - □ Autre : _____

Nombre d'années d'expérience : ____

Horaires de travail :

- □ Jour
- □ Nuit
- □ Mixte

Spécialité du/des service où le travail s'effectue (plusieurs réponses possibles)

□ USI : unité dédiée au COVID-19
□ USI : unité non-dédiée au COVID-19
□ Service non USI : unité dédiée au COVID-19
□ Service non USI : unité non-dédiée au COVID-19
➔ Spécifiez la spécialité du service (plusieurs réponses possibles) :
□ ambulance
□ anesthésie
□ cardiologie
□ centre de tri
□ chirurgie cardiaque
□ chirurgie générale/digestive
□ chirurgie vasculaire et thoracique
□ consultation ambulatoire
□ endocrinologie
□ gastroentérologie
□ gériatrie
□ gynécologie-obstétrique
□ infectiologie
□ laboratoire
□ médecine interne générale
□ néonatologie
□ néphrologie/dialyse
□ oncologie pédiatrique
□ oncologie-hématologie
□ ophtalmologie
□ orthopédie
□ oto-rhino-laryngologie
□ pédiatrie
□ pneumologie
□ psychiatrie
□ radiologie
□ rééducation
□ soins palliatifs
□ Stomatologie/dentisterie
□ transplantation
□ urgences
□ urologie
□ Autre : _____
□ Autre : _____

□ Contact avec un patient confirmé COVID-19 **avec** précautions recommandées
□ Contact avec un patient confirmé COVID-19 **sans** précautions recommandées

Section 3 : Données de santé

Avez-vous été diagnostiqué « COVID-19 positif » avec des tests de laboratoires confirmés depuis le début de l'épidémie ?

□ Non
□ Oui
Si Oui :
Date du prélèvement diagnostique : __/__/____

Conditions médicales sous-jacentes, comorbidités et facteurs de risque. Plusieurs réponses possibles.

□ Grossesse (trimestre: ____)
□ Post-partum (<6 semaines)
□ Maladie cardiovasculaire
□ Hypertension artérielle
□ Diabète
□ Maladie rénale chronique
□ Maladie chronique du foie
□ Maladie chronique des poumons
□ Maladie neurologique ou neuromusculaire chronique, excepté les troubles cognitifs
□ Immunodépression (VIH, prise de médicament immunosuppresseurs etc.)
□ Cancer solide
□ Cancer hématologique
□ Fumeur actuel
□ Autre maladie, à spécifier : ____

Prenez vous actuellement un des traitement médicamenteux suivants?
Inhibiteurs de l'enzyme de conversion de l'angiotensine (IECA) : □ Oui □ Non
Antagonistes de l'angiotensine (Sartans) : □ Oui □ Non

Avez-vous expérimenté un ou plusieurs de ces symptômes dans les 14 derniers jours (en ce compris ce jour). Plusieurs réponses possibles.
□ Pas de symptôme
□ Fièvre / frissons
□ Essoufflement
□ Douleur (indiquez localisation):
□ Faiblesse généralisée
□ Diarrhée
( ) Musculaire ( ) Poitrine
□ Toux
□ Nausée/vomissement
( ) Abdominale ( ) Articulation
□ Maux de gorge
□ Mal de tête
□ Eruption dermatologique
□ Ecoulement nasal
□ Anosmie
□ Irritabilité / confusion mentale
□ Autre, précisez _____

Si symptômes :
Date d'apparition des symptômes (jour/mois/année): __/__/____

Nous vous remercions d'avoir répondu à ce questionnaire !
Le laboratoire vous contactera pour vous communiquer vos résultats.

3.5. QUESTIONNAIRE LORS DU SUIVI (J14, J28, M2, M3, M4, M5)

Section 1: Informations de base

Code patient (numéro d'agrément de l'hôpital suivi de 2 chiffres attribués chronologiquement aux participants) : _ _ _/_ _ _
Date du jour : (jour/mois/année): __/__/____

Section 2 : Informations professionnelles

□ Contact avec un patient confirmé COVID-19 **avec** précautions recommandées
□ Contact avec un patient confirmé COVID-19 **sans** précautions recommandées

Section 3 : Données de santé

Depuis le dernier suivi, avez-vous été diagnostiqué « COVID-19 positif » avec des tests de laboratoires confirmés depuis le début de l'épidémie ?

□ Non
□ Oui
Si Oui :
Date du prélèvement diagnostique : __/__/____

Avez-vous expérimenté un ou plusieurs de ces symptômes dans les 30 derniers jours (en ce compris ce jour). Plusieurs réponses possibles.

□ Pas de symptôme
□ Fièvre / frissons
□ Faiblesse généralisée
□ Toux
□ Maux de gorge
□ Ecoulement nasal
□ Autre, précisez _____
□ Essoufflement
□ Diarrhée
□ Nausée/vomissement
□ Mal de tête
□ Anosmie
□ Douleur (indiquez localisation):
( ) Musculaire ( ) Poitrine
( ) Abdominale ( ) Articulation
□ Eruption dermatologique
□ Irritabilité / confusion mentale

Si symptômes :
Date d'apparition des symptômes (jour/mois/année): __/__/____

Nous vous remercions d'avoir répondu à ce questionnaire !
Le laboratoire vous contactera pour vous communiquer vos résultats.

3.6. APPENDIX 4: INFORMED CONSENT (FRENCH VERSION)

Prévalence, séroprévalence et séroconversion du SARS-CoV-2 chez les professionnels de la santé en Belgique lors de l'épidémie de COVID-19 en 2020

Établissement de recherche : Sciensano, DO Santé publique et Surveillance, Épidémiologie – chercheurs : Laure Mortgat et Els Duysburgh

Formulaire d'information à l'intention du participant

Cher participant,

Des foyers d'un nouveau coronavirus ont été signalés pour la première fois à Wuhan le 31 décembre 2019. Cette épidémie a rapidement touché le monde entier et constitue actuellement une pandémie. Le 12 février 2020, le nouveau coronavirus a été appelé SRAS-CoV-2 et la maladie associée COVID-19. Ce nouveau virus n'est pas le SRAS, ni le MERS, ni la grippe. Les caractéristiques de ce virus sont encore largement méconnues. La transmission interhumaine du virus a été confirmée, mais nous ne connaissons pas encore ni l'importance du portage du virus par des personnes asymptomatiques ni leur faculté à transmettre le virus. Il existe également peu de données concernant le développement et la durée de l'immunité induite par le virus chez les personnes infectées. Il n'existe actuellement aucun vaccin et aucun traitement spécifique.

Dans ce contexte, nous vous invitons à participer à une étude organisée par Sciensano (ancien Institut scientifique de Santé publique) en collaboration avec l'Institut de Médecine Tropicale d'Anvers (ITM), portant sur les professionnels de la santé du secteur hospitalier, maillons clé dans la transmission du virus.

Avant de décider si vous souhaitez participer, il est important que vous compreniez le but de ce projet de recherche et ce que cela implique pour vous pour pouvoir exprimer un consentement éclairé. Le coordinateur de l'étude ou un membre de son équipe va parcourir ce document avec vous. Prenez également le temps de poser des questions s'il y a des incertitudes ou si vous avez besoin d'informations supplémentaires. Quand vous avez décidé(e) de participer à l'étude, il vous sera demandé(e) de signer le formulaire de consentement à la fin du document.

Objectif de l'étude

L'objectif de cette étude est d'élargir les connaissances sur le COVID-19 en Belgique et de contribuer à la recherche scientifique afin de soutenir la lutte contre cette épidémie. Notamment :

- En évaluant la présence (prévalence, incidence) du virus SRAS-CoV-2 et/ou des anticorps qu'il induit (séroprévalence et séroconversion) chez les travailleurs de la santé du secteur hospitaliser belge, pendant 5 mois.
- En permettant la validation de tests sérologiques pour identifier les anticorps contre SRAS-CoV-2 et de l'échantillon salivaire et du frottis nasal pour la détection du virus.

Déroulement de l'étude

L'étude est une étude de cohorte qui suivra approximativement 800 professionnels de la santé répartis dans 16 hôpitaux pour une durée de 5 mois minimum. L'étude débutera au jour0 (D0), et les suivis se réaliseront 14 jours après (D14), 28 jours après (D28) puis une fois par mois (M2, M3, M4 et M5). Les prélèvements suivants seront réalisés à chaque contact (sept contacts en tout) par une équipe de coordination interne au sein de chaque hôpital :

- Un frottis naso-pharyngé
- Un frottis nasal
- Un prélèvement salivaire

- Une prise de sang (1 tube)

Les trois premiers prélèvements seront utilisés pour détecter la présence du virus SARS-CoV-2 (l'infection actuelle), tandis que la prise de sang servira à détecter la présence d'anticorps spécifiques au virus (indiquant un contact récent ou moins récent avec le virus).

Ces échantillons seront envoyés au laboratoire de Sciensano/ITM. Ils se verront attribuer un code unique, afin que le laboratoire puisse transmettre vos résultats aux chercheurs de façon strictement confidentielle. Votre souhait d'être informé de vos résultats doit être signalé au bas de document. En raison de la capacité limitée du testing actuellement, les résultats de vos premiers prélèvements seront disponibles dans la semaine pour la PCR (testant l'infection actuelle) et après quelques semaines pour la sérologie (testant la présence d'anticorps).

Vos échantillons seront stockés dans la Plateforme biobanque centrale de Sciensano (numéro d'attribution FAGG : BB190134). Une biobanque est une installation dans laquelle des prélèvements corporels humains (sang, urine, échantillons de tissus, etc.) sont stockés avec des données supplémentaires relatives à ces échantillons. Vos échantillons seront conservés pendant une période de 10 ans et seront utilisés pour effectuer les analyses spécifiques à l'étude. À la fin de cette période, vos échantillons seront détruits. L'administrateur médical de cette biobanque est le Dr Els Duysburgh (Coordonnées de l'administrateur médical : Els Duysburgh, 02/642 57 44, elza.duysburgh@sciensano.be). Cependant, vous restez le "propriétaire" de vos échantillons. Cela signifie que vous pouvez toujours demander à la biobanque de détruire vos échantillons stockés. Pour cela, vous devez contacter l'administrateur médical de la biobanque (Els Duysburgh, 02/642 57 44, elza.duysburgh@sciensano.be), qui s'assurera que vos échantillons stockés soient détruits. Vos échantillons prélevés et analysés dans le cadre de cette étude seront toujours pseudonymisées après collecte. Merci d'indiquer dans le bas de ce document si vous ne souhaitez pas accepter ceci.

De plus, vous serez invités à répondre à un court questionnaire à chaque contact, concernant votre état de santé et vos conditions de travail (demandé une fois au début de l'étude) et la présence éventuelle de symptômes récents (demandé à chaque contact). Ce questionnaire se remplira online via une application sécurisée et sera transmis directement aux chercheurs. Le même code unique lui sera attribué, afin de faire le lien avec vos résultats biologiques tout en protégeant votre identité.

Risques associés à l'étude

Les risques sont minimes et sont liés aux effets secondaires de la procédure de prélèvement. Le prélèvement nasopharyngé est une procédure désagréable et la prise de sang peut parfois entraîner un hématome, rarement un malaise vagal. Les risques sont minimisés par l'expertise des personnes chargées du prélèvement des échantillons.

Vous ne percevrez aucune compensation ou autre avantage pour votre participation à l'étude, autre que les informations sur vos résultats si vous le souhaitez. Votre participation à cette étude n'entraîne aucun coût supplémentaire pour vous.

Comme décrit dans la rubrique précédente, les résultats des tests réalisés ainsi que les questionnaires seront collectés et analysés par les chercheurs de Sciensano de façon strictement pseudonymisée. Le coordinateur local de l'étude dans votre hôpital sera en charge de coder votre identité et aura lui accès à vos données personnelles (pas aux résultats de vos tests). Celles-ci seront effacées dès que toutes les données auront été collectées.

Votre participation se fait sur une base volontaire et vous pouvez décider à tout moment d'y mettre un terme. Votre décision de ne plus prendre part à l'étude n'aura aucune incidence sur le traitement et les soins dont vous bénéficiez.

L'étude a été approuvée par le comité d'éthique médicale de l'Hôpital universitaire de Gand (UZ Gent).

Même en l'absence de faute, Sciensano est responsable des dommages éventuels imputables à la participation à l'étude que vous subiriez en votre qualité de participant. Sciensano assure avoir conclu un contrat d'assurance pour couvrir ces risques conformément à la loi sur les expériences sur la personne humaine du 7 mai 2004 (Ethias. Référence : 45.433.271 - SARS-CoV-2). (nom de l'assureur, numéro de police, coordonnées).

Confidentialité

Conformément au règlement général sur la protection des données (ou GDPR) (UE) 2016/679 du 27 avril 2016 (en vigueur à partir du 25 mai 2018) et à la loi belge du 30 juillet 2018 relative à la protection des personnes physiques à l'égard du traitement des données à caractère personnel et à la libre circulation de ces données, votre vie privée sera respectée et vous pourrez, si vous le souhaitez, accéder aux données collectées. Toute donnée incorrecte peut être corrigée à votre demande. Votre consentement à participer à l'étude signifie que nous traitons vos données pour les besoins de l'étude clinique. La base juridique du traitement de vos données est le consentement (voir l'article 6 § 1 a) du GDPR et l'article 9 § 2 a) du GDPR).

Toutes les informations collectées au cours de cette étude seront pseudonymisées (vos données peuvent être liées à votre nom pour permettre un suivi pendant l'étude pendant 5 mois). La clé du code qui vous est assigné ne sera accessible qu'au(x) coordinateur(s) local(aux) des études hospitalières. Dans cette étude, vous pouvez également choisir de recevoir les résultats des tests de laboratoire. À cette fin, vous devrez appeler un numéro de téléphone à Sciensano lors de plages horaires prédéfinies, et fournir à votre interlocuteur votre code unique, sur base duquel vos résultats vous seront transmis. Seules les données pseudonymisées seront utilisées pour l'analyse des données et dans tout type de documentations, rapports ou publications (dans la littérature scientifique médicale et/ou lors de conférences médicales) concernant cette étude. La confidentialité des données est donc toujours garantie. La personne responsable du traitement des données est l'investigateur principal de l'étude, le Dr Isabelle Desombere, Dr Laure Mortgat et le Dr Els Duysburgh (Sciensano) et le Dr Kevin Arien (ITG).

Si vous le souhaitez, le délégué à la protection des données (Data Protection Officer) peut vous fournir de plus amples informations sur la protection de vos données personnelles. Coordonnées : Melissa Van Bossuyt, Sciensano (melissa.vanbossuyt@sciensano.be).

L'autorité de surveillance belge responsable de l'application de la législation sur la protection des données peut être contactée via les coordonnées suivantes:

Autorité de protection des données (APD)
Rue de la Presse 35 – 1000 Bruxelles
Tel: +32 2 274 48 00
E-mail: contact@apd-gba.be
Site web: www.autoriteprotectiondonnees.be

D'avance nous tenons à vous remercier pour votre contribution précieuse à cette étude et à la médecine.

Contact

Si vous souhaitez des informations complémentaires, si vous rencontrez un quelconque problème ou si vous éprouvez des inquiétudes, vous pouvez vous adresser aux responsables de l'étude Laure Mortgat (FR) et Els Duysburgh (NL) via le 02.642 57 42 ou le 02.642.54.42 ou via e-mail: laure.mortgat@sciensano.be ou els.duysburgh@sciensano.be.

Séroprévalence et séroconversion du SARS-CoV-2 chez les professionnels de la santé en Belgique lors de l'épidémie de COVID-19 en 2020
Établissement de recherche : Sciensano, DO Santé publique et Surveillance, Épidémiologie – chercheurs : Laure Mortgat et Els Duysburgh

Formulaire de consentement

Participant

Cochez la case si vous êtes d'accord

J'ai lu et compris le document intitulé « Formulaire d'information à l'intention du participant» pages 1 à 4 et j'en ai reçu une copie. J'ai été informé(e) de la nature, du but, de la durée, des effets prévisibles de l'étude et de ce que l'on attend de moi. J'ai été informé(e) des risques et des avantages possibles de l'étude.	
J'ai eu la possibilité et le temps de réfléchir et de discuter à propos de ma participation à cette étude avec la personne de mon choix. J'ai eu la possibilité de poser toutes les questions que je souhaitais et j'ai reçu une réponse satisfaisante à mes questions, y compris mes questions d'ordre médical.	
Je comprends que la participation à l'étude est volontaire et que je peux me retirer de l'étude à tout moment sans donner de justification de cette décision et sans que cela n'affecte d'une manière ou d'une autre mon traitement ultérieur.	
Je comprends que des données me concernant seront collectées pendant ma participation à cette étude et que les chercheurs et les institutions de recherche impliquées (Sciensano à Bruxelles et l'Institut de médecine tropicale à Anvers) assureront la confidentialité de ces données. J'aurai accès aux données collectées	
Je consens au traitement de mes données personnelles et je consens à l'utilisation de mes données pseudonymisées..	
J'ai reçu un exemplaire du « formulaire d'information à l'intention du participant » et du « formulaire de consentement ».	
J'ai été informé que les données personnelles et les données relatives à ma santé sont traitées et stockées pendant 10 ans. Je suis conscient que j'ai le droit d'accéder à cette information et de la corriger. Comme ces données sont traitées à des fins médico-scientifiques, je comprends que l'accès à mes données peut être différé jusqu'à la fin de l'étude. Si je veux accéder à mes données, je m'adresserai au responsable de l'étude.	

☐ Je souhaite recevoir les résultats de mes tests biologiques
☐ J'accepte qu'à l'issue de cette étude, mes échantillons soient stockés dans une biobanque pendant 10 ans

Pour l'Institut Scientifique de Santé Publique

Je soussigné …… (tel : …….. ou e-mail:……), faisant partie de l'équipe de coordination interne de l'étude, déclare avoir fourni les informations nécessaires concernant cette étude par oral, et avoir remis un exemplaire du « formulaire d'information à l'intention du participant ».	
J'assure n'avoir exercé aucune pression sur le participant afin de lui faire consentir à prendre part à l'étude et je suis disposé à répondre à toute question complémentaire éventuelle.	
Je confirme travailler conformément aux principes éthiques prévus dans la Déclaration d'Helsinki, dans les bonnes pratiques cliniques et dans la loi belge du 7 mai 2004 relative aux expérimentations sur la personne humaine.	

Nom et prénom du participant :	Signature	Date
Nom et prénom du chercheur:	Signature	Date

Deux exemplaires doivent être remplis. L'original sera conservé par le chercheur dans l'hôpital participant, la copie sera remise au participant.

4. References

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