

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Prevention of COVID-19 with Oral Vitamin D Supplemental Therapy in Essential Healthcare Teams (PROTECT trial): protocol for a multicentre randomized placebo-controlled, triple-blind trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064058
Article Type:	Protocol
Date Submitted by the Author:	20-Apr-2022
Complete List of Authors:	Ducharme, Francine; CHU Sainte-Justine, Departments of Pediatrics and of Social and preventive medicine Tremblay, Cécile; University of Montreal, Microbiologie Golchi, Shirin; McGill University Hosseini, Banafsheh ; University of Montreal, Longo, Cristina; University of Montreal White, John; McGill University, Physiology Coveillo, Decio; HEC Montreal Quach, Caroline; University of Montreal Ste- Marie, Louis- Georges; University of Montreal Platt, Robert; McGill University
Keywords:	COVID-19, PREVENTIVE MEDICINE, Clinical trials < THERAPEUTICS

SCHOLARONE™
Manuscripts

1
2
3 1 **Prevention of COVID-19 with Oral Vitamin D Supplemental Therapy in Essential**
4
5 2 **Healthcare Teams (PROTECT trial): protocol for a multicentre randomized placebo-**
6
7 **controlled, triple-blind trial**
8
9

10 4
11
12 5 Ducharme FM^{1,2,3}, Tremblay CL⁴, Golchi S⁵, Hosseini B¹, Longo C^{3,6}, White JH⁷, Coviello D⁸,
13
14 6 Quach C⁹, Ste-Marie LG¹⁰, Platt RW^{5,11}
15
16

17 7
18
19 8 This work was funded by the Canadian Institute of Health Research grant # 447317 of the
20
21 9 COVID-19 May 2020 Rapid Response Funding Opportunity.
22
23

24 10
25
26 11 **Correspondence to:**

27
28 12 Dr. Francine M. Ducharme
29 13 Professor, Department of Pediatrics and of Social and Preventive Medicine
30 14 University of Montreal
31 15 Sainte-Justine University Hospital Centre
32 16 3175 Côte Ste-Catherine, room 17-B-000
33 17 Montreal, Quebec, H3T 1C5, Canada
34 18 francine.m.ducharme@umontreal.ca
35
36
37

38
39 1 Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada

40 2 Department of Social and Preventive Medicine, School of Public Health, University of Montreal,
41 Montreal, Quebec, CA

42 3 Clinical Research and Knowledge Transfer Unit on Childhood Asthma (CRUCA), Research Centre,
43 Sainte-Justine University Hospital Centre, Montreal, Quebec, CA

44 4 Department of Microbiology and Infectious disease, Centre Universitaire de santé de Montréal,
45 University of Montreal, Quebec, Canada

46 5 Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Quebec,
47 Canada

48 6 Faculty of Pharmacy, University of Montreal, Montréal, Quebec, Canada

49 7 Department of Physiology, McGill University, Montreal, Quebec, Canada

50 8 Department of Applied Economics, HEC Montreal, Montreal, Quebec, Canada

51 9 Department of Microbiology, Infectious Diseases & Immunology, University of Montreal, Montreal,
52 Quebec, Canada

53 10 Department of Medicine, University of Montreal, Montreal, Quebec, Canada

54 11 Department of Pediatrics, McGill University, Montreal, Quebec, Canada
55
56
57

1
2
3 **19 ABSTRACT**
4

5 **20 Introduction:** In the COVID-19 pandemic, health care workers (HCWs) have been at high-risk of
6
7
8 **21** infection due to their exposure with COVID infections. HCWs are the backbone of our health care
9
10 **22** response to this pandemic, and every health care worker withdrawn or lost due to infection has an
11
12 **23** exponential impact on our capacity to deliver care. Primary prevention is a key approach to reduce
13
14 **24** infection. Vitamin D insufficiency is highly prevalent in Canadians and worldwide. Vitamin D
15
16 **25** supplementation has been shown to significantly decrease the risk of respiratory infections.
17
18 **26** Whether this risk reduction would apply to the COVID-19 infection remains to be determined.
19
20 **27** This study aims to determine the impact of high-dose vitamin D supplementation on incidence of
21
22 **28** laboratory-confirmed COVID19 infection in HCWs working in high COVID incidence areas.
23
24

25 **29 Methods and analysis:** This is a triple-blind, placebo-controlled, parallel-group multicentre trial
26
27
28 **30** of vitamin D supplementation in HCWs at high-risk of infection. Participants were randomly
29
30 **31** allocated in a 1:1 ratio in variable block size to: Intervention—1 oral loading dose of 100,000 IU
31
32 **32** vitamin D3 + 10000 IU weekly vitamin D3 or control—identical placebo loading dose + weekly
33
34 **33** placebo. The primary outcome is incidence of laboratory-confirmed COVID-19 infection,
35
36 **34** documented by RT-qPCR on salivary (or nasopharyngeal) specimens obtained for screening or
37
38 **35** diagnostic purposes, as well as self-obtained salivary specimens obtained at endpoint and COVID-
39
40 **36** 19 seroconversion at endpoints. Secondary outcomes include disease severity; duration of COVID-
41
42 **37** 19 related symptoms; COVID-19 seroconversion documented at endpoint; duration of work
43
44 **38** absenteeism; duration of unemployment support; and adverse health events.
45
46

47 **39 Ethics and dissemination:** This study was approved by the Research Ethics Board of the CHU
48
49 **40** Sainte-Justine and participating institutions. If proven effective in reducing the risk and morbidity
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

41 of COVID-19 infection, vitamin D supplementation could offer the cheapest, most easily
42 implementable primary prevention strategy for HCWs.

43

44

For peer review only

45 **Strengths and limitations of this study**

- 46 • This multicentre randomised controlled trial will be the largest adult study testing the impact
47 of high-dose vitamin D supplementation, compared to placebo, on the risk of infection and
48 severity of COVID-19 in health care professionals.
- 49 • This trial was designed as a hybrid study enabling partially or totally remote screening,
50 randomisation, follow-up, as well as outcome documentation by use of home capillary blood
51 sampling, rapid capillary SARS-CoV2 serology, salivary self-sampling, videoconference,
52 electronic reminders and questionnaires and communication by phone, text messaging or
53 emails.
- 54 • This trial used a pragmatic subject selection and easily applicable intervention to maximise
55 subsequent implementation in practice.
- 56 • The main outcome is clinically meaningful as it explores the primary prevention impact of
57 vitamin D on the risk of laboratory-confirmed infection; it is likely to change practice if a 20%
58 reduction is documented.
- 59 • Because of the variability in diet, vitamin D supplement use, sun exposure, and skin color, it
60 is impossible to control all factors that may affect circulating 25-hydroxyvitamin D levels;
61 however, it is expected that these factors will be balanced between groups due to
62 randomization.
- 63 • A loading and regular doses have been shown to lead to rapid and sustained increase in
64 serum level of 25-hydroxy-vitamin D and ensure adequate group separation. A rapid increase
65 is particularly desired in the context of a rapidly expanding epidemic while weekly doses
66 facilitate adherence in exhausted frontline health workers.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 67 • Given the uncertainty in the progression of the infection rate, the use of a Bayesian adaptive
68 design allows for adaptations (early stopping or prolongation of duration of follow-up) at the
69 interim analysis according to the projection of infection rates.

70

For peer review only

71 Introduction

72 The Coronavirus 2 (SARS-CoV-2) disease (COVID-19) outbreak has rapidly expanded to a global
73 pandemic. Healthcare workers (HCWs) play a crucial role in the fight against the COVID-19
74 pandemic. It was therefore a public health priority to develop strategies to decrease the risk and
75 severity of COVID-19 in this vulnerable population. Indeed, there was rising concern as HCW are
76 overrepresented in terms of infections (3.8% of infected individuals in Wuhan, China,¹ 10% in
77 Italy and 12% in Spain, 10-20% in US)^{2,3} and perhaps severity. Working in long-term care facilities
78 (LTCF) and with aerosol generating medical procedures (e.g., hospitals) further increased the risk
79 (Odds Ratio [OR]: 2.3).⁴ The risk of reporting COVID-19 infection in front-line HCWs, defined
80 as those in direct contact with patients, was 10-fold greater than the general population at the
81 beginning of the pandemic (Hazard Ratio [HR]= 11.61).⁵ Recent research also indicated that
82 HCWs who were Blacks, Asians, or other minority ethnic populations, had a higher likelihood of
83 contracting COVID-19.⁵ Compared to those unexposed to COVID-19 patients; the risk was two
84 to five-fold higher in HCWs exposed to COVID-19 suspected (HR= 2.39) or confirmed (HR=
85 4.83) patients, even with adequate personal protection equipment (PPE).⁵ Although infections may
86 be due to contact with infected patients, community, or family acquired disease, cases were rapidly
87 emerging from cross-infection with asymptomatic infected HCW.

88 Vitamin D is an immunomodulatory micronutrient, and its levels in the body may vary due to diet
89 and environmental conditions. Vitamin D insufficiency has been associated with increased risk of
90 respiratory infections, and possibly COVID-19,⁶ asthma exacerbations, and acute respiratory
91 distress syndrome (ARDS) among others.⁷⁻⁹ Optimal pro-immune and anti-inflammatory impacts
92 likely occur at 25-hydroxyvitamin D (25OHD) levels above 75 nmol/L (30 ng/mL).^{10,11} In a
93 systematic review of 25 randomized controlled trials (RCT) of 11321 individuals, daily/weekly
94 vitamin D supplementation decreased by 19% the rate of acute respiratory infections (two-step

1
2
3 95 analysis; OR 0.81, 95% CI 0.72 to 0.91),^{12,13} with a stronger effect in subjects with baseline
4
5 96 25OHD <25 nmol/L. Whereas subgroup analyses suggested a protective effect primary in
6
7 97 individuals receiving daily or weekly vitamin D supplement without an additional bolus, but not
8
9
10 98 in those with bolus,¹⁴ other important differences in population (e.g., malnutrition),^{15,16} age
11
12 99 (infant),¹⁶ chronic disease (e.g. asthma, COPD)¹⁷⁻²¹ and type of infection (e.g. bacterial)^{15,16} could
13
14 100 have contributed to the apparent lesser effect. Vitamin D supplementation was also found to be
15
16 101 associated with a decreased load of rhinovirus (common cold), consistent with an increased
17
18 102 antiviral immune response.²² A systematic review and several studies reported an inverse
19
20 103 association between serum vitamin D levels and COVID-19 severity, inpatient mortality, as well
21
22 104 as serum levels of C-reactive protein (CRP) and lymphocyte percentage.^{23,24} These findings
23
24 105 suggest that vitamin D status is linked with the severity and mortality of the COVID-19 infection
25
26 106 in the general population, particularly in severe COVID-19 cases.
27
28
29 107 The vitamin D receptor is expressed on innate and adaptive immune cells which also synthesize
30
31 108 the active metabolite 1,25-hydroxyvitamin D₃ (1,25(OH)₂D₃); thus, vitamin D can strengthen
32
33 109 innate and adaptive cellular immunity by increasing local production of antimicrobial peptides,
34
35 110 decreasing secretion of pro-inflammatory cytokines, inhibiting dendritic cell activation,
36
37 111 suppressing T helper cell type 1 response, and promoting T regulatory cells induction. These
38
39 112 cellular effects are crucial for host responses against infection and can reduce the survival and
40
41 113 replication of respiratory viruses.^{13,24} 1,25(OH)₂D₃ is also produced locally in bronchial epithelial
42
43 114 cells and downregulates inflammatory cytokines (e.g. interleukin-8) and chemokines (e.g.
44
45 115 leucocyte attracting CXCL10) expression from stimulated cells.²⁵
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 117 The protocol of a placebo-controlled parallel-group triple-blind RCT to explore the impact of
4
5 118 vitamin D₃ supplementation on reducing the risk of laboratory-confirmed COVID-19 infection in
6
7 119 HCWs is described herein, as per Standard Protocol Items: Recommendation for Intervention
8
9
10 120 Trials guidelines (**Online supplemental file 1**). After funding, but prior to the start of recruitment,
11
12 121 the protocol underwent four amendments (8 protocol versions) in view of the rapidly evolving
13
14 122 science, multiple challenges faced with conducting a large scale COVID-19 trial of high-risk
15
16 123 health-care workers during the pandemic, including difficulty in obtaining large-scale supplies, as
17
18 124 well as favorable pilot results of two novel technologies (**Table 1**). These original and final (1.8,
19
20 125 January 18, 2021) protocol versions are described below.
21
22

23 24 126 **Research questions and study hypothesis**

25 26 127 **Objectives**

27
28 128 The primary question was whether one oral dose of 100,000 IU vitamin D₃ (administered at
29
30 129 baseline) plus weekly supplement of 10,000 IU vitamin D₃ can decrease the risk of laboratory-
31
32 130 confirmed COVID-19 infection, versus placebo, in frontline HCWs in high COVID-19 incidence
33
34 131 areas.
35
36

37
38 132 Additionally, the study aimed to examine if, compared with placebo, the vitamin D intervention
39
40 133 reduces: (i) illness severity, (ii) symptom duration, (iii) work absenteeism, and (iv) unemployment
41
42 134 among frontline health care workers (HCW) in high COVID-19 incidence areas. This study will
43
44 135 also assess various exploratory outcomes.
45
46

47 136 **Hypothesis**

48
49 137 We hypothesised that compared to placebo, vitamin D supplementation would decrease the
50
51 138 incidence of laboratory-confirmed symptomatic COVID-19 infection by 20% in frontline HCWs
52
53 139 working in high COVID-19 incidence area.
54
55
56
57
58
59
60

140 **Table 1. Study Amendments and Notifications**

Version number	Clinical trial Application (CTA)				Investigational testing Authorization (ITA)	
	Changes	Description	Submitted	Approved	Submitted	Approval
Version 0.0 11-05-2020						
Version 1.0 23-08-2020	<ul style="list-style-type: none"> Eligibility Outcomes & Covariates 	<ul style="list-style-type: none"> Strengthening of exclusion of 'suspected or previously undocumented COVID-19 infection' by adding: (i) a questionnaire of symptoms elaborated by Menni et al. and (ii) a rapid (15-minute) serology test, not yet approved nor tested in Canada, <u>to be pre-tested in a pilot study.</u> Addition of capillary blood self-collection with Tasso-SST device (<u>to be pre-tested in a pilot study.</u>) 	23-08-2020	16-09-2020	N/A	N/A
Amendment 1 Version 1.1 23-10-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes Main outcome 	<ul style="list-style-type: none"> Clarification of the NADAL® COVID-19 IgG/IgM Rapid Test (Teracero Pharma Inc., Lachine Canada) on venous whole blood as the rapid serology test to exclude prior infection (<u>following pilot comparative study</u>) Validation of Nadal serology test compared to IgG SARS-CoV2 serology as endpoint exploratory outcome Salivary COVID-19 RT-PCR test method prioritized over nasopharyngeal samples for twice-monthly self-collection or accepted for clinical diagnostic by qPCR 	23-10-2020 (CTA-A) †	02-11-2020	23-10-2020 (NADAL)	14-11-2020
Amendment 2 Versions 1.2-1.4 Version 1.5 27-11-2020	<ul style="list-style-type: none"> Primary Outcome Outcomes 	<ul style="list-style-type: none"> Removal of q2 weeks saliva sample for COVID-19 rt-PCR analysis due to supply problem with 50 mL Falcon centrifuge tube caused by a global plastics shortage combined with un-acceptable delay for public tender for a contract with courier service for q2weeks biological samples Addition of questions and procedures to account for the possible effect of the Vitamin D supplementation on immune response to COVID-19 vaccine, including a saliva sampling for COVID-19 by RT-PCR and a blood test for serology (and vitamin D) prior to vaccination 	27-11-2020 (CTA-A) †	30-11-2020	23-11-2020 (TASSO & NADAL)	2-12-2020

Version number	Clinical trial Application (CTA)				Investigational testing Authorization (ITA)	
	Changes	Description	Submitted	Approved	Submitted	Approval
	<ul style="list-style-type: none"> Covariates & Outcome (Device) 	<ul style="list-style-type: none"> Specification of the TASSO SST on Demand (Tasso Inc, Seattle, USA) as choice for capillary blood self-sampling (following pilot comparative study) 				
Amendment 3 versions 1.6 & Version 1.7 12-12-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes 	<ul style="list-style-type: none"> Exclusion of health care workers who have received the COVID-19 vaccine prior to enrolment Addition of (i) effect of high-dose vitamin D on SARS-CoV-2 IgG titers before & after 2nd dose of COVID-19 vaccine and (ii) the long-term infection rate up to 12 months after end-of-study Modifying exploratory outcome to allow exploration of modulating effect of vitamin D, not only on the risk of COVID-19 infection but also on response to vaccine 	12-12-2020 (CTA-A) †	16-12-2020	12-12-2020 (TASSO & NADAL)	23-12-2020
Amendment 4 Version 1.8 18-01-2021	<ul style="list-style-type: none"> Exploratory Outcomes Eligibility 	<ul style="list-style-type: none"> Clarification that the serology to be done just prior the second dose of a COVID-19 vaccine may not always be at 3 or 4 weeks (as recommended by vaccine manufacturer) to reflect the recent governmental decision to delay the timing of the 2nd vaccine dose to 12 to 16 weeks Slightly modifying wording to target healthcare workers at risk of contact with infected individuals that were not suspected of being infected (e.g., patients, colleagues, students, etc.) 	18-01-2021 (CTA-N) ‡	N/A	18-01-2020 ¶	01-02-2021

† CTA-A, Clinical Trial Application -Amendment

‡ CTA-N, Clinical Trial Application -Notification

¶ As there is no 'notification' category for the Investigational Testing Authorization (ITA), each amendment or notification to the Clinical Trial Application (CTA) must be submitted as a new amendment for the devices to be reviewed by ITA.

146 **Methods and analysis**

147 **Study design**

148 This was a pragmatic 16-week, triple-blind, placebo-controlled, parallel-group, randomized trial
149 comparing supplemental vitamin D3 and placebo in HCWs with the possibility of extending the
150 study up to 24 weeks, depending on infection rate progression during an interim analysis (**Figure**
151 **1**).

153 **Subjects**

154 HCWs (i.e., physicians, allied health care workers, orderlies, etc.) were eligible if they: (i) were
155 aged ≥ 18 and < 70 years old; (ii) were authorized to practice in Quebec; (iii) were working or
156 scheduled to work over the next 16 weeks in a setting at high-risk of contact with COVID-19
157 infected individuals, particularly (but not only) those involved with aerosol generating medical
158 procedures in hospitals and/or caring for patients in long-term care facilities; (iv) were working in
159 high COVID incidence areas in the greater Montreal area and surroundings; (v) were covered by
160 the provincial universal public health insurance (*Régie de l'assurance-maladie du Québec*
161 [RAMQ] for medical services and hospitalisations; (vi) had a personal email or phone (to which
162 to send reminders and questionnaire by email or text messages); (vii) had a fixed address (to which
163 to send the material) in the greater Montreal or surrounding areas.

164 HCWs were excluded if they met any of the following criteria: vitamin D supplementation
165 (cholecalciferol or calcitriol) intake > 400 IU/day (or $> 12,000$ IU/month) in past 3 months;
166 intention to take > 400 IU per day during the study period; suspected or previously documented
167 COVID-19 infection; history of nephrolithiasis, hypercalcemia, hyperphosphatemia,
168 hyperparathyroidism, granulomatosis disease (e.g., tuberculosis, sarcoidosis), renal failure, or

1
2
3 169 active cancer; current intake of medications that may cause hypercalcemia such as lithium,
4
5 170 teriparatide, or digoxin; anticipated prolonged absence from work during the study period (i.e.,
6
7 171 pregnancy); anticipated difficult follow-up; enrollment in a concurrent interventional randomized
8
9 172 trial; have already received the vaccine against COVID-19. Participation in this trial did not
10
11 173 preclude subsequent enrollment in a COVID-19 therapeutic (but not preventive) trial, which would
12
13 174 be documented.
14
15
16
17 175

19 176 **Study intervention**

21 177 Participants in the intervention group received 100,000 IU vitamin D₃ at randomization followed
22
23 178 by a weekly dose of 10,000 IU vitamin D₃ for 16 weeks. Participants in the control groups received
24
25 179 an identical placebo bolus followed by placebo weekly supplement for 16 weeks. Sufficient supply
26
27 180 was provided for 24 weeks, in case of prolongation study based on the interim analysis.
28
29 181 Participants in both groups were asked to take the study intervention with their most copious meal.
30
31 182 Treatment of co-morbidities were permitted. Vitamin D intake up to 400 IU per day was allowed.
32
33
34
35 183

37 184 **Randomization**

39 185 Randomization was implemented using a computer-generated random list stratified by one of 11
40
41 186 workplaces (*Centre Hospitalier Universitaire* [CHU]) or health region (*Centre Intégré*
42
43 187 *Universitaire de Santé et Services Sociaux* [CIUSSS] or *Centre Intégré de Santé et Services*
44
45 188 *Sociaux* [CISSS]). HCWs were allocated (1:1) using permuted block randomization to enhance
46
47 189 concealment. Group allocation codes for each stratum was held in a secure location with restricted
48
49 190 access by the Central Pharmacy and Data Management.
50
51
52
53
54 191

192 **Patient and public involvement**

193 Participant burden of research measures was assessed using feedback from patients participating
194 in one pilot round. Patients were not involved in study design, recruitment of participants or conduct
195 of the study. Results of this study will be disseminated through public fora.

196

197 **Outcomes**

198 *Primary outcome*

199 The original primary outcome, incidence of laboratory-confirmed COVID-19 infection, was
200 originally based on (i) bi-monthly self-obtained mid-turbinate nasopharyngeal (NP) swabs,
201 complemented by (ii) NP swabs obtained clinically for screening or diagnostic purposes
202 throughout the study, both analysed by RT-qPCR approved by Health Canada. Faced with the
203 unexpected cancellation of our large order of Falcon tubes to collect saliva sample for qPCR
204 combined with the unacceptable additional delay for a public tender to securing a contract with a
205 private courier service and in view of the uniform protocol for screening symptomatic or COVID-
206 19 exposed health care workers throughout the Province of Quebec and the reliability of IgG
207 serology, we decided to forgo the twice-monthly saliva sampling for qPCR analysis. The revised
208 definition of the primary outcome became the incidence of laboratory-confirmed COVID-19
209 infection, documented by RT-qPCR based on salivary (or nasopharyngeal) specimens (i) obtained
210 for screening or diagnostic purposes throughout the study and (ii) self-obtained salivary specimens
211 obtained at endpoint as well as (ii) COVID-19 IgG seroconversion at endpoint (in COVID-
212 unvaccinated individuals: ≥ 15 UA on the anti-S SARS-CoV-2 IgG Diasorin on Liaison XL
213 platform; in COVID-vaccinated individuals : ≥ 1.40 index (S/C) on the anti-N SARS-CoV-2 IgG
214 on ARCHITECT platform)

1
2
3 215
4
5 216 *Secondary outcomes*
6
7
8 217 (i) *Distribution of disease severity* on a 5-category ordinal scale [asymptomatic; mild (managed at
9
10 218 home); moderate (hospitalisation without supplemental oxygen); severe (oxygen
11
12 219 supplementation); critical (mechanical ventilation/death)], (self-reported, RAMQ); (ii) *Duration*
13
14 220 *of COVID-19 positivity* between 1st COVID+ to first COVID- test) revised to *Duration of COVID-*
15
16 221 *19 related symptoms* in individuals with laboratory confirmation of COVID infection, (self-
17
18 222 reported on diary); (iii) COVID-19 IgG seroconversion documented at endpoint (see above); (iv)
19
20 223 duration of work absenteeism (self-reported, medical records or human resources databases); (iv)
21
22 224 duration of unemployment support (human resource databases); (v) Adverse health events (self-
23
24 225 reported). Several *exploratory outcomes* pertained to the: incidence of post-acute and chronic
25
26 226 symptoms; long-term (1-year) morbidity and work absence related to COVID-19; change in gene
27
28 227 expression of ACE2 and TMPRSS2 in saliva cells; change in inflammatory markers (i.e., CRP),
29
30 228 immune response post vaccination; other viral infections; and genetic markers (including changes
31
32 229 in gene expression).

33
34
35
36
37
38 230

39 40 231 **Study Procedures**

41
42 232 To facilitate the recruitment of participants, this study is conceived as hybrid trial enabling partially
43
44 233 or totally remote trial participation including screening, randomization, follow-up, and end-of-
45
46 234 study visit.

47
48
49 235

50 51 236 *Pre-Screening*

1
2
3 237 Advertisements were placed in health institutions, newspapers, social media and online, where
4
5 238 participants were invited to complete an online pre-screening form, read and download the consent
6
7
8 239 forms; and if eligible and interested, to book a virtual screening appointment (via a secured
9
10 240 videoconferencing platform) with research team who would confirm eligibility, explain the study,
11
12 241 obtain informed consent, and schedule a virtual or in-person randomization visit.
13
14
15 242

16 17 243 *Screening*

18
19 244 At the virtual screening visit by videoconferencing, research coordinators completed with the
20
21 245 individual a more extensive eligibility questionnaire, which included additional questions about:
22
23 246 anticipated work exposure over the next 16 weeks to COVID-infected or suspected individuals
24
25
26 247 and to high-risk medical procedures; work place (*Centre Hospitalier Universitaire* [CHU]) or
27
28 248 *Centre Hospitalier Universitaire Sainte-Justine*) or health region (CIUSSS or CISSS), serving as
29
30 249 randomization stratum; prior laboratory-confirmed or physician-suspected COVID-19 infection;
31
32
33 250 assessment of the likelihood of prior/current, yet undocumented, COVID-19 infection using the 5-
34
35 251 item questionnaire developed by Menni et al²⁶ (score >0.50 interpreted as high likelihood of prior
36
37 252 infection); and finally, the comfort level with the study design and procedures, including saliva
38
39 253 and capillary blood sampling self-collection demonstrated by instructional videos. Eligible and
40
41 254 consenting individuals electronically signed an online consent form (with the signed PDF consent
42
43 255 form automatically emailed to participants). Then, two additional questionnaires were completed
44
45 256 on line with the research coordinators namely: (i) the baseline questionnaire collecting information
46
47 257 about household, ethnicity, part- vs. full-time work, personal health, skin color (measured with the
48
49 258 Fitzpatrick scale),²⁷ concomitant medications or supplements, and (ii) the nominative CRF
50
51 259 collecting demographic information essential to opening a medical and pharmaceutical research
52
53
54
55
56
57
58
59
60

1
2
3 260 record (i.e., public health insurance number, allergies) and maintaining contact with the research
4
5 261 team throughout the study (preferred means to receive electronic reminders/questionnaires and to
6
7 262 be notified of positive test results; address to receive study material or for biological sample pick-
8
9 263 up; and next-of-kin contact in case of inability to respond to questionnaire due to illness), and to
10
11 264 document work absence (employee number).
12
13
14
15

16 265
17 266 Finally, at the screening visit, the participant was asked to choose an appointment for a *virtual* (via
18
19 267 a secured videoconferencing platform) or *in-person* randomization visit at one of several locations.
20
21 268 To help select their preferred visit format, videos of key procedures (such as home blood
22
23 269 collection) were shown. Only in participants with a significant likelihood of a current or past
24
25 270 undocumented (Menni score > 0.5) was an *in-person* randomization visit mandatory to receive the
26
27 271 rapid COVID-19 serology test, prior to randomisation.
28
29
30
31

32 272
33 273 *Preparation and shipment of Study drug by Research Pharmacy*
34
35 274 The list of new participants approved by one of the PIs was sent daily by email to the CHUM
36
37 275 research team to be open a medical chart and send an electronically signed prescription for the
38
39 276 Study medication, to the Research Pharmacy for preparation of study drug.
40
41
42

43 277
44
45 278 Prior to randomization, a list of all consenting and eligible participants was automatically sent
46
47 279 every night to the one of the co-principal investigators (FMD or CT) who screening and baseline
48
49 280 questionnaires to approve or refuse study entry and electronically signed their decision. The list
50
51 281 daily list of new PI-approved participants was sent electronically daily to the CHUM research
52
53 282 team. Medical and pharmaceutical records were opened and an electronically signed prescription
54
55
56
57
58
59
60

283 for the Study medication sent to the Research Pharmacy for preparation of study drug for a given
284 target date.

285 To enable remote randomization, the randomization took place about one week prior to the
286 randomization visit to allow enough time for the preparation and shipment of patient-specific study
287 supplement to the research team and, in turn, the shipment of the Study supplement and all
288 materials required for the randomization visit by the research team to the participant.

290 *Randomization visit*

291 Seventy-two and 24 hours prior to, and at, the randomisation visit, participants were screened by
292 questionnaire for recent travel, symptoms suggestive of SARS-Cov2 infection, or exposure to
293 COVID-19 infected individuals. Those who responded positively were asked to get tested, notify
294 their institutional health service and await end of quarantine and/or confirmed negative test to
295 reschedule the randomisation visit.

296
297 Randomization visit (week 0) was performed *in person* (60 minutes) or *remotely* (90 minutes),
298 depending on the availability and preference of participants as well as their likelihood of a past
299 COVID-19 infection.

300
301 *In-person* visits were conducted—by appointment only—in designated rooms with restricted
302 access. The research coordinators wore personal protection equipment (PPE), and all procedures,
303 from participant arrival to departure, were approved by the institutional Infection Control and
304 Safety committee. The *in-person* visit entailed (i) ascertainment of the signed consent form, (ii)
305 capillary blood sample collection to perform NADAL® COVID-19 IgG/IgM Rapid Test (Teracero

1
2
3 306 Pharma Inc., Lachine Canada), (iii) venous blood sample collection for baseline serum 25(OH)D
4
5 307 and SARS-CoV-2 IgG serology analyses and if genetic consent, DNA; (iv) viewing of the saliva
6
7 308 collection video and instruction pamphlet, (v) collection of the first specimen under supervision,
8
9
10 309 (vi) a final verification of the eligibility and exclusion criteria; (vii) randomization; (viii) oral
11
12 310 administration of 100,000 IU vitamin D₃ or an identical placebo, and (ix) distribution of the study
13
14 311 material including study supplement, saliva sampling kit for end-of-study, biohazard and sampling
15
16 312 bag, and, if a remote visit was anticipated at week 16, capillary blood collection kits (TASSO
17
18 313 OnDemand SST device). Any patient with a positive NADAL COVID-19 IgM/IgG Rapid Test
19
20 314 serology test were excluded prior to randomisation.
21
22
23

24 315
25
26 316 The *remote* randomization visit, conducted by video-conference, was similar to the *in-person*
27
28 317 randomization visit with the following additions: (a) viewing of the capillary blood collection
29
30 318 video and instructional pamphlet; (b) remote capillary sampling under guidance using the TASSO-
31
32 319 SST device (TASSO Inc., Seattle, USA); (c) viewing of the saliva collection video and
33
34 320 instructional pamphlet (OG-600 Oragene DNA Collection Kit, DNA Genotek Inc., Ottawa,
35
36 321 Canada); (d) remote DNA salivary sampling under guidance; (e) preparation of biological samples
37
38 322 for shipment with phase change and insulated envelopes under guidance and (f) organising
39
40 323 collection of biological specimens by approved courier service to respective laboratories. Note that
41
42 324 a Nadal serology test was not conducted remotely.
43
44
45
46

47 325
48
49 326 *Follow-up*
50
51 327 Participants received *weekly electronic (SMS or email) reminders* to take their weekly Study
52
53 328 Supplement (10,000 IU vitamin D or an identical placebo) and to start completing an *online daily*
54
55
56
57
58
59
60

1
2
3 329 *Diary* if they tested positive to SARS-CoV2 or they developed symptoms suggestive of COVID-
4
5 330 19 infection.

6
7
8 331 Every two weeks, participants received a link to complete a *brief online questionnaire* asking to
9
10 332 report: their adherence to weekly Study Supplement intake; health status including recent COVID-
11
12 333 19 related exposure, symptoms, or testing; adverse health events or new co-morbidities; change in
13
14 334 concomitant medications or supplement intake; work status (active duty, quarantined, holiday,
15
16 335 sick) and work setting (ED, ICU, etc.); as well as expected/recent COVID-19 vaccination (date
17
18 336 and vaccine name) if any; the latter question served to enable timely shipment of materials for
19
20 337 additional sampling prior to vaccination, as COVID-19 vaccination was permitted during the
21
22 338 study. In participants who planned to get vaccinated during the study, three additional blood, and
23
24 339 one additional saliva, samplings, either *on-site* or *remotely*, were planned including: saliva (for
25
26 340 COVID-19 qPCR analysis) and blood (for SARS-CoV-2 anti-S IgG serology) sampled prior to
27
28 341 vaccination, a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology) collected just prior
29
30 342 to the second vaccine dose, and a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology)
31
32 343 collected one month of after second vaccine dose and endpoint. Regardless of their vaccination
33
34 344 status, participants were asked to continue taking the weekly Study Supplement and complete the
35
36 345 bi-monthly questionnaire until the end of the study. If questionnaires were not completed within 2
37
38 346 days of the target date, the research coordinator reached out the participant to complete the
39
40 347 information.

41 42 43 44 45 46 47 348 48 49 349 *End-of-Study visit*

50
51 350 An end-of-study visit was conducted either in-person (60 minutes) or remotely (90 minutes),
52
53 351 depending on the availability and preference of participants and likelihood of a current COVID-
54
55
56
57
58
59
60

1
2
3 352 19 infection. The *in-person end-of-study visit* entailed the collection of a (i) venous blood sample
4
5 353 for serum 25(OH)D and SARS-CoV-2 anti-S IgG serological results and in vaccinated participants
6
7 354 a SARS-CoV-2 anti-N IgG serology, (ii) capillary blood sample to perform the NADAL®
8
9 355 COVID-19 IgG/IgM Rapid Test, (iii) a saliva sample for SARS-CoV-2 qPCR analysis as well as
10
11 356 guessing of allocation and return of the study supplement bottle to assess adherence and any
12
13
14 357 unused material.
15
16

17 358
18
19 359 The *remote end-of-study visit* conducted via videoconference entailed the same procedures as the
20
21 360 in-person end-of-study visit with one exception: the self-collection of a capillary (instead of
22
23 361 venous) blood using TASSO-SST devices (for the serum 25(OH)D and SARS-CoV-2 anti-S
24
25 362 with/without anti-N serology). Individuals were guided into self-performing the pinprick capillary
26
27 363 method to perform the NADAL® COVID-19 IgG/IgM Rapid Test and return of biological samples
28
29 364 and materials by pre-paid approved courier.
30
31
32

33 365 34 35 366 *Covariates*

36
37 367 Several covariates that may act as confounders or interaction variables in the magnitude of effect
38
39 368 associated with the intervention were documented, namely: baseline serum 25OHD level;
40
41 369 smoking; concomitant supplements or drug(s) that alter calcium or vitamin D absorption or
42
43 370 metabolism such as diuretics and anti-epileptics (reported at baseline and every 2 weeks); skin
44
45 371 color (documented at baseline); obesity (documented by height & weight [BMI] at baseline); other
46
47 372 comorbidities (i.e., diabetes, hypertension, etc.) that may affect the severity of COVID-19
48
49 373 infection and receipt of a COVID-19 vaccine (documented by verbal report bi-monthly). All
50
51
52
53
54
55
56
57
58
59
60

1
2
3 374 external (governmental and institutional) databases were to be obtained 3 months before, and up
4
5 375 to 16 months following, randomization (as well as 12 months after then study endpoint).
6
7

8 376

9
10 377 *During an event*

11
12 378 During COVID-19 related symptoms or documented SARS-CoV infection, participants were
13
14 379 instructed to complete a daily symptom diary from date of onset of symptoms or positive test, until
15
16 380 two days with no symptoms or 14 days if asymptomatic,
17
18

19 381

20
21 382 *Risk management*

22
23 383 Clinical and biochemical adverse health events (AHEs) were monitored throughout the study and
24
25 384 reported for all patients at the end of the study. No specific laboratory safety monitoring was
26
27 385 planned given the established safety of the loading and weekly doses.²⁸ Adverse Health Events
28
29 386 (AHE) were recorded via electronic questionnaires throughout the study. Participant who reported
30
31 387 symptoms suggestive of vitamin D intoxication had a venous blood sampling (total and ionised
32
33 388 calcium, phosphorus, alkaline phosphatase, albumin, and creatinine). Any abnormal laboratory
34
35 389 values was interpreted as ‘clinically significant’ or ‘not clinically significant’ by the Site
36
37 390 endocrinologist blinded to study allocation. Further investigation or action for individual
38
39 391 participants (including interruption, cessation, or unblinding of the study drug via pharmacy or by
40
41 392 analysis of serum 25OHD) was be determined by the Site endocrinologist, if indicated to ensure
42
43 393 participant safety. The AHE’s occurrence was reviewed periodically by the Data and Safety
44
45 394 Monitoring Board (DSMB). Code breaking was allowed only if deemed essential for participant
46
47 395 management. If relevant, summary reports aggregating (or not if requested) both groups were to
48
49 396 be provided to the DSMB.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 3974
5 3986
7 3998
9
10 400 **Data management and monitoring**

11
12 401 The principle investigator (FMD) and statistical group (SG, RP) oversaw randomization, data
13
14 402 management, progress monitoring, and all analyses, including those for Data Monitoring Safety
15
16 403 Board (DMSB). The DSMB membership included: Lehana Thabane, biostatistician (Chair), Gary
17
18 404 Kobinger, infectious disease specialist, Kevin Thorpe, biostatistician, and Edgar Delvin,
19
20 405 biochemist & expert in Vitamin D. DACIMA was used for online data entry and management.
21
22
23

24 406

25
26 407 A combination of *remote* monitoring activities and *in-person* routine monitoring visits were
27
28 408 conducted by an independent Study Monitor with the first randomised participants at each site and
29
30 409 on a bi-monthly basis, to ensure that each Site adhered to the study protocol, Good Clinical Practice
31
32 410 guidelines and data collection completeness.
33
34

35 411

36
37
38 412 **Sample size calculation**

39
40 413 Given uncertainties in infection progression, a Bayesian adaptive design was used where the
41
42 414 posterior probability of effectiveness, i.e., $P(OR < 1 | \text{data})$ was the basis of inference and decision
43
44 415 making.²⁹ Assuming an expected OR of 0.80 and 1:1 treatment allocation, a total net sample size
45
46 416 of 2100 was required to identify a 20% reduction in the risk of COVID-19 in the vitamin D vs.
47
48 417 control group, with 80% power with the design described above. Considering a drop-out rate of
49
50 418 15%, 2414 participants were targeted. An interim analysis was planned when 75% of participants
51
52
53 419 reached week 12, at which time the following assessments were made: the *progression over time*

1
2
3 420 *in the incidence of infection* (slope of the curve of infection) was updated and if the probability of
4
5 421 effectiveness exceeded 0.95 [$p(\text{OR}<1)>0.95$], the trial should be terminated for efficacy at the
6
7 422 interim point (12 weeks); otherwise, the study would continue to 16 weeks. Simulation results
8
9 423 showed that, with the net sample size of 2100 (assuming an expected OR of 0.80 and 1:1 treatment
10
11 424 allocation), there was about a 55% chance that the trial would be terminated for efficacy at the
12
13 425 interim analysis. The overall infection rate was monitored on a monthly basis: note that the study
14
15 426 could have been extended to 24 weeks based on the progress of the infection rate, if required.
16
17
18
19 427

21 428 **Statistical analysis**

23 429 *Primary outcome*

24 430 An intention-to-treat (ITT) analysis was to be carried out with all randomized participants. For the
25
26 431 primary outcome, the posterior distribution of the odds ratio of COVID-19 infection (OR) was the
27
28 432 basis of inference in interim and final analyses. The posterior distribution of the OR was to be
29
30 433 estimated by drawing samples from the posterior risks under each arm, which could be obtained
31
32 434 analytically in a Beta-binomial model. Flat prior distributions were assumed for the risks
33
34 435 (Beta(1,1)). Posterior 95% credible intervals were to be reported as interval estimates for the OR.
35
36 436 Crude analyses as well as analyses adjusted for important covariates (i.e., potential confounders,
37
38 437 effect modification, and baseline group imbalances) were to be conducted. Subgroup analyses
39
40 438 would be conducted on baseline 25OHD, age, sex, BMI, occupational risk, and COVID-19
41
42 439 vaccination. A stratified analysis on geographical infection rate would be explored; sensitivity
43
44 440 analysis censoring to date of COVID-19 vaccination, would be conducted if applicable.
45
46
47
48
49
50

51 441

53 442 *Secondary outcomes*

54
55
56
57
58
59
60

1
2
3 443 Distribution of disease severity defined as a 5-level ordinal outcome would be examined with a
4
5 444 Bayesian analysis using a proportional odds (PO) model; the posterior probability of OR would be
6
7
8 445 obtained by Markov chain Monte Carlo sampling implemented in Stan.²⁹ Duration of symptoms,
9
10 446 duration of workday absences and of unemployment would be examined by a zero-inflated Poisson
11
12 447 distribution.
13

14 448

17 449 **Ethics and dissemination**

19 450 This study has been reviewed and approved by the research ethics board (REB) of the CHU Sainte-
20
21 451 Justine, serving as the local REB of all participating institutions. A non-objection letter (NOL)
22
23 452 from Health Canada has been obtained to use high-dose Vitamin D loading dose as well as Tasso
24
25 453 OnDemand device for home blood sampling and the NADAL COVID-19 IgM/IgG Rapid serology
26
27 454 test. Written informed consent for study participation, for biobanking specimens for ancillary
28
29 455 studies, and for subsequent publication of results was obtained from all participants, with the
30
31 456 knowledge that participation is voluntary and can be withdrawn at any time with no effect on their
32
33 457 current/future medical care. As part of the informed consent, enrolees had the option to participate
34
35 458 in the HostSeq COVID-19 Canadian biobank conducted under the supervision of CGen, a national
36
37 459 Canadian platform for sequencing and genome analysis (**Supplementary file 2**). In Canada, health
38
39 460 care is provided to those who suffer harm from trial participation.
40
41

42 461 All protocol amendments were submitted to Health Canada, investigators and REB; if these
43
44 462 changes implied a revision of consent forms, ongoing trial participants were informed of new
45
46 463 modifications to provide informed consent. All information obtained during the study were and
47
48 464 will be kept confidential as per the law. Data was collected directly by electronic data capture on
49
50 465 Dacima Clinical Suite (DACIMA Software Inc., Montreal, Canada). Data safety and
51
52
53
54
55
56
57
58
59
60

1
2
3 466 confidentiality was upheld at all data collection stages by assigning a unique subject ID to each
4
5 467 participant, with data and samples kept under lock and key, electronic password protection and
6
7
8 468 access restricted to study personnel. Samples collected during the study were labelled with the
9
10 469 unique research code, prior to transfer and storage at the CHUSJ biobank, with access restricted to
11
12 470 authorised personnel.

13
14 471 This trial uses pragmatic patient (irrespective of baseline 25OHD level) and intervention to
15
16 472 maximise subsequent implementation into practice. If effective in reducing infection and
17
18 473 morbidity, this approach would be readily implementable and could markedly influence practice
19
20 474 during the COVID-19 pandemic. No participant identifiers will be used in the dissemination of
21
22 475 this research. Health care professionals serving as partners informed the study design and pre-test
23
24 476 all questionnaires and will contribute to a disseminating plan. Results will be disseminated to the
25
26 477 medical community and public health departments via national/international conferences and
27
28 478 publications in peer-reviewed journals as well as to the public and study participants via the
29
30 479 Direction Collaboration-Partenariat Patient of the University of Montreal and the Canadian
31
32 480 Respiratory Research Network (CRRN) patient platform who would contribute to a disseminating
33
34 481 plan to reach as many individuals as possible.

35
36
37
38
39
40 482

41 42 43 483 **Trial Status**

44
45 484 The study was conducted as per version 1.8 (January 18, 2021). The recruitment started on
46
47 485 February 9, 2021. Upon the DSMB recommendation, recruitment was stopped prematurely on
48
49 486 March 18, 2021 after 34 participants enrolled due to the inability to target sample size of 2415
50
51 487 participants. The DSMB advised that the continuation of the trial, as originally designed, would not be
52
53 488 able to answer the research question and recommended that recruitment be stopped for futility. Recruitment
54
55
56
57
58
59
60

1
2
3 489 difficulties were attributed in part to the high use of vitamin D and high concurrent vaccination rate
4
5 490 among our target population, healthcare workers, the first targeted to be vaccinated from January 2021
6
7 491 onwards. Based on the recommendations of the study's endocrinologist, a premature cessation of
8
9 492 follow-up after a minimum of 4 weeks from randomization to monitor the safety of intervention
10
11 493 in all participants. The timeframe was deemed sufficient to ensure participant safety while learning
12
13 494 for the study, that is, transforming the PROTECT study into a pilot study to document the impact
14
15 495 of the Study intervention on the rise in Vitamin D serum level, participants' adherence the Study
16
17 496 intervention and procedures in the context of a hybrid study, etc. The last end-of-visit was
18
19 497 conducted on May 4 202.

20
21
22 498
23
24
25
26 499 Potential redirection of the study were discussed. The first option was to change the main outcome for an
27
28 500 immunogenicity study in the general adult population. However, after strong consideration of the amount
29
30 501 of changes to be made to the protocol and related documents (standards of procedures, case report
31
32 502 forms, participant' instructions and notification, etc.), the expected delay in obtaining approval by
33
34 503 all regulatory and ethical authorities, the impossible logistic of recruiting participants after the
35
36 504 same duration of exposure to the Study intervention prior to their vaccination, combined with the
37
38 505 government of Quebec announcement that all willing adults would be vaccinated by June 24, 2021,
39
40 506 the PI judged that it would be unfeasible to perform a scientific solid and feasible trial on
41
42 507 immunogenicity if one could not control the timing of immunization, combined with the expected
43
44 508 very short recruitment timeframe.

45
46
47 509
48
49
50
51 510 A second option that received very strong consideration was to replicate the PROTECT trial in
52
53 511 children aged 9 years and over. Again, after considering changes to be made to the protocol and
54
55
56
57
58
59
60

1
2
3 512 related documents, the expected delay for obtaining approval by all regulatory and ethical
4
5 513 authorities including school boards, combined with the Pfizer-BioNtech announcement that their
6
7 514 vaccine was not only 100% successful for preventing COVID-19 infection in adolescents aged 12
8
9 515 to 15 years but that they forecast vaccinating teenagers in time for September 2021 school entry,
10
11 516 the PI judge it was unrealistic to aim for the large recruitment target within such a short timeframe.
12
13
14

15 517

16
17 518 The protocol was submitted after the last patient end-of-study visit, due to the incredible amount of
18
19 519 work done to conducted to set-up and initiate this large hybrid trial, including two pilot studies
20
21 520 testing two experimental devices to enable partially or totally remote participation, in the context
22
23 521 of the pandemic which imposed large protocol and space restrictions for recruiting on-site
24
25 522 potentially COVID-19 infected health care workers, several protocol amendments to facilitate and
26
27 523 adjust the trial in the context of emerging science and anticipated vaccination campaign and their
28
29 524 impact of all electronic documents, manual of procedures, and regulatory approvals, coupled with
30
31 525 the premature end-of-follow-up in enrolled participants. The publication of this protocol is meant
32
33 526 to share our experience, enables protocol uptake in the context of another epidemic/pandemic, and
34
35 527 serves as reference for the publication of pilot studies and lessons learned from this experience.
36
37
38
39

40 528

41
42 529
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

530 **Authors' contribution**

531 FMD designed the study protocol, secured funding, and oversaw the overall conduct of the project.
532 CLT contributed to the protocol and amendments, directed the study implementation at the
533 CHUM, and coordinated the prescription of study drug, pharmacy dispensation, as well as salivary
534 sample reception and interpretation. SG conceived the statistical approach and sample size
535 calculation and along with RWP, oversaw randomization and statistical analysis. CL, JHW and
536 CQ contributed to the study design and amendments. BH wrote the first manuscript draft. LGSM
537 oversaw the safety assessment. DC is responsible for the work absenteeism analysis. All co-
538 authors approved the manuscript. Authorship eligibility on resulting manuscripts will follow
539 standard guidelines.³⁰

540

541 **Competing interests**

542 The authors have no competing interests.

543

544 **Funding**

545 This study is funded by a grant awarded through a peer-reviewed process of the COVID-19 May
546 2020 Rapid Response Funding Opportunity by the Canadian Institute of Health Research, 160
547 Elgin Street, Ottawa, ON K1A 0W9, Canada (grant number # 447317)

548

549 **Data access.**

550 The datasets used and analyzed during the current study will be made available by the
551 corresponding author on reasonable request.

1
2
3 552 **Provenance and peer review**
4
5

6 553 This study was not commissioned. It was peer-reviewed for funding and ethical approval.
7
8

9 554

10
11 555 **Acknowledgements**
12

13 556 We acknowledge the precious collaboration of Danny Germain from Quebec Riva Laboratories
14
15 557 who agree to provide free of charge Study Preparations (vitamin D and matching placebo),
16
17 558 available in bottles of 60 tablets, allowing for study prolongation. We sincerely thank Benoit
18
19 559 Hebert of Teracero Pharma Inc, for providing free-of-charge the NADAL COVID-19 IgM/IgG
20
21 560 Rapid serology test kits. We are indebted to Martin Sauvageau for implementing and coordinating
22
23 561 the RT-qPCR analysis of saliva samples at the Montreal Clinical Research Institute, Christian
24
25 562 Renaud for coordinating the COVID-19 serology analysis, and Claude Bourassa for coordinating
26
27 563 all other blood analyses at the Sainte-Justine University Health Centre. We acknowledge the
28
29 564 precious collaboration of Raymond Loyer from EFS Solution Santé who adapted their appointment
30
31 565 software for our needs as well as John Padoba, Rabie Razgallah, and Mustapha Gharb who
32
33 566 programmed and revised the eCRF to our needs. We sincerely thank Anna Smyrnova for
34
35 567 coordinating the development of the eCRF and data management. We are indebted to Catherine
36
37 568 Lamontague from Orokom Communication Marketing who developed the communication
38
39 569 strategy and tools and oversaw the publicity campaign with Marie-Line B nard-Cyr of the CHUSJ
40
41 570 who also developed the PROTECT website and Laureanne Marceau of the CHUM. We sincerely
42
43 571 thank the members of the Data Monitoring Safety Board namely Lehana Thabane (Chair), Gary
44
45 572 Kobinger, Kevin Thorpe and Edgar Delvin.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

573 **References:**

- 574 1. Zhang Y. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases
575 (COVID-19) in China. *CCDC weekly* 2020;41(2):145-151.
576 (<http://weekly.chinacdc.cn/en/article/id/e53946e2-c6c4-41e9-9a9b-fea8db1a8f51>).
- 577 2. Flaxman SM, Swapnil; Gandy, Alex: et al. Estimating the number of infections and the impact of
578 non-pharmaceutical interventions on COVID-19 in 11 European countries. 2020.
- 579 3. CDC COVID-19 Response Team. Characteristics of Health Care Personnel with COVID-19 — United
580 States, February 12–April 9, 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:477-481. DOI:
581 <http://dx.doi.org/10.15585/mmwr.mm6915e6>.
- 582 4. Ran L, Chen X, Wang Y, Wu W, Zhang L, Tan X. Risk Factors of Healthcare Workers with Corona
583 Virus Disease 2019: A Retrospective Cohort Study in a Designated Hospital of Wuhan in China. *Clin*
584 *Infect Dis* 2020 (In eng). DOI: 10.1093/cid/ciaa287.
- 585 5. Nguyen LH, Drew DA, Graham MS, et al. Risk of COVID-19 among front-line health-care workers
586 and the general community: a prospective cohort study. *The Lancet Public Health*. DOI:
587 10.1016/S2468-2667(20)30164-X.
- 588 6. Ilie PCS, Simina; Smith, Lee The role of Vitamin D in the prevention of Coronavirus Disease 2019
589 infection and mortality (pre-print). *Research Square* 2020. DOI: 10.21203/rs.3.rs-21211/v1.
- 590 7. Hughes DA, Norton R. Vitamin D and respiratory health. *Clin Exp Immunol* 2009;158(1):20-5. DOI:
591 10.1111/j.1365-2249.2009.04001.x.
- 592 8. Herr C, Greulich T, Koczulla RA, et al. The role of vitamin D in pulmonary disease: COPD, asthma,
593 infection, and cancer. *Respir Res* 2011;12:31. DOI: 10.1186/1465-9921-12-31.
- 594 9. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin D deficiency causes deficits in
595 lung function and alters lung structure. *Am J Respir Crit Care Med* 2011;183(10):1336-43. DOI:
596 10.1164/rccm.201010-1596OC.
- 597 10. Hewison M. An update on vitamin D and human immunity. *Clinical Endocrinology* 2012;76(3):315-
598 325. DOI: 10.1111/j.1365-2265.2011.04261.x.
- 599 11. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune
600 system and the clinical implications of vitamin D deficiency. *Mol Nutr Food Res* 2011;55(1):96-
601 108. (In eng). DOI: 10.1002/mnfr.201000174.
- 602 12. Martineau A. Vitamin D supplementation to prevent asthma exacerbations – Authors'
603 reply. *The Lancet Respiratory Medicine* 2018;6(6):e26-e27. DOI: 10.1016/S2213-2600(18)30199-
604 1.
- 605 13. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute
606 respiratory tract infections: systematic review and meta-analysis of individual participant data.
607 *Bmj* 2017;356:i6583. (In eng). DOI: 10.1136/bmj.i6583.
- 608 14. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute
609 respiratory infections: individual participant data meta-analysis. *Health Technol Assess*
610 2019;23(2):1-44. (In eng). DOI: 10.3310/hta23020.
- 611 15. Manaseki-Holland S, Qader G, Isaq Masher M, et al. Effects of vitamin D supplementation to
612 children diagnosed with pneumonia in Kabul: a randomised controlled trial. *Trop Med Int Health*
613 2010;15(10):1148-55. DOI: 10.1111/j.1365-3156.2010.02578.x.
- 614 16. Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D
615 supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority
616 trial. *Lancet* 2012;379(9824):1419-1427. DOI: 10.1016/S0140-6736(11)61650-4.
- 617 17. Jensen ME, Mailhot G, Alos N, et al. Vitamin D intervention in preschoolers with viral-induced
618 asthma (DIVA): a pilot randomised controlled trial. *Trials* 2016;17(1):353. DOI: 10.1186/s13063-
619 016-1483-1.

- 1
2
3 620 18. Castro M, King TS, Kunselman SJ, et al. Effect of vitamin D3 on asthma treatment failures in adults
4 621 with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *Jama*
5 622 2014;311(20):2083-91. (Multicenter Study
6
7 623 Randomized Controlled Trial
8
9 624 Research Support, N.I.H., Extramural) (In English). DOI: <http://dx.doi.org/10.1001/jama.2014.5052>.
10 625 19. Denlinger LC, King TS, Cardet JC, et al. Vitamin D Supplementation and the Risk of Colds in Patients
11 626 with Asthma. *Am J Respir Crit Care Med* 2016;193(6):634-41. (In eng). DOI: 10.1164/rccm.201506-
12 627 1169OC.
13 628 20. Martineau AR, MacLaughlin BD, Hooper RL, et al. Double-blind randomised placebo-controlled
14 629 trial of bolus-dose vitamin D³ supplementation in adults with asthma (ViDiAs).
15 630 *Thorax* 2015;70(5):451-457. DOI: 10.1136/thoraxjnl-2014-206449.
16 631 21. Martineau AR, James WY, Hooper RL, et al. Vitamin D3 supplementation in patients with chronic
17 632 obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial.
18 633 *Lancet Respir Med* 2015;3(2):120-130. (In eng). DOI: 10.1016/s2213-2600(14)70255-3.
19 634 22. Goodall EC, Granados AC, Luinstra K, et al. Vitamin D3 and gargling for the prevention of upper
20 635 respiratory tract infections: a randomized controlled trial. *BMC Infect Dis* 2014;14:273. (In eng).
21 636 DOI: 10.1186/1471-2334-14-273.
22 637 23. Maghbooli Z, Sahraian MA, Ebrahimi M, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin
23 638 D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection.
24 639 *PLoS One* 2020;15(9):e0239799. (In eng). DOI: 10.1371/journal.pone.0239799.
25 640 24. Ismailova A, White JH. Vitamin D, infections and immunity. *Reviews in Endocrine and Metabolic*
26 641 *Disorders* 2021. DOI: 10.1007/s11154-021-09679-5.
27 642 25. Pfeiffer PE, Hawrylowicz CM. Vitamin D and lung disease. *Thorax* 2012;67(11):1018. DOI:
28 643 10.1136/thoraxjnl-2012-202139.
29 644 26. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict
30 645 potential COVID-19. *Nature medicine* 2020;26(7):1037-1040. DOI: 10.1038/s41591-020-0916-2.
31 646 27. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol*
32 647 1988;124(6):869-71. (In eng). DOI: 10.1001/archderm.124.6.869.
33 648 28. McNally JD, Iliriani K, Pojsupap S, et al. Rapid normalization of vitamin D levels: a meta-analysis.
34 649 *Pediatrics* 2015;135(1):e152-66. DOI: 10.1542/peds.2014-1703.
35 650 29. Harrell FL, Chris. Statistical Design and Analysis Plan for Randomized Trial of Hydroxychloroquine
36 651 for Treatment of COVID-19: ORCHID. (<http://hbiostat.org/proj/covid19/bayesplan.html>).
37 652 30. International Committee of Medical Journal Editors. Recommendations for the Conduct,
38 653 Reporting, Editing, and Publication of Scholarly Work in Medical Journals.
39 654 <http://www.icmje.org/icmje-recommendations.pdf>: 2018.

655

1
2
3 657 **Figure Legend**
4

5 658 **Figure 1-** Study outline
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

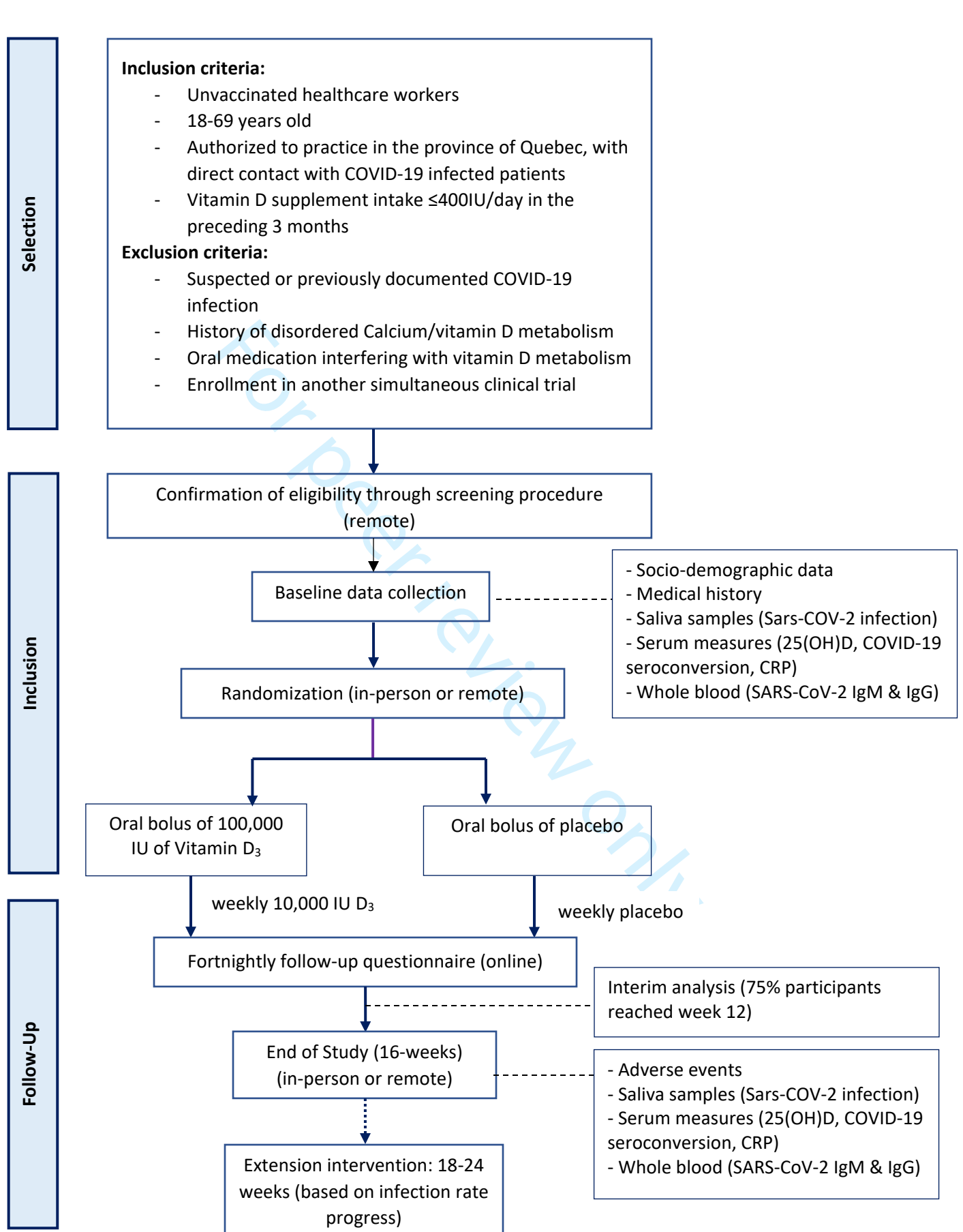


Figure 1



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	8 (line 138)
Funding	4	Sources and types of financial, material, and other support	1, 25, supplemental funding file
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 26-27
	5b	Name and contact information for the trial sponsor	1, 26
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	26

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) 19, 20, 26,27

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
	6b	Explanation for choice of comparators	6, 7
Objectives	7	Specific objectives or hypotheses	8, 9
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	9, 10

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
---------------	---	--	----

1				
2				
3	Eligibility criteria	10	Inclusion and exclusion criteria	9, 10
4			for participants. If applicable,	
5			eligibility criteria for study	
6			centres and individuals who	
7			will perform the interventions	
8			(eg, surgeons,	
9			psychotherapists)	
10				
11				
12	Interventions	11a	Interventions for each group	10, 11
13			with sufficient detail to allow	
14			replication, including how and	
15			when they will be administered	
16				
17		11b	Criteria for discontinuing or	10
18			modifying allocated	
19			interventions for a given trial	
20			participant (eg, drug dose	
21			change in response to harms,	
22			participant request, or	
23			improving/worsening disease)	
24				
25				
26		11c	Strategies to improve	17, 18
27			adherence to intervention	
28			protocols, and any procedures	
29			for monitoring adherence (eg,	
30			drug tablet return, laboratory	
31			tests)	
32				
33				
34		11d	Relevant concomitant care	10
35			and interventions that are	
36			permitted or prohibited during	
37			the trial	
38				
39	Outcomes	12	Primary, secondary, and other	11, 12
40			outcomes, including the	
41			specific measurement variable	
42			(eg, systolic blood pressure),	
43			analysis metric (eg, change	
44			from baseline, final value, time	
45			to event), method of	
46			aggregation (eg, median,	
47			proportion), and time point for	
48			each outcome. Explanation of	
49			the clinical relevance of	
50			chosen efficacy and harm	
51			outcomes is strongly	
52			recommended	
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Participant	13	Time schedule of enrolment,	15, 16, 17, 18, Figure 1
4	timeline		interventions (including any	
5			run-ins and washouts),	
6			assessments, and visits for	
7			participants. A schematic	
8			diagram is highly	
9			recommended (see Figure)	
10				
11				
12	Sample size	14	Estimated number of	20-21
13			participants needed to achieve	
14			study objectives and how it	
15			was determined, including	
16			clinical and statistical	
17			assumptions supporting any	
18			sample size calculations	
19				
20				
21	Recruitment	15	Strategies for achieving	12,13
22			adequate participant	
23			enrolment to reach target	
24			sample size	
25				

Methods: Assignment of interventions (for controlled trials)

Allocation:

31	Sequence	16a	Method of generating the	11
32	generation		allocation sequence (eg,	
33			computer-generated random	
34			numbers), and list of any	
35			factors for stratification. To	
36			reduce predictability of a	
37			random sequence, details of	
38			any planned restriction (eg,	
39			blocking) should be provided	
40			in a separate document that is	
41			unavailable to those who enrol	
42			participants or assign	
43			interventions	
44				
45				
46				
47	Allocation	16b	Mechanism of implementing	11
48	concealment		the allocation sequence (eg,	
49	mechanism		central telephone; sequentially	
50			numbered, opaque, sealed	
51			envelopes), describing any	
52			steps to conceal the sequence	
53			until interventions are	
54			assigned	
55				
56				
57				
58				
59				
60				

1				
2				
3	Implementation	16c	Who will generate the	11
4			allocation sequence, who will	
5			enrol participants, and who will	
6			assign participants to	
7			interventions	
8				
9				
10	Blinding	17a	Who will be blinded after	5, 19
11	(masking)		assignment to interventions	
12			(eg, trial participants, care	
13			providers, outcome assessors,	
14			data analysts), and how	
15				
16		17b	If blinded, circumstances	19
17			under which unblinding is	
18			permissible, and procedure for	
19			revealing a participant's	
20			allocated intervention during	
21			the trial	
22				
23				
24	Methods: Data collection, management, and analysis			
25				
26	Data collection	18a	Plans for assessment and	12 to 19
27	methods		collection of outcome,	
28			baseline, and other trial data,	
29			including any related	
30			processes to promote data	
31			quality (eg, duplicate	
32			measurements, training of	
33			assessors) and a description	
34			of study instruments (eg,	
35			questionnaires, laboratory	
36			tests) along with their reliability	
37			and validity, if known.	
38			Reference to where data	
39			collection forms can be found,	
40			if not in the protocol	
41				
42				
43				
44		18b	Plans to promote participant	12 to 19
45			retention and complete follow-	
46			up, including list of any	
47			outcome data to be collected	
48			for participants who	
49			discontinue or deviate from	
50			intervention protocols	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Data	19	Plans for data entry, coding,	20
4	management		security, and storage,	
5			including any related	
6			processes to promote data	
7			quality (eg, double data entry;	
8			range checks for data values).	
9			Reference to where details of	
10			data management procedures	
11			can be found, if not in the	
12			protocol	
13				
14				
15	Statistical	20a	Statistical methods for	21, 22
16	methods		analysing primary and	
17			secondary outcomes.	
18			Reference to where other	
19			details of the statistical	
20			analysis plan can be found, if	
21			not in the protocol	
22				
23				
24		20b	Methods for any additional	22
25			analyses (eg, subgroup and	
26			adjusted analyses)	
27				
28				
29		20c	Definition of analysis	21-22
30			population relating to protocol	
31			non-adherence (eg, as	
32			randomised analysis), and any	
33			statistical methods to handle	
34			missing data (eg, multiple	
35			imputation)	
36				

Methods: Monitoring

37				
38				
39	Data monitoring	21a	Composition of data	20,
40			monitoring committee (DMC);	
41			summary of its role and	
42			reporting structure; statement	
43			of whether it is independent	
44			from the sponsor and	
45			competing interests; and	
46			reference to where further	
47			details about its charter can be	
48			found, if not in the protocol.	
49			Alternatively, an explanation of	
50			why a DMC is not needed	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	20
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17, 19, 20, 23
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20

Ethics and dissemination

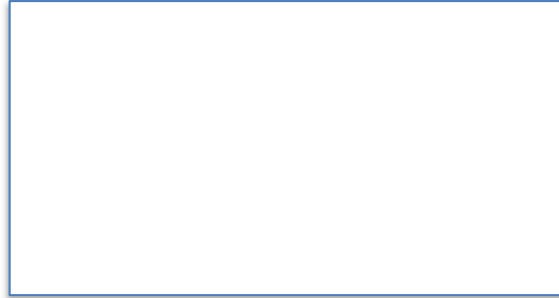
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A

1				
2				
3	A	27	How personal information	14-15, 25
4			about potential and enrolled	
5			participants will be collected,	
6			shared, and maintained in	
7			order to protect confidentiality	
8			before, during, and after the	
9			trial	
10				
11				
12	Declaration of	28	Financial and other competing	27
13	interests		interests for principal	
14			investigators for the overall	
15			trial and each study site	
16				
17	Access to data	29	Statement of who will have	27
18			access to the final trial dataset,	
19			and disclosure of contractual	
20			agreements that limit such	
21			access for investigators	
22				
23				
24	Ancillary and	30	Provisions, if any, for ancillary	25
25	post-trial care		and post-trial care, and for	
26			compensation to those who	
27			suffer harm from trial	
28			participation	
29				
30	Dissemination	31a	Plans for investigators and	23
31	policy		sponsor to communicate trial	
32			results to participants,	
33			healthcare professionals, the	
34			public, and other relevant	
35			groups (eg, via publication,	
36			reporting in results databases,	
37			or other data sharing	
38			arrangements), including any	
39			publication restrictions	
40				
41				
42				
43		31b	Authorship eligibility guidelines	25
44			and any intended use of	
45			professional writers	
46				
47		31c	Plans, if any, for granting	27
48			public access to the full	
49			protocol, participant-level	
50			dataset, and statistical code	
51				
52				

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary file 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supplementary file 2

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.



INFORMATION AND CONSENT FORM

Project Title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

Protocol Number: PROTECT 2020

- **Principal investigators at CHUSJ:** Dr. Francine M. Ducharme, MD, FRCPC, Paediatrician, Centre hospitalier universitaire Sainte-Justine (CHUSJ)
- **Principal investigator at the CHUM:** Dr. Cecile Tremblay, MD, FRCPC, Microbiologist/Infectiologist, Centre hospitalier universitaire de Montréal (CHUM)

Co-investigators:

- Decio Coviello, health economist, Hautes-Études Commerciales, University of Montreal
- Shirin Golchi, biostatistician, McGill university
- Cristina Longo, epidemiologist, University of Montreal
- Robert Platt, biostatistician, McGill University
- Caroline Quach, MD, Paediatrician microbiologist, CHUSJ
- Christian Renaud, pediatric microbiologist, CHUSJ
- John White, biochemist, McGill University

Co-investigators at the CHUM:

- Dr. Louis-Georges Sainte-Marie, MD, endocrinologist, CHUM
- Dr. Emil Toma, MD, Dsc, FRCPC, CHUM

Industrial Collaborators: Laboratoires Riva, Blainville, Quebec

Funding source: Canadian Institutes of Health Research (CIHR), in the context of the COVID-19 Rapid Research Funding Opportunity

Multicenter identifier: MP-21-2021-3044

CHUM project number : 20.319

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

Today, we are inviting you to participate in this research study because you are a healthcare worker who is working in a high COVID-19 incidence area and in a setting with a high risk of contact with COVID-19 infected cases. Please read this information to help you decide if you want to participate in this research project. It is important that you understand this information. We encourage you to ask questions. Please take all the time you need to make your decision. You may also want to discuss this study with your family doctor, a family member or a close friend.

WHY IS THIS STUDY BEING DONE?

During the current COVID-19 pandemic, many healthcare workers are working in an environment which increases their probability of contracting this viral infection. Healthcare workers are more frequently infected than the rest of the population. Infected healthcare workers can infect their family, their patients, and their contacts. In addition to being withdrawn from work, they could have transmitted the disease to other colleagues, which further impedes our ability to deliver care to the population.

Vitamin D supplementation can decrease the risk of having the common cold, but it is not known if it could have an effect on the COVID-19 infection. Vitamin D is produced in our bodies from exposure to the sun and can be obtained from supplements and certain foods. However, many Canadians do not have an adequate intake of vitamin D throughout the year.

However, studies testing supplementation with other seemingly harmless vitamins, such as beta carotene and vitamin E, have shown unexpected important adverse reactions. Therefore, it is necessary to properly assess the benefits and the potential unexpected adverse reactions in the context of a clinical study.

This study will investigate whether a high-dose vitamin D supplementation could reduce the risk and severity of COVID-19 infection and work absence in healthcare workers.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

We will be recruiting 2414 graduate healthcare workers, men and women, aged 18 to 69 years old, actively working and scheduled to continue working in a setting at high-risk of contact with people infected with COVID-19 and are working in high COVID incidence areas.

WHAT DOES THE STUDY INVOLVE?

If you agree to participate in this study, you will be assigned by chance to one of two groups. One group will receive one dose of 100,000 IU of vitamin D by mouth at the first visit and then take at home 1 pill of 10,000 IU of vitamin D once a week. The other group will receive a placebo dose at the first visit and then a placebo pill once a week. The vitamin D supplement is the active substance, meaning it could have an effect in the body. A placebo is an inactive substance, meaning it has no effect in the body. A placebo is used in clinical studies, such as this one, to ensure that observed changes are due to the active treatment and not to chance. You will have an equal chance of being assigned to each

group. The placebo and the vitamin D supplement look and taste exactly the same, so no one will know which treatment you are given, including the people involved in the study. In this informed consent form, we will use “Study supplement” to refer either to the vitamin D or the placebo.

This study should last 16 weeks and involves two visits. But prior to the first visit, we will conduct a screening/enrolment visit remotely by videoconferencing (or phone). If eligible and consenting, there will be a randomisation visit and an end-of-study visit, the latter two could be conducted in-person or remotely by videoconferencing, at your preference. Because of the pandemic, we wish to reduce the need and/or length of any in-person visit by doing as much as possible remotely.

Note that the study could finish earlier or be prolonged to 24 weeks, depending on the evolution of the pandemic. We ask that you don't change your usual diet or intake of vitamin supplements (if any) during the study.

Screening/Enrolment (pre-visit: about 45-60 minutes)

- ❖ We will review the eligibility questionnaire you have completed online, complete it with additional questions, explain the study in detail, and answer your questions.
- ❖ If eligible and consenting, you will be asked to sign the consent form, complete a few short study questionnaires and provide your contact information.
- ❖ We will ask you questions about your demographics (household, ethnicity), work-related activities and personal health (weight, height, skin color, smoking, medication, vitamins, supplements, health problems).
- ❖ To enable the creation of a medical and research pharmacy records, obtain information on COVID test, and ensure optimal contact with you throughout the study, we will ask personal information namely your RAMQ number, names (yours, your parents, your spouse), any drug allergy, your employee number (or practice number for physicians), your postal and email addresses, phone numbers and that of a next of kin and your preferred means to reach you.
- ❖ We could show you videos of key procedures (e.g. home blood collection) to help you chose your preferred type of randomisation visit.
- ❖ We will schedule the randomisation visit at a mutually convenient time and place given your choice of in-person or remote visit by videoconferencing. However, in case of a suspected prior COVID-19 infection, we would prefer you do an in-person visit to perform a rapid screening test for COVID-19 antibodies.

Randomisation visit (First visit: Week 0)

During the visit, which will last approximately an hour,

- ❖ If not already done, we will ask you to sign the consent forms, complete missing study questionnaires and your contact information.
- ❖ We will take a venous blood sample of about 15 mL (3 teaspoons) to measure the level of vitamin D, look for COVID-19 antibodies and to do an optional genetic analysis to examine a possible genetic predisposition to respond to vitamin D and to severity of COVID-19 symptoms.

- ❖ We would like to obtain a small drop of blood either from the venous puncture or via a finger-prick to look for COVID-19 antibodies in your blood using NADAL® COVID-19 IgG/IgM Rapid Test: if positive, you would not be eligible for this study. This test is not yet licensed for use in Canada and its use in this study is investigational. It has been selected for use prior to enrolment because it provides antibody results in 15 minutes. The results of this investigational test will be shared with you, acknowledging the risk of false positive or negative results. They will also be subsequently compared to the approved (Liaison IgG COVID-19) serology test.
- ❖ We will show you how a video on the TASSO home blood sampling kit at home for the last visit; if interested, we will show you how to use it, identify your sample, package it, and send it back to us (see below under First Remote visit). This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling.
- ❖ We will show you how to take a saliva sample by spitting into a tube. You will receive a pamphlet with instructions and could watch a video. You should not brush your teeth, eat, drink, smoke or chew gum for 30 minutes before spitting a small volume of saliva (2 mL). If you prefer to do an oro-nasopharyngeal sample, you would need to insert a swab (a small tube with a cotton tip) into the back of your mouth, then in one of your nostrils gently rotating the swab for about 5 seconds. We will ask you to take the saliva (or oro-nasopharyngeal) sample under our guidance. We will then show you how to identify it with our prepared labels, record the date and time, package it, and sent it back for analysis for COVID-19, and possibly other viruses and cells.
- ❖ You will be asked to take ten (10) pills of the Study supplement at this first visit only in front of the research personnel (in person or by videoconference). You will take home the bottle of Study supplement and be asked to take one (1) pill once a week until the end of the study.
- ❖ We will send you by text message or email as per your preference, a first reminder with a link to a questionnaire to confirm that you have received it and are able to complete and submit the brief questionnaire. The same approach will be used every week.
- ❖ We will give you all the other study materials including the saliva collection tube pre-printed labels, biohazard bags, insulated envelopes or boxes for shipment, prepaid courier waybills, and if you are interested in a **Remote end-of-study visit**, the TASSO home blood collection kit. It is possible that we ask you to use the NADAL® COVID-19 IgG/IgM Rapid Test at the last visit for validation purposes.

For participants choosing to have a **Remote First Visit**, in whom there is a suspicion that you may have had a prior undiagnosed COVID-19 infection in the past, we would prefer that you come for an in-person visit. Alternatively, we may send you first a finger-prick test kit to look for COVID-19 antibodies in your blood; if so, we would ask that you use it on your fingertip in front of us by videoconference. If positive, you would not be eligible for this study. If negative, the Study supplement and required materials would then be sent to the participant's home prior to the randomisation visit. We will ask you to take in front of us by videoconference, the Study supplement, the saliva sample, as well as the blood test and 2nd optional saliva sample (for genetic analysis).

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
- ❖ We will show you how to take a small sample (<1 mL) of capillary blood, using a blood collection kit specifically conceived for home collection, called TASSO-SST OnDemand. This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling. We will ask you to watch a short video and read the brochure explaining the procedure, then ask you to use it under our guidance. Briefly, you will need to warm the skin of your upper arm by rubbing it for about 45 seconds, disinfecting it, applying the little device on your arm, pressing on a button that will puncture a very small hole in the skin, then leave the device in place for about 5 minutes while blood flows slowly in a small tube. As only a small sample of blood can be obtained, it is very likely that we ask you to repeat this with a second kit. We will show you how to remove the small tube, close it with a small cap, identify the sample with our prepared labels, record the sampling date and time, package it, and prepare it to be sent for analysis for vitamin D and COVID-19 antibodies. We will ask your feedback on this type of blood collection method.
 - ❖ If you wish to participate in the optional genetic analysis, we will ask you to collect 2 mL of saliva in another small tube (as the blood sample made by TASSO is not enough for this analysis), identify and date the sample with our prepared labels, and send it to us.

28 **Between visits**

- 29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
- ❖ For the following weeks 0 to 16 (or later, should the study be extended up to 24 weeks) participants will take every week one (1) Study pill.
 - ❖ You will receive a message via email or text message, according to your indicated preference, to remind you
 - Once a week to take the Study supplement
 - Once every two weeks to take the Study supplement, and fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose.
 - ❖ If there is no response from you within a few days of sending the electronic questionnaire, we will contact you; if there is still no response from you within 7 days of us sending the electronic questionnaire, we will contact your next-of-kin indicated by you.

50 **If you are infected during the study**

51
52
53
54
55
56
57
58

If we obtain a positive COVID result from one of your saliva (or oro-nasopharyngeal) samples, you will be notified by a Study team member, by the preferred communication means you have indicated (phone, text message or email). As all positive results will be reported to the public health authorities you will be contacted as soon as possible by them for an assessment and instructions.

If you receive a positive COVID result from a test done outside this study (i.e., for clinical reasons), we ask that you inform us immediately and to indicate it in the follow-up questionnaire.

At the reception of a positive COVID test result,

- ❖ We will ask you to complete the daily diary of symptoms (duration of 1-2 minutes)
 - Until 48 hours after resolution of symptoms
 - Or if you remain asymptomatic, for a minimum of 14 days;
- ❖ If symptoms reappear, we will ask you to restart documenting them in the daily diary of symptoms
 - Until 48 hours after resolution of symptoms;
- ❖ If your symptoms continue beyond 14 days, we will ask you to complete the weekly diary of symptoms (duration of 1-2 minutes), once a week, until resolution of symptoms
- ❖ We will ask you to continue taking your weekly supplement and completing the follow-up questionnaire once every two weeks.

In case of an imminent vaccination against COVID-19

- ❖ If you expect to receive a vaccine against COVID-19 in the next few weeks, we will ask you to notify us immediately or via the questionnaire every two weeks.
 - We will rapidly organise a visit in person or remotely, before the scheduled date of the vaccination, to obtain a saliva sample to test for COVID-19 infection and a blood sample either in your vein (9 mL) or with the TASSO device at home to look for COVID-19 antibodies and level of vitamin D prior to the vaccination.
 - Just before, and about 1 month after, your second vaccine dose against COVID-19, we will ask you for another blood sample either in your vein (4.5 mL) or with the TASSO device at home to look for COVID-19 antibodies.
- ❖ We will ask you to continue
 - Once a week to take the Study supplement
 - Once every two weeks to fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose, whether you have been vaccinated or not.

End-of-study – Week 16 (or later if the study is prolonged)

At the end of the study, you will be invited to a last in-person or remote visit. This research visit will take approximately 30 minutes and involve the following:

- ❖ You will be asked to return the Study supplement bottle containing the unused pills.

- ❖ A venous (or capillary blood if done remotely) sample and, if you have not tested positive at COVID-19 before, a saliva (or oro-nasopharyngeal) sample will be collected.
- ❖ A rapid COVID-19 antibody test (NADAL®) may also be done on a blood drop (finger-prick or from the venous puncture) for validation purposes. If done remotely, this test may be done on blood sampled by finger-prick under our guidance by videoconference.
- ❖ You will complete the last few short study questionnaires and any missing information in the previous ones, if applicable.
- ❖ If conducted remotely, the samples, Study supplement bottle and unused material should also be shipped to the Coordinating Center.

Collecting information on COVID-19 tests made for clinical reasons

The results for a COVID-19 test performed for clinical reasons outside this study will be documented by you in the follow-up questionnaire (*faster means to inform us*) as well as in your institution's (Pandemic) or provincial database of COVID-19 cases, namely Trajectoire Santé publique (TSP) including all individuals who tested positive and all healthcare workers who tested positive under the supervision of the Ministère de la santé et des services sociaux (MSSS). If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we have complete information on the primary outcome of the study and thus allow us to determine accurately the impact of the intervention on the risk of infection with COVID-19.

Collecting information on healthcare services

The date, diagnosis, type of professional and of health care services which you have received during medical visits and hospitalisations will be obtained from the administrative databases of the Régie de l'assurance maladie du Québec (RAMQ) and Quebec hospital discharges (MED-ECHO). This will allow us to accurately determine the impact of the intervention on the severity of COVID-19 infection and other concomitant illnesses.

Collecting information of work absence

The number of days of work absence, overall, by type (i.e., holiday, illness, etc.), and specifically due to COVID-19, including absences due to an infection acquired at work or outside of work, preventive withdrawal due to pregnancy or other health conditions, awaiting test results/investigation, or other reason for quarantine will be collected from you via the follow-up questionnaire (*faster and most detailed means*), as well as from your institution's *Direction of Health Resources* or, if you are an attending physician, from the *Direction of professional services*. If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we accurately ascertain the impact of the intervention on work absences.

BIOBANK

For the purposes of this study, we will keep the biological samples collected (blood, saliva and/or oro-nasopharyngeal) in a biobank as well as the clinical and administrative data collected during the course of this study in order to complete the study's objectives, and to

1
2
3 conduct research on vitamin D, COVID-19 and its treatments and other related diseases.
4 We would like to quantify specific cellular receptors which allow entry of COVID-19 into
5 cells (for example, the angiotensin converting enzyme-ACE2) and inflammatory markers
6 (such as the C-reactive protein). The collected samples will be kept in a biobank in the
7 Research Center of CHU Sainte-Justine under the supervision of Dr Francine M.
8 Ducharme. The samples will be kept as long as the research team can guarantee their proper
9 management. Confidentiality of the identity of the samples will be guaranteed by assigning
10 them a specific code. Your sample will not be identified by your name and cannot be used
11 to identify you directly. After 5 years, the code key will be destroyed, and the samples will
12 become completely anonymous. Your samples could possibly be shared with other
13 researchers in other institutions. However, the access to data will only be allowed for
14 approved projects by an independent research ethics board.
15
16
17
18

19 **GENETIC ANALYSIS (optional)**

20
21 Each person has their own set of unique genes or “genome”. Genetic research aims to
22 determine if there are genetic predispositions which make you more susceptible to a
23 COVID-19 infection, to respond to vitamin D, to modulate disease severity and the
24 interaction of these factors.
25
26

27 If you accept to participate in the genetic analysis, these analyses will be done on a small
28 part (4 mL) of the venous blood sample provided during the first visit. If you decide to
29 participate remotely, we will ask you to provide a saliva sample in a small tube.
30
31

32 We would like to sequence your entire genome and conduct gene expression analyses. We
33 would also like to share your genetic data as well as other collected clinical data during the
34 PROTECT study with the Canadian database HostSeq COVID-19 for use for COVID-19
35 related research and other aspects of human health. This biobank will serve as a centralized
36 resource in Canada for COVID-19 research and other health-related studies. The data in
37 the HostSeq database are under the supervision of CGen, a national Canadian platform
38 financed by the federal government for sequencing and genome analysis. The principal
39 investigators of the PROTECT study as well as the administrators of the HostSeq biobank
40 COVID-19 will share your genetic and clinical information with other Canadian and
41 international researchers whom are approved by CGen (the sponsor). The data could also
42 be used for commercial use. However, your data will not be shared with until after an
43 examination by a data access committee. This committee will verify that the use of the
44 proposed research is in line with the objectives of the database HostSeq and that the
45 research team which requests access has already been granted the required approval in
46 accordance in terms of research ethics requirements. Approved researchers will sign
47 agreements. These agreements will control how the data will be used. Individual results of
48 any research conducted using your samples or any individual incidental findings will not
49 be shared with you, as the research conducted on your data will have no individual
50 diagnostic or therapeutic significance to you.
51
52
53
54
55
56

57 **WHAT ARE THE BENEFITS AND RISKS OF THIS STUDY?**

Benefits:

You may not benefit directly from the study intervention if it is not efficacious or if you have been assigned in the placebo group. However, the screening may identify earlier an active or past COVID-19 infection that was not apparent. Your participation will help advance our knowledge on vitamin D and on the prevention of COVID-19 infection in healthcare workers and other individuals at risk of infection.

Each positive COVID-19 result from the saliva (or oro-nasopharyngeal) or blood sample will be shared with you according to your preferred way of communication: telephone, text message or email. All positive saliva (or oro-nasopharyngeal) results will also be shared by the Microbiology Laboratory of CHUM with the Public health authorities and will be added into your file at the CHUM and Dossier Santé Québec. No other research result will be provided to you. Research findings resulting from your participation to this study could potentially contribute to creating commercial products from which you would not be able to claim any financial benefit.

Risks:**• Related to study medication:**

The vitamin D dose used in this study has been shown to be safe in adults. This dose is approved by Health Canada for the purpose of this study only, but not for clinical use yet. It is unlikely that you will have any side effects because of the amount of vitamin D used in this study as when combining the first and weekly doses, the total remains below the maximum amount allowed.

However, we will ask you to notify us immediately if you have any of the following, as they could be signs of an acute excess intake of vitamin D: mainly, a marked increase in thirst or an increase in the volume and frequency of urination (with or without fatigue, loss of appetite, nausea or vomiting, headaches, drowsiness, cardiac arrhythmias, constipation, muscle or bone or chest pain, mouth dryness or a metallic taste).

Later signs and symptoms that may indicate a chronic excess intake of vitamin D are: a marked increase in thirst, an increase in the volume and frequency of urination including during the night, loss of appetite, weight loss, red eye or conjunctivitis, inflammation of the pancreas, light sensitivity, runny nose, itching, fever, reduced libido, kidney stones, increased concentration of some analytes in the blood (BUN, AST, ALT, cholesterol), or in urine (albumin), ectopic calcification, hypertension, cardiac arrhythmias and rarely, a psychosis.

It's possible that other currently unknown risks are associated with Vitamin D intake.

One of the reasons we collect a blood sample is to measure the concentration of vitamin D in the blood at the start and end of the study. this will allow us to see if the vitamin D blood level is linked to the number and severity of COVID-19 confirmed cases.

• Related to study procedures:

1
2
3 The salivary collection sample is painless. If done, an oro-nasopharyngeal swab may cause
4 slight discomfort during collection that will subside after its removal. The side effects of
5 having blood collected by venous puncture or TASSO can include bleeding, bruising,
6 discomfort and pain at the sample site. It is possible that the NADAL COVID-19 IgG/IgM
7 Test may give false positive or false negative results. In case of divergence of results, we
8 will communicate to you the results of the approved IgG test when available.
9
10

11
12
13 **• Related to confidentiality:**

14 There is always a small risk that your data could one day be re-identified. The genetic
15 information is unique to each person, just as your fingerprint. This means that theoretically,
16 you could be identified using your genetic code; however, this is not easy to do.
17 Considering the advances in technology, there could be new ways to link you to data that
18 we have not foreseen today, despite the strict confidentiality measures in place. Possible
19 re-identification or unintentional disclosure of your genetic and clinical research data could
20 lead to a loss in confidentiality and a possible future discrimination against yourself or your
21 biological parents. But all security measures will be put in place to protect your privacy.
22
23

24 **WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?**

25
26 The Study supplements are provided free of charge by the manufacturer, Laboratoire
27 RIVA.
28

29
30 **WHAT ARE THE OTHER FINANCIAL ASPECTS?**

31 For each completed visit (0 and 16 weeks), you will receive a \$25 check by mail to
32 compensate for your time. The check may arrive at your home between 4 and 8 weeks after
33 the visit.
34
35

36 **HOW IS PRIVACY INSURED?**

37
38 During your participation in this research study, the investigators responsible for this study
39 as well as the members of their research team will collect, in a research file, the required
40 personal information to answer the scientific objectives of this research project.
41

42 These information could include your demographic data (name, sex, date of birth, ethnic
43 origin, weight and height), your past and present health status, your health-related habits,
44 medication you take, your work absences, and the results of all tests, exams, and procedures
45 which you will participate in. Your personal file will include your address, email, telephone
46 numbers, RAMQ number, and employee or practice number be kept in a separate file with
47 restricted access; this information is required to create a medical and pharmacy file at the
48 CHUM and for communication purposes during the study.
49

50
51 The coded blood, saliva (and/or oro-nasopharyngeal) samples will be sent to the biobank
52 located at the Research Center of CHU Sainte-Justine under the supervision of Dr Francine
53 M. Ducharme. The coded results of completed analyses will be kept on a protected server
54 with restricted access at DACIMA company during the study, and thereby transferred to a
55 secure server with restricted access in the Research Center of CHU Sainte-Justine under
56 the supervision of Dr Francine M. Ducharme. During the study, the personal information
57
58

1
2
3 used to arrange virtual and in-person study visit appointments will be kept on a protected
4 server with restricted access at the company providing the appointment-making software.
5 Following the conclusion of the study, this information of yours will be transferred to
6 a secure server with restricted access in the Research Center of CHU Sainte-Justine under
7 the supervision of Dr Francine M. Ducharme. The database of HostSeq will be kept on
8 secure cloud servers (online) that are based in Canada and will be indefinitely kept or until
9 they are not useful for research.
10

11
12 To ensure your privacy, a copy of the consent form as well as the results to the diagnostic
13 tests required for conducting the research project, will be copied in the research and
14 medical file of the CHUM. Therefore, each person or company which you authorize to
15 consult your medical file, will have access to this information.
16

17 The research data will be kept for at least 25 years by the principle investigator. The data
18 collected could be published or discussed during scientific meetings, but it would not be
19 possible to identify you.
20

21 All collected information will remain confidential within the limits provided by law. You
22 will only be identified by a code number. The key to the code linking your name to your
23 research file will be kept by the investigator responsible for this research project.
24

25
26 To ensure your safety, a copy of the consent form as well as the results of the diagnostic
27 tests required for research purposes will be placed in the research file and the medical file
28 of the CHUM. Consequently, any person or company to whom you give access to your
29 medical file will have access to this information.
30

31 Research data will be kept for at least 25 years by the investigator responsible for this
32 research project. Research data may be published or be the subject of scientific discussion,
33 but it will not be possible to identify you.
34

35
36
37 For the purposes of surveillance, control, safety and marketing of the Study drug, your
38 research as well as your medical files could be consulted by a person mandated by a
39 regulatory organization, in Canada or elsewhere, such as Health Canada, as well as sponsor
40 representatives of the company manufacturing the vitamin D pills for this project
41 (Laboratoire RIVA), the institution or research ethics committee. These people and
42 organizations adhere to a strict confidentiality agreement.
43

44 You have the right to consult your research file to verify the collected data and to correct
45 them, if needed. Moreover, access to certain information before the end of the study could
46 mean your removal from this study in order to maintain the study's integrity.
47
48
49

50 **IS YOUR PARTICIPATION VOLUNTARY?**

51
52 Yes. Taking part in this study is voluntary. You may choose not to be in this study. You
53 can decide to stop being in the study at any time, without needing to provide any reason,
54 but simply informing the research team.
55
56
57
58

1
2
3 Your decision to refuse participation or to stop participating in the study at a later time,
4 will have no effect on the quality of care or services to which you are entitled or on your
5 relationship with the people that provide them.
6

7
8 The principal investigators of this study, the research ethics board, the funding agency or
9 the sponsor could decide to end your participation in the study without your consent. This
10 could happen if there are new information or findings that indicate your participation is no
11 longer in the best of your interests, or if you have not been following the study instructions
12 as explained, or if there are other administrative-related reasons to stop the project.
13

14 If you stop participating in the study or if you have been removed from it, the collected
15 information and material already received will be kept (as well as the data pertaining to
16 healthcare services and work absences will continue to be collected) and analysed to ensure
17 the validity of this project, unless you specifically ask for them to be destroyed. If this is
18 the case, these data and/or material will be removed from the biobank provided that the
19 code key (linking between nominal data and the study code) is still available, that is, up to
20 5 years after the end of the study.
21
22

23 If you decide to drop out of the HostSeq database, your data will no longer be shared, and
24 no new data will be collected. The data already in the HostSeq database will be destroyed
25 once informed about this decision. However, it could be impossible to remove the results
26 once they have been compiled with the results of other participants or if they have been
27 published. Moreover, if certain data have been shared with other researchers, it could be
28 possible not to be able to remove this part of the data. In such a case of unsuccessful
29 withdrawal from the study, your identity will always be protected.
30
31

32 All new information acquired during the course of the study which could have an impact
33 on your decision to continue participation will be shared with you rapidly, which is the
34 reason why we would like to keep your personal information and have your approval to
35 communicate with you after the end of the study (optional).
36
37
38
39
40
41
42
43
44

45 **WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**

46
47 If you have any questions about the research project or if you have any problems that you
48 believe are related to your participation in the project, you can call the researchers
49 responsible for the project:
50

- 51 • Dr. Francine M. Ducharme at 514 345 4931, extension 4398
 - 52 • Dr. Cecile Tremblay at 514 890-8000, extension 14645
- 53
54
55
56
57
58

1
2
3 If you would like information about your rights related to your participation in the research,
4 you may contact the Ombudsman - complaints and quality services of the CHU Sainte-
5 Justine at 514 345-4749, of the CHUM at 514 890-8484 or your CIUSSS/CIUSSS:
6

- 7 • CIUSSS de l'Est-de-l'Île-de-Montréal : 514 252-3510
- 8 • CIUSSS de l'Ouest-de-l'Île-de-Montréal : 514-989-1885, extension: 1010
- 9 • CIUSSS du Centre-Sud-de-l'Île-de-Montréal : 514 593-3600
- 10 • CIUSSS de la Montérégie-Est : 450-468-8447
- 11 • CIUSSS de la Montérégie-Centre : 450-466-5434
- 12
- 13

14 **RESEARCH ETHICS COMMITTEE**

15 The Research Ethics Board of CHU Sainte-Justine has approved this study and will
16 continue to monitor it for all participating institutions of the Quebec Health and Social
17 Services network.
18

19 **LIABILITY**

20 This research is not funded by a private industry. In case of side effects resulting from the
21 study medication or from procedures required for this research project, you will receive all
22 necessary medical care covered by the Quebec's provincial health insurance plan (RAMQ)
23 or by your private drug insurance plan. You will be responsible for paying the portion of
24 any costs not covered.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CONSENT FORM

Research project title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

The nature and procedures of this research project were explained to me. I have read the information and consent forms and I kept a copy, or a copy has been provided to me. I was able to ask my questions and they were answered to my satisfaction. After consideration, I agree to participate in this research project.

I authorize the research team to consult the collected data about me in the COVID infection database (Pandemic) of my institution and/or the provincial TSP database, the medical and hospitalisation services database (RAMQ and MED-ECHO), and the workplace absenteeism database (Human Resources Directorate or Professional Services Directorate) to obtain information that is pertinent to this project.

By agreeing to participate in this study, you are not waiving any of my rights under the law. You are not releasing the investigators from their legal and professional liability.

Name of participant (Print)

Signature

Date

1. I consent to the analysis of gene expression and the sequencing of the whole genome of my coded biological material (blood, saliva, and/or oro-nasopharyngeal). The whole genome sequence could be hosted in the Canadian HostSeq COVID-19 biobank and linked to a database containing the viral genome. This would serve to explore any genetic predisposition to COVID-19, the severity of the disease and response to vaccine.

Yes _____ (Initials) No _____ (Initials)

2. I consent to prolonging the access to my coded data on healthcare use, COVID-19 infections and work absenteeism for 12 months following the study end date, to explore the long-term impact of COVID-19 infection and vaccination.

Yes _____ (Initials) No _____ (Initials)

3. I consent to being contacted to update my personal information, obtain additional information about my health or to be invited to participate in new research.

Yes _____ (Initials) No _____ (Initials)

4. In case I receive a vaccine against COVID-19 during the study, I agree to do the blood samples before the first and second vaccine dose as well as 1 month after the 2nd vaccine dose, even if these samples were to be done after the end-of-study's visit planned at week 16 (or 24).

Yes _____ (Initials) No _____ (Initials)

Participant's signature: _____

1
2 I have explained the research study and the terms of this information and consent form to the research
3 participant, and I answered all his/her questions. I explained that participation in a research project is
4 free and voluntary and could be stopped at any time they choose.
5
6
7

8
9 Name of person obtaining consent (Print) Signature Date
10
11

12
13 **(FOR THE CHUM PARTICIPANTS ONLY)**
14

15 **COMMITMENT OF THE PRINCIPAL INVESTIGATOR AT THE CHUM**
16

17 I certify that this information and consent form was explained to the research participant, and that the
18 questions the participant had were answered.
19

20
21 I undertake, together with the research team, to respect what was agreed upon in the information and
22 consent form, and to give a signed and dated copy of this form to the research participant.}
23
24
25
26
27

28 Name (Print) Signature of the principal
29 investigator at the CHUM Date
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

BMJ Open

Prevention of COVID-19 with oral vitamin D supplemental therapy in essential healthcare teams (PROTECT trial): protocol for a multicentre, randomized, placebo-controlled, triple-blind trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064058.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Nov-2022
Complete List of Authors:	Ducharme, Francine; CHU Sainte-Justine, Departments of Pediatrics and of Social and preventive medicine Tremblay, Cécile; University of Montreal, Microbiologie Golchi, Shirin; McGill University Hosseini, Banafsheh ; University of Montreal, Longo, Cristina; University of Montreal White, John; McGill University, Physiology Coviello, Decio; HEC Montreal Quach, Caroline; University of Montreal Ste- Marie, Louis- Georges; University of Montreal Platt, Robert; McGill University
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Infectious diseases
Keywords:	COVID-19, PREVENTIVE MEDICINE, Clinical trials < THERAPEUTICS

SCHOLARONE™
Manuscripts

1
2
3 1 **Prevention of COVID-19 with oral vitamin D supplemental therapy in essential healthcare**
4
5 2 **teams (PROTECT trial): protocol for a multicentre, randomized, placebo-controlled, triple-**
6
7 3 **blind trial**
8
9

10 4
11
12 5 Ducharme FM^{1,2,3}, Tremblay CL⁴, Golchi S⁵, Hosseini B¹, Longo C^{3,6}, White JH⁷, Coveillo D⁸,
13
14 6 Quach C⁹, Ste-Marie LG¹⁰, Platt RW^{5,11}
15
16
17 7

18
19 8 **Correspondence to:**
20

21 9 Dr. Francine M. Ducharme
22 10 Professor, Department of Pediatrics and of Social and Preventive Medicine
23 11 University of Montreal
24 12 Sainte-Justine University Hospital Centre
25 13 3175 Côte Ste-Catherine, room 17-B-000
26 14 Montreal, Quebec, H3T 1C5, Canada
27 15 francine.m.ducharme@umontreal.ca
28
29
30
31
32
33
34
35
36
37
38

39 ¹ Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada

40 ² Department of Social and Preventive Medicine, School of Public Health, University of Montreal,
41 Montreal, Quebec, CA

42 ³ Clinical Research and Knowledge Transfer Unit on Childhood Asthma (CRUCA), Research Centre,
43 Sainte-Justine University Hospital Centre, Montreal, Quebec, CA

44 ⁴ Department of Microbiology and Infectious disease, Centre Universitaire de santé de Montréal,
45 University of Montreal, Quebec, Canada

46 ⁵ Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Quebec,
47 Canada

48 ⁶ Faculty of Pharmacy, University of Montreal, Montréal, Quebec, Canada

49 ⁷ Department of Physiology, McGill University, Montreal, Quebec, Canada

50 ⁸ Department of Applied Economics, HEC Montreal, Montreal, Quebec, Canada

51 ⁹ Department of Microbiology, Infectious Diseases & Immunology, University of Montreal, Montreal,
52 Quebec, Canada

53 ¹⁰ Department of Medicine, University of Montreal, Montreal, Quebec, Canada

54 ¹¹ Department of Pediatrics, McGill University, Montreal, Quebec, Canada
55
56
57
58
59
60

16 ABSTRACT

17 **Introduction:** In the COVID-19 pandemic, health care workers (HCWs) have been at high-risk of
18 infection due to their exposure with COVID infections. HCWs are the backbone of our health care
19 response to this pandemic, and every health care worker withdrawn or lost due to infection has an
20 exponential impact on our capacity to deliver care. Primary prevention is a key approach to reduce
21 infection. Vitamin D insufficiency is highly prevalent in Canadians and worldwide. Vitamin D
22 supplementation has been shown to significantly decrease the risk of respiratory infections.
23 Whether this risk reduction would apply to the COVID-19 infection remains to be determined.
24 This study aims to determine the impact of high-dose vitamin D supplementation on incidence of
25 laboratory-confirmed COVID19 infection rate and severity in HCWs working in high COVID
26 incidence areas.

27 **Methods and analysis:** This is a triple-blind, placebo-controlled, parallel-group multicentre trial
28 of vitamin D supplementation in HCWs at high-risk of infection. Participants were randomly
29 allocated in a 1:1 ratio in variable block size to: Intervention—1 oral loading dose of 100,000 IU
30 vitamin D3 + 10000 IU weekly vitamin D3 or control—identical placebo loading dose + weekly
31 placebo. The primary outcome is incidence of laboratory-confirmed COVID-19 infection,
32 documented by RT-qPCR on salivary (or nasopharyngeal) specimens obtained for screening or
33 diagnostic purposes, as well as self-obtained salivary specimens obtained at endpoint and COVID-
34 19 seroconversion at endpoints. Secondary outcomes include disease severity; duration of COVID-
35 19 related symptoms; COVID-19 seroconversion documented at endpoint; duration of work
36 absenteeism; duration of unemployment support; and adverse health events.

37 **Ethics and dissemination:** This study was approved by the Research Ethics Board of the CHU
38 Sainte-Justine and participating institutions. If proven effective in reducing the risk and morbidity

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

39 of COVID-19 infection, vitamin D supplementation could offer the cheapest, most easily
40 implementable primary prevention strategy for HCWs. [NCT04483635]

41
42

For peer review only

43 **Strengths and limitations of this study**

- 44 • This trial was designed as a hybrid study enabling partially or totally remote screening,
45 randomisation, follow-up, as well as outcome documentation by use of home capillary blood
46 and saliva sampling, visits conducted by videoconference, monitoring by electronic reminders
47 and questionnaires, and communication by phone, text messaging or emails.
- 48 • This trial used a pragmatic subject selection and easily applicable intervention to maximise
49 subsequent implementation in practice.
- 50 • The main outcome was clinically meaningful as it explored the primary prevention impact of
51 vitamin D on the risk of laboratory-confirmed infection and would likely change practice if a
52 20% reduction was documented.
- 53 • A single loading dose followed by regular doses have been shown to lead to rapid and
54 sustained increase in serum level of 25-hydroxy-vitamin D and ensure adequate group
55 separation, both properties desired in the context of a rapidly expanding epidemic while
56 facilitating adherence in exhausted frontline health workers.
- 57 • Given the uncertainty in the progression of the infection rate, the use of a Bayesian adaptive
58 design allows for adaptations (early stopping or prolongation of duration of follow-up) at the
59 interim analysis according to the projection of infection rates.

60

61 Introduction

62 The Coronavirus 2 (SARS-CoV-2) disease (COVID-19) outbreak has rapidly expanded to a global
63 pandemic. Healthcare workers (HCWs) play a crucial role in the fight against the COVID-19
64 pandemic. It was therefore a public health priority to develop strategies to decrease the risk and
65 severity of COVID-19 in this vulnerable population. Indeed, there was rising concern as HCW are
66 overrepresented in terms of infections (3.8% of infected individuals in Wuhan, China,¹ 10% in
67 Italy and 12% in Spain, 10-20% in US)^{2,3} and perhaps severity. Working in long-term care facilities
68 (LTCF) and with aerosol generating medical procedures (e.g., hospitals) further increased the risk
69 (Odds Ratio [OR]: 2.3).⁴ The risk of reporting COVID-19 infection in front-line HCWs, defined
70 as those in direct contact with patients, was 10-fold greater than the general population at the
71 beginning of the pandemic (Hazard Ratio [HR]= 11.61).⁵ Recent research also indicated that
72 HCWs who were Blacks, Asians, or other minority ethnic populations, had a higher likelihood of
73 contracting COVID-19.⁵ Compared to those unexposed to COVID-19 patients; the risk was two
74 to five-fold higher in HCWs exposed to COVID-19 suspected (HR= 2.39) or confirmed (HR=
75 4.83) patients, even with adequate personal protection equipment (PPE).⁵ Although infections may
76 be due to contact with infected patients, community, or family acquired disease, cases were rapidly
77 emerging from cross-infection with asymptomatic infected HCW.

78 Vitamin D is an immunomodulatory micronutrient, and its levels in the body may vary due to diet
79 and environmental conditions. Vitamin D insufficiency has been associated with increased risk of
80 respiratory infections, and possibly COVID-19,⁶ asthma exacerbations, and acute respiratory
81 distress syndrome (ARDS) among others.⁷⁻⁹ Optimal pro-immune and anti-inflammatory impacts
82 likely occur at 25-hydroxyvitamin D (25OHD) levels above 75 nmol/L (30 ng/mL).^{10,11} In a
83 systematic review of 25 randomized controlled trials (RCT) of 11321 individuals, daily/weekly
84 vitamin D supplementation decreased by 19% the rate of acute respiratory infections (two-step

1
2
3 85 analysis; OR 0.81, 95% CI 0.72 to 0.91),^{12,13} with a stronger effect in subjects with baseline
4
5 86 25OHD <25 nmol/L. Whereas subgroup analyses suggested a protective effect primary in
6
7 87 individuals receiving daily or weekly vitamin D supplement without an additional bolus, but not
8
9
10 88 in those with bolus,¹⁴ other important differences in population (e.g., malnutrition),^{15,16} age
11
12 89 (infant),¹⁶ chronic disease (e.g. asthma, COPD)¹⁷⁻²¹ and type of infection (e.g. bacterial)^{15,16} could
13
14 90 have contributed to the apparent lesser effect. Of interest, Vitamin D supplementation significantly
15
16 91 reduced the rate of severe exacerbations (i.e., requiring rescue systemic corticosteroids), a
17
18 92 condition association with airway inflammation, with no impact of bolus use or not.¹⁴ Vitamin D
19
20 93 supplementation was also found to be associated with a decreased load of rhinovirus (common
21
22 94 cold), consistent with an increased antiviral immune response.²² A systematic review and several
23
24 95 studies reported an inverse association between serum vitamin D levels and COVID-19 severity,
25
26 96 inpatient mortality, as well as serum levels of C-reactive protein (CRP) and lymphocyte
27
28 97 percentage.^{23,24} These findings suggest that vitamin D status was linked with the severity and
29
30 98 mortality of the COVID-19 infection in the general population, particularly in severe COVID-19
31
32 99 cases. Whether Vitamin D could prevent or lessen infection and/or the inflammatory response
33
34 100 associated with the COVID-19 remained to be explored.²⁵ At the time of the funding in June 2020
35
36 101 and study initiation (February 2021), no other primary prevention trials were published. Since then,
37
38 102 one positive and two negative trials testing different vitamin D intervention were published.²⁶⁻²⁸
39
40
41
42
43
44
45
46

47 104 The vitamin D receptor is expressed on innate and adaptive immune cells which also synthesize
48
49 105 the active metabolite 1,25-hydroxyvitamin D₃ (1,25(OH)₂D₃); thus, vitamin D can strengthen
50
51 106 innate and adaptive cellular immunity by increasing local production of antimicrobial peptides,
52
53
54 107 decreasing secretion of pro-inflammatory cytokines, inhibiting dendritic cell activation,
55
56
57
58
59

1
2
3 108 suppressing T helper cell type 1 response, and promoting T regulatory cells induction. These
4
5 109 cellular effects are crucial for host responses against infection and can reduce the survival and
6
7
8 110 replication of respiratory viruses.^{13,24} 1,25(OH)₂D₃ is also produced locally in bronchial epithelial
9
10 111 cells and downregulates inflammatory cytokines (e.g. interleukin-8) and chemokines (e.g.
11
12 112 leucocyte attracting CXCL10) expression from stimulated cells.²⁹
13
14
15 113

16
17 114 The protocol of a placebo-controlled parallel-group triple-blind RCT to explore the impact of
18
19 115 vitamin D₃ supplementation on reducing the risk and severity of laboratory-confirmed COVID19
20
21 116 infection in HCWs is described herein, as per Standard Protocol Items: Recommendation for
22
23 117 Intervention Trials guidelines (**Online supplemental file 1**). After funding, but prior to the start
24
25
26 118 of recruitment, the protocol underwent four amendments (8 protocol versions) in view of the
27
28 119 rapidly evolving science, multiple challenges faced with conducting a large scale COVID-19 trial
29
30
31 120 of high-risk health-care workers during the pandemic, including difficulty in obtaining large-scale
32
33 121 supplies, as well as favorable pilot results of two novel technologies (**Table 1**). These original and
34
35 122 final (1.8, January 18, 2021) protocol versions are described below. The trial was initiated but
36
37
38 123 interrupted prematurely due to recruitment difficulty.
39

40 124

41 42 125 **Research questions and study hypothesis**

43 44 126 **Objectives**

45
46
47 127 The primary question was whether one oral dose of 100,000 IU vitamin D₃ (administered at
48
49 128 baseline) plus weekly supplement of 10,000 IU vitamin D₃ can decrease the risk of laboratory-
50
51 129 confirmed COVID-19 infection, versus placebo, in frontline HCWs in high COVID-19 incidence
52
53
54 130 areas.
55
56
57
58
59
60

1
2
3 131 Additionally, the study aimed to examine if, compared with placebo, the vitamin D intervention
4
5 132 reduces: (i) illness severity, (ii) symptom duration, (iii) work absenteeism, and (iv) unemployment
6
7 133 among frontline health care workers (HCW) in high COVID-19 incidence areas. This study will
8
9 134 also assess various exploratory outcomes.
10
11
12
13

135

136 **Hypothesis**

137 We hypothesised that compared to placebo, vitamin D supplementation would decrease the
18
19 138 incidence of laboratory-confirmed symptomatic COVID-19 infection by 20% in frontline HCWs
20
21 139 working in high COVID-19 incidence area.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

140 **Table 1. Study Amendments and Notifications**

Version number	Clinical trial Application (CTA)				Investigational Testing Authorization (ITA)	
	Changes	Description	Submitted	Approved	Submitted	Approval
Version 0.0 11-05-2020						
Version 1.0 23-08-2020	<ul style="list-style-type: none"> Eligibility Outcomes & Covariates 	<ul style="list-style-type: none"> Strengthening of exclusion of ‘suspected or previously undocumented COVID-19 infection’ by adding: (i) a questionnaire of symptoms elaborated by Menni et al. and (ii) a rapid (15-minute) serology test, not yet approved nor tested in Canada, <u>to be pre-tested in a pilot study.</u> Addition of capillary blood self-collection with Tasso-SST device <u>(to be pre-tested in a pilot study).</u> 	23-08-2020	16-09-2020	N/A	N/A
Amendment 1 Version 1.1 23-10-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes Main outcome 	<ul style="list-style-type: none"> Clarification of the NADAL® COVID-19 IgG/IgM Rapid Test (Teracero Pharma Inc., Lachine Canada) on venous whole blood as the rapid serology test to exclude prior infection (<u>following pilot comparative study</u>) Validation of Nadal serology test compared to IgG SARS-CoV2 serology as endpoint exploratory outcome Salivary COVID-19 RT-PCR test method prioritized over nasopharyngeal samples for twice-monthly self-collection or accepted for clinical diagnostic by qPCR 	23-10-2020 (CTA-A) †	02-11-2020	23-10-2020 (NADAL)	14-11-2020
Amendment 2 Versions 1.2-1.4 Version 1.5 27-11-2020	<ul style="list-style-type: none"> Primary Outcome Outcomes 	<ul style="list-style-type: none"> Removal of q2 weeks saliva sample for COVID-19 rt-PCR analysis due to supply problem with 50 mL Falcon centrifuge tube caused by a global plastics shortage combined with un-acceptable delay for public tender for a contract with courier service for q2weeks biological samples Addition of questions and procedures to account for the possible effect of the Vitamin D supplementation on immune response to COVID-19 vaccine, including a saliva sampling for COVID-19 by RT-PCR and a blood test for serology (and vitamin D) prior to vaccination 	27-11-2020 (CTA-A) †	30-11-2020	23-11-2020 (TASSO & NADAL)	2-12-2020

Version number	Clinical trial Application (CTA)				Investigational Testing Authorization (ITA)	
	Changes	Description	Submitted	Approved	Submitted	Approval
	<ul style="list-style-type: none"> Covariates & Outcome (Device) 	<ul style="list-style-type: none"> Specification of the TASSO SST on Demand (Tasso Inc, Seattle, USA) as choice for capillary blood self-sampling (following pilot comparative study) 				
Amendment 3 versions 1.6 & Version 1.7 12-12-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes 	<ul style="list-style-type: none"> Exclusion of health care workers who have received the COVID-19 vaccine prior to enrolment Addition of (i) effect of high-dose vitamin D on SARS-CoV-2 IgG titers before & after 2nd dose of COVID-19 vaccine and (ii) the long-term infection rate up to 12 months after end-of-study Modifying exploratory outcome to allow exploration of modulating effect of vitamin D, not only on the risk of COVID-19 infection but also on response to vaccine 	12-12-2020 (CTA-A) †	16-12-2020	12-12-2020 (TASSO & NADAL)	23-12-2020
Amendment 4 Version 1.8 18-01-2021	<ul style="list-style-type: none"> Exploratory Outcomes Eligibility 	<ul style="list-style-type: none"> Clarification that the serology to be done just prior the second dose of a COVID-19 vaccine may not always be at 3 or 4 weeks (as recommended by vaccine manufacturer) to reflect the recent governmental decision to delay the timing of the 2nd vaccine dose to 12 to 16 weeks Slightly modifying wording to target healthcare workers at risk of contact with infected individuals that were not suspected of being infected (e.g., patients, colleagues, students, etc.) 	18-01-2021 (CTA-N) ‡	N/A	18-01-2020 ¶	01-02-2021

† CTA-A, Clinical Trial Application -Amendment

‡ CTA-N, Clinical Trial Application -Notification

¶ As there is no 'notification' category for the Investigational Testing Authorization (ITA), each amendment or notification to the Clinical Trial Application (CTA) must be submitted as a new amendment for the devices to be reviewed by ITA.

146 **Methods and analysis**

147 **Study design**

148 This was a pragmatic 16-week, triple-blind, placebo-controlled, parallel-group, randomized trial
149 comparing supplemental vitamin D3 and placebo in HCWs with the possibility of extending the
150 study up to 24 weeks, depending on infection rate progression during an interim analysis (**Figure**
151 **1**).

153 **Subjects**

154 HCWs (i.e., physicians, allied health care workers, orderlies, etc.) were eligible if they: (i) were
155 aged ≥ 18 and < 70 years old; (ii) were authorized to practice in Quebec; (iii) were working or
156 scheduled to work over the next 16 weeks in a setting at high-risk of contact with COVID-19
157 infected individuals, particularly (but not only) those involved with aerosol generating medical
158 procedures in hospitals and/or caring for patients in long-term care facilities; (iv) were working in
159 high COVID incidence areas in the greater Montreal area and surroundings; (v) were covered by
160 the provincial universal public health insurance (*Régie de l'assurance-maladie du Québec*
161 [RAMQ] for medical services and hospitalisations; (vi) had a personal email or phone (to which
162 to send reminders and questionnaire by email or text messages); (vii) had a fixed address (to which
163 to send the material) in the greater Montreal or surrounding areas.

164 HCWs were excluded if they met any of the following criteria: vitamin D supplementation
165 (cholecalciferol or calcitriol) intake > 400 IU/day (or $> 12,000$ IU/month) in past 3 months;
166 intention to take > 400 IU per day during the study period; suspected or previously documented
167 COVID-19 infection; history of nephrolithiasis, hypercalcemia, hyperphosphatemia,
168 hyperparathyroidism, granulomatosis disease (e.g., tuberculosis, sarcoidosis), renal failure, or

1
2
3 169 active cancer; current intake of medications that may cause hypercalcemia such as lithium,
4
5 170 teriparatide, or digoxin; anticipated prolonged absence from work during the study period (i.e.,
6
7 171 pregnancy); anticipated difficult follow-up; enrollment in a concurrent interventional randomized
8
9 172 trial; have already received the vaccine against COVID-19. Participation in this trial did not
10
11 173 preclude subsequent enrollment in a COVID-19 therapeutic (but not preventive) trial, which would
12
13 174 be documented.
14
15
16
17 175
18

19 176 **Study intervention**

20
21 177 Participants in the intervention group received 100,000 IU vitamin D₃ (cholecalciferol) at
22
23 178 randomization followed by a weekly dose of 10,000 IU vitamin D₃ for 16 weeks. Participants in
24
25 179 the control groups received an identical placebo bolus followed by placebo weekly supplement for
26
27 180 16 weeks. Sufficient supply was provided for 24 weeks, in case of prolongation study based on the
28
29 181 interim analysis. Participants in both groups were asked to take the study intervention with their
30
31 182 most copious meal. Treatment of co-morbidities were permitted. Vitamin D intake up to 400 IU
32
33 183 per day was allowed.
34
35
36
37
38 184

39 185 **Randomization**

40
41 186 Randomization was implemented using a computer-generated random list stratified by one of 11
42
43 187 workplaces (*Centre Hospitalier Universitaire* [CHU]) or health region (*Centre Intégré*
44
45 188 *Universitaire de Santé et Services Sociaux* [CIUSSS] or *Centre Intégré de Santé et Services*
46
47 189 *Sociaux* [CISSS]). HCWs were allocated (1:1) using permuted block randomization to enhance
48
49 190 concealment. Group allocation codes for each stratum was held in a secure location with restricted
50
51 191 access by the Central Pharmacy and Data Management.
52
53
54
55
56
57
58
59
60

192

193 Patient and public involvement

194 Participant burden of research measures was assessed using feedback from patients participating
195 in one pilot round. Patients were not involved in study design, recruitment of participants or conduct
196 of the study. Results of this study will be disseminated through public fora.

197

198 Outcomes*199 Primary outcome*

200 The original primary outcome, incidence of laboratory-confirmed COVID-19 infection, was
201 originally based on (i) bi-monthly self-obtained mid-turbinate nasopharyngeal (NP) swabs,
202 complemented by (ii) NP swabs obtained clinically for screening or diagnostic purposes
203 throughout the study, both analysed by RT-qPCR approved by Health Canada. Faced with the
204 unexpected cancellation of our large order of Falcon tubes to collect saliva sample for qPCR
205 combined with the unacceptable additional delay for a public tender to securing a contract with a
206 private courier service and in view of the uniform protocol for screening symptomatic or COVID-
207 19 exposed health care workers throughout the Province of Quebec and the reliability of IgG
208 serology, we decided to forgo the twice-monthly saliva sampling for qPCR analysis. The revised
209 definition of the primary outcome became the incidence of laboratory-confirmed COVID-19
210 infection, documented by RT-qPCR based on salivary (or nasopharyngeal) specimens (i) obtained
211 for screening or diagnostic purposes throughout the study and (ii) self-obtained salivary specimens
212 obtained at endpoint as well as (ii) COVID-19 IgG seroconversion at endpoint (in COVID-
213 unvaccinated individuals: ≥ 15 UA on the anti-S SARS-CoV-2 IgG Diasorin on Liaison XL

1
2
3 214 platform; in COVID-vaccinated individuals : ≥ 1.40 index (S/C) on the anti-N SARS-CoV-2 IgG
4
5 215 on ARCHITECT platform)
6
7
8 216

9
10 217 *Secondary outcomes*

11
12 218 (i) *Distribution of disease severity* on a 5-category ordinal scale [asymptomatic; mild (managed at
13
14 219 home); moderate (hospitalisation without supplemental oxygen); severe (oxygen
15
16 220 supplementation); critical (mechanical ventilation/death)], (self-reported, RAMQ); (ii) *Duration*
17
18 221 *of COVID-19 positivity* between 1st COVID+ to first COVID- test) revised to *Duration of COVID-*
19
20 222 *19 related symptoms* in individuals with laboratory confirmation of COVID infection, (self-
21
22 223 reported on diary); (iii) COVID-19 IgG seroconversion documented at endpoint (see above); (iv)
23
24 224 duration of work absenteeism (self-reported, medical records or human resources databases); (iv)
25
26 225 duration of unemployment support (human resource databases); (v) Adverse health events (self-
27
28 226 reported). Several *exploratory outcomes* pertained to the: incidence of post-acute and chronic
29
30 227 symptoms; long-term (1-year) morbidity and work absence related to COVID-19; change in gene
31
32 228 expression of ACE2 and TMPRSS2 in saliva cells; change in inflammatory markers (i.e., CRP),
33
34 229 immune response post vaccination; other viral infections; and genetic markers (including changes
35
36 230 in gene expression).
37
38
39
40
41
42
43
44

45 232 **Study Procedures**

46
47 233 To facilitate the recruitment of participants, this study is conceived as hybrid trial enabling partially
48
49 234 or totally remote trial participation including screening, randomization, follow-up, and end-of-
50
51 235 study visit.
52
53
54
55
56
57
58
59
60

1
2
3 237 *Pre-Screening*
4

5 238 Advertisements were placed in health institutions, newspapers, social media and online, where
6
7 239 participants were invited to complete an online pre-screening form, read and download the consent
8
9
10 240 forms; and if eligible and interested, to book a virtual screening appointment (via a secured
11
12 241 videoconferencing platform) with research team who would confirm eligibility, explain the study,
13
14 242 obtain informed consent, and schedule a virtual or in-person randomization visit.
15
16

17 243
18
19 244 *Screening*
20

21 245 At the virtual screening visit by videoconferencing, research coordinators completed with the
22
23 246 individual a more extensive eligibility questionnaire, which included additional questions about:
24
25 247 anticipated work exposure over the next 16 weeks to COVID-infected or suspected individuals
26
27 248 and to high-risk medical procedures; work place (*Centre Hospitalier Universitaire* [CHU]) or
28
29 249 *Centre Hospitalier Universitaire Sainte-Justine*) or health region (CIUSSS or CISSS), serving as
30
31 250 randomization stratum; prior laboratory-confirmed or physician-suspected COVID-19 infection;
32
33 251 assessment of the likelihood of prior/current, yet undocumented, COVID-19 infection using the 5-
34
35 252 item questionnaire developed by Menni et al³⁰ (score >0.50 interpreted as high likelihood of prior
36
37 253 infection); and finally, the comfort level with the study design and procedures, including saliva
38
39 254 and capillary blood sampling self-collection demonstrated by instructional videos. Eligible and
40
41 255 consenting individuals electronically signed an online consent form (with the signed PDF consent
42
43 256 form automatically emailed to participants). Then, two additional questionnaires were completed
44
45 257 on line with the research coordinators namely: (i) the baseline questionnaire collecting information
46
47 258 about household, ethnicity, part- vs. full-time work, personal health, skin color (measured with the
48
49 259 Fitzpatrick scale),³¹ concomitant medications or supplements, and (ii) the nominative CRF
50
51
52
53
54
55
56
57
58
59
60

1
2
3 260 collecting demographic information essential to opening a medical and pharmaceutical research
4
5 261 record (i.e., public health insurance number, allergies) and maintaining contact with the research
6
7 262 team throughout the study (preferred means to receive electronic reminders/questionnaires and to
8
9 263 be notified of positive test results; address to receive study material or for biological sample pick-
10
11 264 up; and next-of-kin contact in case of inability to respond to questionnaire due to illness), and to
12
13 265 document work absence (employee number).
14
15
16
17 266

18
19 267 Finally, at the screening visit, the participant was asked to choose an appointment for a *virtual* (via
20
21 268 a secured videoconferencing platform) or *in-person* randomization visit at one of several locations.
22
23 269 To help select their preferred visit format, videos of key procedures (such as home blood
24
25 270 collection) were shown. Only in participants with a significant likelihood of a current or past
26
27 271 undocumented (Menni score > 0.5) was an *in-person* randomization visit mandatory to receive the
28
29 272 rapid COVID-19 serology test, prior to randomisation.
30
31
32
33 273

34 35 274 *Preparation and shipment of Study drug by Research Pharmacy*

36
37 275 The list of new participants approved by one of the PIs was sent daily by email to the CHUM
38
39 276 research team to be open a medical chart and send an electronically signed prescription for the
40
41 277 Study medication, to the Research Pharmacy for preparation of study drug.
42
43
44
45 278

46
47 279 Prior to randomization, a list of all consenting and eligible participants was automatically sent
48
49 280 every night to the one of the co-principal investigators (FMD or CT) who screening and baseline
50
51 281 questionnaires to approve or refuse study entry and electronically signed their decision. The list
52
53 282 daily list of new PI-approved participants was sent electronically daily to the CHUM research
54
55
56
57
58
59
60

1
2
3 283 team. Medical and pharmaceutical records were opened and an electronically signed prescription
4
5 284 for the Study medication sent to the Research Pharmacy for preparation of study drug for a given
6
7
8 285 target date.

9
10 286 To enable remote randomization, the randomization took place about one week prior to the
11
12 287 randomization visit to allow enough time for the preparation and shipment of patient-specific study
13
14 288 supplement to the research team and, in turn, the shipment of the Study supplement and all
15
16
17 289 materials required for the randomization visit by the research team to the participant.

18
19 290

20
21 291 *Randomization visit*

22
23 292 Seventy-two and 24 hours prior to, and at, the randomisation visit, participants were screened by
24
25
26 293 questionnaire for recent travel, symptoms suggestive of SARS-Cov2 infection, or exposure to
27
28 294 COVID-19 infected individuals. Those who responded positively were asked to get tested, notify
29
30
31 295 their institutional health service and await end of quarantine and/or confirmed negative test to
32
33 296 reschedule the randomisation visit.

34
35 297

36
37
38 298 Randomization visit (week 0) was performed *in person* (60 minutes) or *remotely* (90 minutes),
39
40 299 depending on the availability and preference of participants as well as their likelihood of a past
41
42 300 COVID-19 infection.

43
44 301

45
46
47 302 *In-person* visits were conducted—by appointment only—in designated rooms with restricted
48
49 303 access. The research coordinators wore personal protection equipment (PPE), and all procedures,
50
51 304 from participant arrival to departure, were approved by the institutional Infection Control and
52
53 305 Safety committee. The *in-person* visit entailed (i) ascertainment of the signed consent form, (ii)

1
2
3 306 capillary blood sample collection to perform NADAL® COVID-19 IgG/IgM Rapid Test (Teracero
4
5 307 Pharma Inc., Lachine Canada), (iii) venous blood sample collection for baseline serum 25(OH)D
6
7 308 and SARS-CoV-2 IgG serology analyses and if genetic consent, DNA; (iv) viewing of the saliva
8
9 309 collection video and instruction pamphlet, (v) collection of the first specimen under supervision,
10
11 310 (vi) a final verification of the eligibility and exclusion criteria; (vii) randomization; (viii) oral
12
13 311 administration of 100,000 IU vitamin D₃ or an identical placebo, and (ix) distribution of the study
14
15 312 material including study supplement, saliva sampling kit for end-of-study, biohazard and sampling
16
17 313 bag, and, if a remote visit was anticipated at week 16, capillary blood collection kits (TASSO
18
19 314 OnDemand SST device). Any patient with a positive NADAL COVID-19 IgM/IgG Rapid Test
20
21 315 serology test were excluded prior to randomisation.
22
23
24
25

26 316
27
28 317 The *remote* randomization visit, conducted by video-conference, was similar to the *in-person*
29
30 318 randomization visit with the following additions: (a) viewing of the capillary blood collection
31
32 319 video and instructional pamphlet; (b) remote capillary sampling under guidance using the TASSO-
33
34 320 SST device (TASSO Inc., Seattle, USA); (c) viewing of the saliva collection video and
35
36 321 instructional pamphlet (OG-600 Oragene DNA Collection Kit, DNA Genotek Inc., Ottawa,
37
38 322 Canada); (d) remote DNA salivary sampling under guidance; (e) preparation of biological samples
39
40 323 for shipment with phase change and insulated envelopes under guidance and (f) organising
41
42 324 collection of biological specimens by approved courier service to respective laboratories. Note that
43
44 325 a Nadal serology test was not conducted remotely.
45
46
47
48

49 326
50
51 327 *Follow-up*
52
53
54
55
56
57
58
59
60

1
2
3 328 Participants received *weekly electronic (SMS or email) reminders* to take their weekly Study
4
5 329 Supplement (10,000 IU vitamin D or an identical placebo) and to start completing an *online daily*
6
7 330 *Diary* if they tested positive to SARS-CoV2 or they developed symptoms suggestive of COVID-
8
9 331 19 infection.

10
11
12 332 Every two weeks, participants received a link to complete a *brief online questionnaire* asking to
13
14 333 report: their adherence to weekly Study Supplement intake; health status including recent COVID-
15
16 334 19 related exposure, symptoms, or testing; adverse health events or new co-morbidities; change in
17
18 335 concomitant medications or supplement intake; work status (active duty, quarantined, holiday,
19
20 336 sick) and work setting (ED, ICU, etc.); as well as expected/recent COVID-19 vaccination (date
21
22 337 and vaccine name) if any; the latter question served to enable timely shipment of materials for
23
24 338 additional sampling prior to vaccination, as COVID-19 vaccination was permitted during the
25
26 339 study. In participants who planned to get vaccinated during the study, three additional blood, and
27
28 340 one additional saliva, samplings, either *on-site* or *remotely*, were planned including: saliva (for
29
30 341 COVID-19 qPCR analysis) and blood (for SARS-CoV-2 anti-S IgG serology) sampled prior to
31
32 342 vaccination, a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology) collected just prior
33
34 343 to the second vaccine dose, and a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology)
35
36 344 collected one month of after second vaccine dose and endpoint. Regardless of their vaccination
37
38 345 status, participants were asked to continue taking the weekly Study Supplement and complete the
39
40 346 bi-monthly questionnaire until the end of the study. If questionnaires were not completed within 2
41
42 347 days of the target date, the research coordinator reached out the participant to complete the
43
44 348 information.

45
46
47 349
48
49
50
51
52
53 350 *End-of-Study visit*

1
2
3 351 An end-of-study visit was conducted either in-person (60 minutes) or remotely (90 minutes),
4
5 352 depending on the availability and preference of participants and likelihood of a current COVID-
6
7 353 19 infection. The *in-person end-of-study visit* entailed the collection of a (i) venous blood sample
8
9 354 for serum 25(OH)D and SARS-CoV-2 anti-S IgG serological results and in vaccinated participants
10
11 355 a SARS-CoV-2 anti-N IgG serology, (ii) capillary blood sample to perform the NADAL®
12
13 356 COVID-19 IgG/IgM Rapid Test, (iii) a saliva sample for SARS-CoV-2 qPCR analysis as well as
14
15 357 guessing of allocation and return of the study supplement bottle to assess adherence and any
16
17 358 unused material.
18
19
20
21
22

23
24 360 The *remote end-of-study visit* conducted via videoconference entailed the same procedures as the
25
26 361 in-person end-of-study visit with one exception: the self-collection of a capillary (instead of
27
28 362 venous) blood using TASSO-SST devices (for the serum 25(OH)D and SARS-CoV-2 anti-S
29
30 363 with/without anti-N serology). Individuals were guided into self-performing the pinprick capillary
31
32 364 method to perform the NADAL® COVID-19 IgG/IgM Rapid Test and return of biological samples
33
34 365 and materials by pre-paid approved courier.
35
36
37
38
39

40 367 *Covariates*

41
42 368 Several covariates that may act as confounders or interaction variables in the magnitude of effect
43
44 369 associated with the intervention were documented, namely: baseline serum 25OHD level;
45
46 370 smoking; concomitant supplements or drug(s) that alter calcium or vitamin D absorption or
47
48 371 metabolism such as diuretics and anti-epileptics (reported at baseline and every 2 weeks); skin
49
50 372 color (documented at baseline); obesity (documented by height & weight [BMI] at baseline); other
51
52 373 comorbidities (i.e., diabetes, hypertension, etc.) that may affect the severity of COVID-19
53
54
55
56
57
58
59

1
2
3 374 infection and receipt of a COVID-19 vaccine (documented by verbal report bi-monthly). All
4
5 375 external (governmental and institutional) databases were to be obtained 3 months before, and up
6
7 376 to 16 months following, randomization (as well as 12 months after then study endpoint).
8
9

10 377

11
12 378 *During an event*

13
14 379 During COVID-19 related symptoms or documented SARS-CoV infection, participants were
15
16 380 instructed to complete a daily symptom diary from date of onset of symptoms or positive test, until
17
18 381 two days with no symptoms or 14 days if asymptomatic,
19
20

21 382

22
23
24 383 *Risk management*

25
26 384 Clinical and biochemical adverse health events (AHEs) were monitored throughout the study and
27
28 385 reported for all patients at the end of the study. No specific laboratory safety monitoring was
29
30 386 planned given the established safety of the loading dose of 100,000 IU and weekly dose of 10,000
31
32 387 IU.^{32,33} Adverse Health Events (AHE) were recorded via electronic questionnaires throughout the
33
34 388 study. Participant who reported symptoms suggestive of vitamin D intoxication had a venous blood
35
36 389 sampling (total and ionised calcium, phosphorus, alkaline phosphatase, albumin, and creatinine).
37
38 390 Any abnormal laboratory values was interpreted as ‘clinically significant’ or ‘not clinically
39
40 391 significant’ by the Site endocrinologist blinded to study allocation. Further investigation or action
41
42 392 for individual participants (including interruption, cessation, or unblinding of the study drug via
43
44 393 pharmacy or by analysis of serum 25OHD) was be determined by the Site endocrinologist, if
45
46 394 indicated to ensure participant safety. The AHE’s occurrence was reviewed periodically by the
47
48 395 Data and Safety Monitoring Board (DSMB). Code breaking was allowed only if deemed essential
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 396 for participant management. If relevant, summary reports aggregating (or not if requested) both
4
5 397 groups were to be provided to the DSMB.
6
7

8 398

9
10 399 **Data management and monitoring**

11
12 400 The principle investigator (FMD) and statistical group (SG, RP) oversaw randomization, data
13
14 401 management, progress monitoring, and all analyses, including those for Data Monitoring Safety
15
16 402 Board (DMSB). The DSMB membership included: Lehana Thabane, biostatistician (Chair), Gary
17
18 403 Kobinger, infectious disease specialist, Kevin Thorpe, biostatistician, and Edgar Delvin,
19
20 404 biochemist & expert in Vitamin D. DACIMA was used for online data entry and management.
21
22
23

24 405

25
26 406 A combination of *remote* monitoring activities and *in-person* routine monitoring visits were
27
28 407 conducted by an independent Study Monitor with the first randomised participants at each site and
29
30 408 on a bi-monthly basis, to ensure that each Site adhered to the study protocol, Good Clinical Practice
31
32 409 guidelines and data collection completeness.
33
34

35 410

36
37 411 **Sample size calculation**

38
39 412 Given uncertainties in infection progression, a Bayesian adaptive design was used where the
40
41 413 posterior probability of effectiveness, i.e., $P(OR < 1 | \text{data})$ was the basis of inference and decision
42
43 414 making.³⁴ Assuming an expected OR of 0.80 and 1:1 treatment allocation, a total net sample size
44
45 415 of 2100 was required to identify a 20% reduction in the risk of COVID-19 in the vitamin D vs.
46
47 416 control group, with 80% power with the design described above. Considering a drop-out rate of
48
49 417 15%, 2414 participants were targeted. An interim analysis was planned when 75% of participants
50
51 418 reached week 12, at which time the following assessments were made: the *progression over time*
52
53
54
55
56
57
58
59

1
2
3 419 *in the incidence of infection* (slope of the curve of infection) was updated and if the probability of
4
5 420 effectiveness exceeded 0.95 [$p(\text{OR}<1)>0.95$], the trial should be terminated for efficacy at the
6
7 421 interim point (12 weeks); otherwise, the study would continue to 16 weeks. Simulation results
8
9 422 showed that, with the net sample size of 2100 (assuming an expected OR of 0.80 and 1:1 treatment
10
11 423 allocation), there was about a 55% chance that the trial would be terminated for efficacy at the
12
13 424 interim analysis.³⁵ The overall infection rate was monitored on a monthly basis: note that the study
14
15 425 could have been extended to 24 weeks based on the progress of the infection rate, if required.
16
17
18
19 426

427 **Statistical analysis**

428 *Primary outcome*

429 An intention-to-treat (ITT) analysis was to be carried out with all randomized participants. For the
430 primary outcome, the posterior distribution of the odds ratio of COVID-19 infection (OR) was the
431 basis of inference in interim and final analyses. The posterior distribution of the OR was to be
432 estimated by drawing samples from the posterior risks under each arm, which could be obtained
433 analytically in a Beta-binomial model. Flat prior distributions were assumed for the risks
434 (Beta(1,1)). Posterior 95% credible intervals were to be reported as interval estimates for the OR.
435 Crude analyses as well as analyses adjusted for important covariates (i.e., potential confounders,
436 effect modification, and baseline group imbalances) were to be conducted. Subgroup analyses
437 would be conducted on baseline 25OHD, age, sex, BMI, occupational risk, and COVID-19
438 vaccination. A stratified analysis on geographical infection rate would be explored; sensitivity
439 analysis censoring to date of COVID-19 vaccination, would be conducted if applicable.

440

441 *Secondary outcomes*

1
2
3 442 Distribution of disease severity defined as a 5-level ordinal outcome would be examined with a
4
5 443 Bayesian analysis using a proportional odds (PO) model; the posterior probability of OR would be
6
7 444 obtained by Markov chain Monte Carlo sampling implemented in Stan.³⁴ Duration of symptoms,
8
9 445 duration of workday absences and of unemployment would be examined by a zero-inflated Poisson
10
11 446 distribution.
12
13
14
15 447

16 17 448 **Ethics and dissemination**

18
19 449 This study has been reviewed and approved by the research ethics board (REB) of the CHU Sainte-
20
21 450 Justine, serving as the local REB of all participating institutions. A non-objection letter (NOL)
22
23 451 from Health Canada has been obtained to use high-dose Vitamin D loading dose as well as Tasso
24
25 452 OnDemand device for home blood sampling and the NADAL COVID-19 IgM/IgG Rapid serology
26
27 453 test. Written informed consent for study participation, for biobanking specimens for ancillary
28
29 454 studies, and for subsequent publication of results was obtained from all participants, with the
30
31 455 knowledge that participation is voluntary and can be withdrawn at any time with no effect on their
32
33 456 current/future medical care. As part of the informed consent, enrolees had the option to participate
34
35 457 in the HostSeq COVID-19 Canadian biobank conducted under the supervision of CGen, a national
36
37 458 Canadian platform for sequencing and genome analysis (**Supplementary file 2**). In Canada, health
38
39 459 care is provided to those who suffer harm from trial participation.
40
41
42
43

44
45 460 All protocol amendments were submitted to Health Canada, investigators and REB; if these
46
47 461 changes implied a revision of consent forms, ongoing trial participants were informed of new
48
49 462 modifications to provide informed consent. All information obtained during the study were and
50
51 463 will be kept confidential as per the law. Data was collected directly by electronic data capture on
52
53 464 Dacima Clinical Suite (DACIMA Software Inc., Montreal, Canada). Data safety and
54
55
56
57
58
59
60

1
2
3 465 confidentiality was upheld at all data collection stages by assigning a unique subject ID to each
4
5 466 participant, with data and samples kept under lock and key, electronic password protection and
6
7 467 access restricted to study personnel. Samples collected during the study were labelled with the
8
9 468 unique research code, prior to transfer and storage at the CHUSJ biobank, with access restricted to
10
11 469 authorised personnel.

12
13
14 470 This trial uses pragmatic patient (irrespective of baseline 25OHD level) and intervention to
15
16 471 maximise subsequent implementation into practice. If effective in reducing infection and
17
18 472 morbidity, this approach would be readily implementable and could markedly influence practice
19
20 473 during the COVID-19 pandemic. No participant identifiers will be used in the dissemination of
21
22 474 this research. Health care professionals serving as partners informed the study design and pre-test
23
24 475 all questionnaires and will contribute to a disseminating plan. Results will be disseminated to the
25
26 476 medical community and public health departments via national/international conferences and
27
28 477 publications in peer-reviewed journals as well as to the public and study participants via the
29
30 478 Direction Collaboration-Partenariat Patient of the University of Montreal and the Canadian
31
32 479 Respiratory Research Network (CRRN) patient platform who would contribute to a disseminating
33
34 480 plan to reach as many individuals as possible.

35
36
37
38
39
40 481

41 42 43 482 **Trial status**

44
45 483 The study was conducted as per version 1.8 (January 18, 2021). The recruitment started on
46
47 484 February 9, 2021. Upon the DSMB recommendation, recruitment was stopped prematurely on
48
49 485 March 18, 2021 after 34 participants enrolled due to the inability to recruit approximately 200
50
51 486 participants/week required to meet the target sample size of 2415 participants. The DSMB advised
52
53 487 that the continuation of the trial, as originally designed, would not be able to answer the research question
54
55
56
57
58
59
60

1
2
3 488 and recommended that recruitment be stopped for futility. Recruitment difficulties were attributed in
4
5 489 part to the high use of vitamin D and high concurrent vaccination rate among our target population,
6
7 490 healthcare workers, the first targeted to be vaccinated from January 2021 onwards. Based on the
8
9 491 recommendations of the study's endocrinologist, a premature cessation of follow-up after a
10
11 492 minimum of 4 weeks from randomization to monitor the safety of intervention in all participants.
12
13 493 The timeframe was deemed sufficient to ensure participant safety while learning for the study, that
14
15 494 is, transforming the PROTECT study into a pilot study to document the impact of the study
16
17 495 intervention on the rise in Vitamin D serum level, participants' adherence the study intervention
18
19 496 and procedures in the context of a hybrid study, etc. The last end-of-visit was conducted on May
20
21 497 4, 2022.
22
23
24
25

26 498
27
28 499 Potential redirections of the study were discussed. The first option was to change the main outcome for an
29
30 500 immunogenicity study in the general adult population. However, after strong consideration of the
31
32 501 amount of changes to be made to the protocol and related documents (standards of procedures,
33
34 502 case report forms, participant' instructions and notification, etc.), the expected delay in obtaining
35
36 503 approval by all regulatory and ethical authorities, the impossible logistic of recruiting participants
37
38 504 after the same duration of exposure to the study intervention prior to their vaccination, combined
39
40 505 with the government of Quebec announcement that all willing adults would be vaccinated by June
41
42 506 24, 2021, the research team judged that it would unfeasible to perform a scientific solid and feasible
43
44 507 trial on immunogenicity if one could not control the timing of immunization, combined with the
45
46 508 expected very short recruitment timeframe.
47
48
49
50

51
52 509
53
54
55
56
57
58
59
60

1
2
3 510 A second option that received very strong consideration was to replicate the PROTECT trial in
4
5 511 children aged 9 years and over. Again, after considering changes to be made to the protocol and
6
7 512 related documents, the expected delay for obtaining approval by all regulatory and ethical
8
9 513 authorities including school boards, combined with the Pfizer-BioNtech announcement that their
10
11 514 vaccine was not only 100% successful for preventing COVID-19 infection in adolescents aged 12
12
13 515 to 15 years but that they forecast vaccinating teenagers in time for September 2021 school entry,
14
15 516 the PI judge it was unrealistic to aim for the large recruitment target within such a short timeframe.
16
17
18
19
20

21 517
22 518 The protocol was submitted after the last patient end-of-study visit, due to the incredible amount of
23
24 519 work done to conducted to set-up and initiate this large hybrid trial; the latter included two pilot
25
26 520 studies testing two experimental devices to enable partially or totally remote participation, in the
27
28 521 context of the pandemic which imposed large protocol and space restrictions for recruiting on-site
29
30 522 potentially COVID-19 infected health care workers, several protocol amendments to facilitate and
31
32 523 adjust the trial in the context of emerging science and anticipated vaccination campaign and their
33
34 524 impact of all electronic documents, manual of procedures, and regulatory approvals, coupled with
35
36 525 the premature end-of-follow-up in enrolled participants.
37
38
39
40
41

42 526
43 527 With the gained experience and knowledge, it is crucial that a future trial must begin fast prior to
44
45 528 widespread vaccination and in populations where infection rate is high.²⁸ Permitting study entry
46
47 529 to individuals with prior infection and prior vaccination (given common reinfections and
48
49 530 temporary vaccine protection)³⁶ could have been considered, but it would have significantly
50
51 531 reduced the event rate, required prolongation beyond 24 weeks (and additional funding), and
52
53 532 compromised study power as was noted in other primary prevention trials.^{26,27} Restricting
54
55
56
57
58
59
60

1
2
3 533 eligibility to patients with vitamin D deficiency (<25 nmol/l) would have severely interfere with
4
5 534 recruitment ability in population-based or health care workers studies.²⁶⁻²⁸ Use of calciferol may
6
7
8 535 be associated with more potency and rapid rise in serum 25OHD than expected for
9
10 536 cholecalciferol³⁷ although the choice is debated³⁸ and rapid access to study drug and matching
11
12 537 placebo remain a crucial challenge at the onset of a pandemic. Revisiting the intervention dose and
13
14
15 538 frequency of administration in light of the latest literature on SARS-CoV2 and related virus should
16
17 539 be considered, although current evidence suggest that, with similar doses, high-incidence
18
19 540 population may be more important than dosing in primary prevention^{26,28} and high doses are
20
21 541 effective in tertiary prevention.³⁵ A pragmatic design with fewer outcomes and monitoring via
22
23 542 administrative databases appears theoretically more efficient, but requires rapid access to data
24
25 543 when interim analyses are planned to monitor event rate, a serious challenge. Pursuing a hybrid
26
27 544 approach to facilitate enrollment in the context of a pandemic is feasible, although electronic self-
28
29 545 screening and outcome monitoring required a lot of programming that may delay implementation.
30
31
32
33 546
34
35 547 The publication of this protocol is meant to share our experience, including conducting a hybrid
36
37 548 (virtual and/or in-person) trial and lessons learned, enable protocol uptake and its improvement in
38
39 549 the context of another epidemic/pandemic, and serve as reference for the publication of our pilot
40
41 550 studies that enabled this trial, and lessons learned from this experience.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

553 **Contributors**

554 FMD designed the study protocol, secured funding, and oversaw the overall conduct of the project.
555 CLT contributed to the protocol and amendments, directed the study implementation at the
556 CHUM, and coordinated the prescription of study drug, pharmacy dispensation, as well as salivary
557 sample reception and interpretation. SG conceived the statistical approach and sample size
558 calculation and along with RWP, oversaw randomization and statistical analysis. CL, JHW and
559 CQ contributed to the study design and amendments. BH wrote the first manuscript draft. LGSM
560 oversaw the safety assessment. DC is responsible for the work absenteeism analysis. All co-
561 authors approved the manuscript. Authorship eligibility on resulting manuscripts will follow
562 standard guidelines.

563

564 **Competing interests**

565 The authors declare that they have no competing interests.

566

567 **Funding**

568 This study is funded by a grant awarded through a peer-reviewed process of the COVID-19 May
569 2020 Rapid Response Funding Opportunity by the Canadian Institute of Health Research, 160
570 Elgin Street, Ottawa, ON K1A 0W9, Canada (grant number #447317).

571

572 **Data availability statement**

573 The datasets used and analyzed during the current study will be made available by the
574 corresponding author on reasonable request.

1
2
3 5754
5 576 **Acknowledgements**

6
7
8 577 We acknowledge the precious collaboration of Danny Germain from Quebec Riva Laboratories
9
10 578 who agree to provide free of charge Study Preparations (vitamin D and matching placebo),
11
12 579 available in bottles of 60 tablets, allowing for study prolongation. We sincerely thank Benoit
13
14 580 Hebert of Teracero Pharma Inc, for providing free-of-charge the NADAL COVID-19 IgM/IgG
15
16 581 Rapid serology test kits. We are indebted to Martin Sauvageau for implementing and coordinating
17
18 582 the RT-qPCR analysis of saliva samples at the Montreal Clinical Research Institute, Christian
19
20 583 Renaud for coordinating the COVID-19 serology analysis, and Claude Bourassa for coordinating
21
22 584 all other blood analyses at the Sainte-Justine University Health Centre. We acknowledge the
23
24 585 precious collaboration of Raymond Loyer from EFS Solution Santé who adapted their appointment
25
26 586 software for our needs as well as John Padoba, Rabie Razgallah, and Mustapha Gharb who
27
28 587 programmed and revised the eCRF to our needs. We sincerely thank Anna Smyrnova for
29
30 588 coordinating the development of the eCRF and data management. We are indebted to Catherine
31
32 589 Lamontague from Orokom Communication Marketing who developed the communication
33
34 590 strategy and tools and oversaw the publicity campaign with Marie-Line B nard-Cyr of the CHUSJ
35
36 591 who also developed the PROTECT website and Laureanne Marceau of the CHUM. We sincerely
37
38 592 thank the members of the Data Monitoring Safety Board namely Lehana Thabane (Chair), Gary
39
40 593 Kobinger, Kevin Thorpe and Edgar Delvin.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

594 **References**

- 595 1. Zhang Y. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases
596 (COVID-19) in China. *CCDC weekly* 2020;41:145-51.
- 597 2. Flaxman SM, Swapnil; Gandy, Alex: et al. Estimating the number of infections and the impact of
598 non-pharmaceutical interventions on COVID-19 in 11 European countries. 2020.
- 599 3. CDC COVID-19 Response Team. Characteristics of Health Care Personnel with COVID-19 — United
600 States, February 12–April 9, 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:477-81.
- 601 4. Ran L, Chen X, Wang Y, Wu W, Zhang L, Tan X. Risk Factors of Healthcare Workers with Corona
602 Virus Disease 2019: A Retrospective Cohort Study in a Designated Hospital of Wuhan in China. *Clin Infect*
603 *Dis* 2020.
- 604 5. Nguyen LH, Drew DA, Graham MS, et al. Risk of COVID-19 among front-line health-care workers
605 and the general community: a prospective cohort study. *The Lancet Public Health*.
- 606 6. Ilie PCS, Simina; Smith, Lee The role of Vitamin D in the prevention of Coronavirus Disease 2019
607 infection and mortality (pre-print). *Research Square* 2020.
- 608 7. Hughes DA, Norton R. Vitamin D and respiratory health. *Clin Exp Immunol* 2009;158:20-5.
- 609 8. Herr C, Greulich T, Koczulla RA, et al. The role of vitamin D in pulmonary disease: COPD, asthma,
610 infection, and cancer. *Respir Res* 2011;12:31.
- 611 9. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin D deficiency causes deficits in
612 lung function and alters lung structure. *Am J Respir Crit Care Med* 2011;183:1336-43.
- 613 10. Hewison M. An update on vitamin D and human immunity. *Clinical Endocrinology* 2012;76:315-
614 25.
- 615 11. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune
616 system and the clinical implications of vitamin D deficiency. *Mol Nutr Food Res* 2011;55:96-108.
- 617 12. Martineau A. Vitamin D supplementation to prevent asthma exacerbations; Authors' reply. *The*
618 *Lancet Respiratory Medicine* 2018;6:e26-e7.
- 619 13. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute
620 respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ*
621 2017;356:i6583.
- 622 14. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute
623 respiratory infections: individual participant data meta-analysis. *Health Technol Assess* 2019;23:1-44.
- 624 15. Manaseki-Holland S, Qader G, Isaq Masher M, et al. Effects of vitamin D supplementation to
625 children diagnosed with pneumonia in Kabul: a randomised controlled trial. *Trop Med Int Health*
626 2010;15:1148-55.
- 627 16. Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D
628 supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial.
629 *Lancet* 2012;379:1419-27.
- 630 17. Jensen ME, Mailhot G, Alos N, et al. Vitamin D intervention in preschoolers with viral-induced
631 asthma (DIVA): a pilot randomised controlled trial. *Trials* 2016;17:353.
- 632 18. Castro M, King TS, Kunselman SJ, et al. Effect of vitamin D3 on asthma treatment failures in adults
633 with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *Jama*
634 2014;311:2083-91.
- 635 19. Denlinger LC, King TS, Cardet JC, et al. Vitamin D Supplementation and the Risk of Colds in Patients
636 with Asthma. *Am J Respir Crit Care Med* 2016;193:634-41.
- 637 20. Martineau AR, MacLaughlin BD, Hooper RL, et al. Double-blind randomised placebo-controlled
638 trial of bolus-dose vitamin D³ supplementation in adults with asthma (ViDiAs). *Thorax*
639 2015;70:451-7.

- 1
2
3 640 21. Martineau AR, James WY, Hooper RL, et al. Vitamin D3 supplementation in patients with chronic
4 641 obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet*
5 642 *Respir Med* 2015;3:120-30.
- 6 643 22. Goodall EC, Granados AC, Luinstra K, et al. Vitamin D3 and gargling for the prevention of upper
7 644 respiratory tract infections: a randomized controlled trial. *BMC Infect Dis* 2014;14:273.
- 8 645 23. Maghbooli Z, Sahraian MA, Ebrahimi M, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin
9 646 D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS*
10 647 *One* 2020;15:e0239799.
- 11 648 24. Ismailova A, White JH. Vitamin D, infections and immunity. *Reviews in Endocrine and Metabolic*
12 649 *Disorders* 2021.
- 13 650 25. Jafarzadeh A, Chauhan P, Saha B, Jafarzadeh S, Nemati M. Contribution of monocytes and
14 651 macrophages to the local tissue inflammation and cytokine storm in COVID-19: Lessons from SARS and
15 652 MERS, and potential therapeutic interventions. *Life Sci* 2020;257:118102.
- 16 653 26. Jolliffe DA, Holt H, Greenig M, et al. Effect of a test-and-treat approach to vitamin D
17 654 supplementation on risk of all cause acute respiratory tract infection and covid-19: phase 3 randomised
18 655 controlled trial (CORONAVIT). *BMJ* 2022;378:e071230.
- 19 656 27. Brunvoll SH, Nygaard AB, Ellingjord-Dale M, et al. Prevention of covid-19 and other acute
20 657 respiratory infections with cod liver oil supplementation, a low dose vitamin D supplement: quadruple
21 658 blinded, randomised placebo controlled trial. *BMJ* 2022;378:e071245.
- 22 659 28. Villasis-Keever MA, López-Alarcón MG, Miranda-Novales G, et al. Efficacy and Safety of Vitamin D
23 660 Supplementation to Prevent COVID-19 in Frontline Healthcare Workers. A Randomized Clinical Trial. *Arch*
24 661 *Med Res* 2022;53:423-30.
- 25 662 29. Pfeffer PE, Hawrylowicz CM. Vitamin D and lung disease. *Thorax* 2012;67:1018.
- 26 663 30. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict
27 664 potential COVID-19. *Nature medicine* 2020;26:1037-40.
- 28 665 31. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol*
29 666 1988;124:869-71.
- 30 667 32. Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin d supplementation in adult
31 668 populations: a systematic review. *Endocrine practice : official journal of the American College of*
32 669 *Endocrinology and the American Association of Clinical Endocrinologists* 2014;20:341-51.
- 33 670 33. Vieth R, Holick MF. Chapter 57B - The IOM—Endocrine Society Controversy on Recommended
34 671 Vitamin D Targets: In Support of the Endocrine Society Position. In: Feldman D, ed. *Vitamin D (Fourth*
35 672 *Edition)*: Academic Press; 2018:1091-107.
- 36 673 34. Harrell FL, Chris. Statistical Design and Analysis Plan for Randomized Trial of Hydroxychloroquine
37 674 for Treatment of COVID-19: ORCHID. 2020.
- 38 675 35. Golchi S. Estimating design operating characteristics in Bayesian adaptive clinical trials. *Can J Stat*
39 676 2022;50:417-36.
- 40 677 36. Eythorsson E, Runolfsson HL, Ingvarsson RF, Sigurdsson MI, Palsson R. Rate of SARS-CoV-2
41 678 Reinfection During an Omicron Wave in Iceland. *JAMA Network Open* 2022;5:e2225320-e.
- 42 679 37. Pérez-Castrillón JL, Dueñas-Laita A, Brandi ML, et al. Calcifediol is superior to cholecalciferol in
43 680 improving vitamin D status in postmenopausal women: a randomized trial. *Journal of Bone and Mineral*
44 681 *Research* 2021;36:1967-78.
- 45 682 38. Sosa Henríquez M, Gómez de Tejada Romero MJ. Cholecalciferol or Calcifediol in the Management
46 683 of Vitamin D Deficiency. *Nutrients* 2020;12.
- 47
48
49
50
51
52
53 684
54
55
56
57
58
59
60

1
2
3 686 **Figure legend**
4
5 687 **Figure 1. Study outline**
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

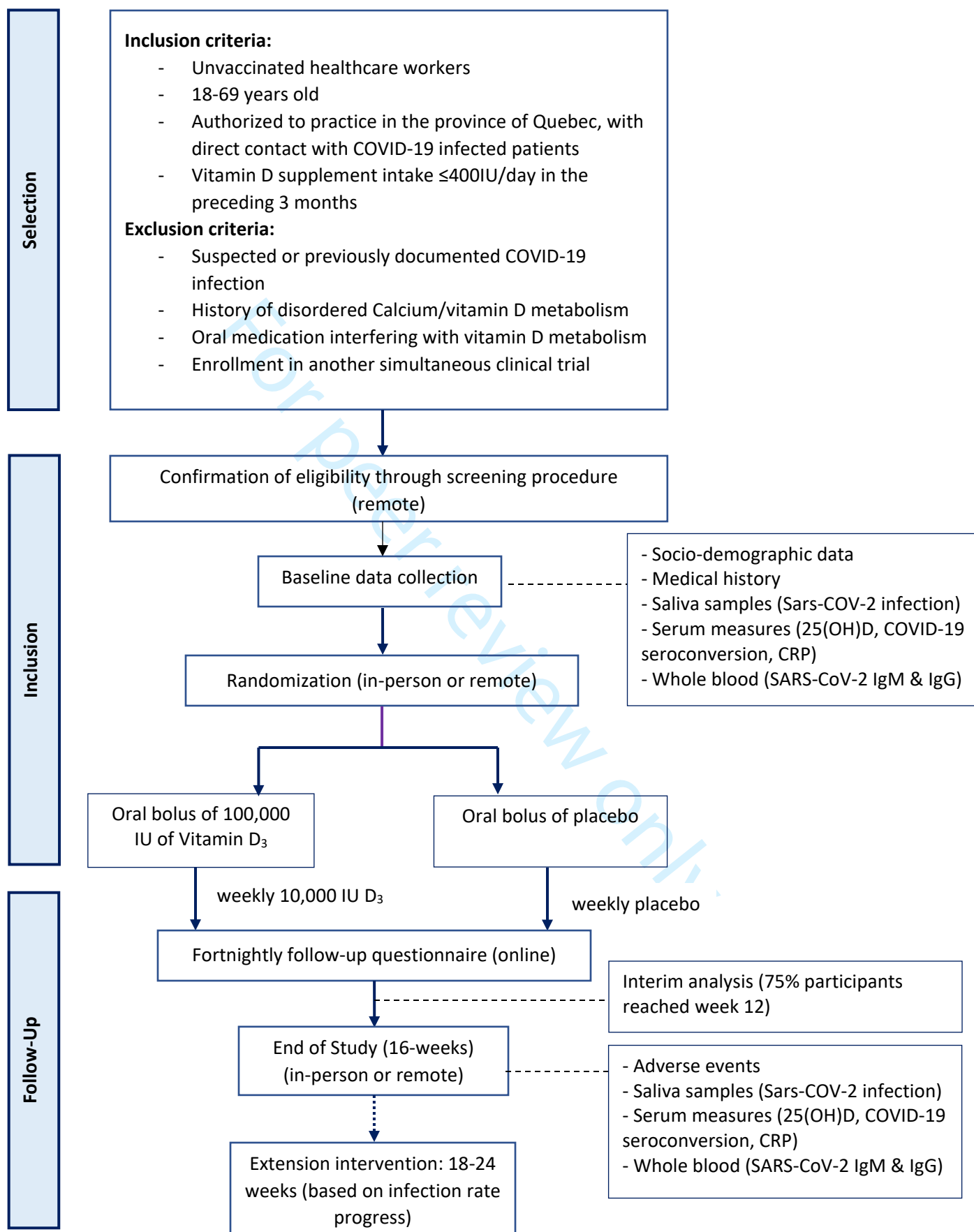


Figure 1



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	8 (line 138)
Funding	4	Sources and types of financial, material, and other support	1, 25, supplemental funding file
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 26-27
	5b	Name and contact information for the trial sponsor	1, 26
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	26

5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19, 20, 26,27
----	--	---------------

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
	6b	Explanation for choice of comparators	6, 7
Objectives	7	Specific objectives or hypotheses	8, 9
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	9, 10

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
---------------	---	--	----

1				
2				
3	Eligibility criteria	10	Inclusion and exclusion criteria	9, 10
4			for participants. If applicable,	
5			eligibility criteria for study	
6			centres and individuals who	
7			will perform the interventions	
8			(eg, surgeons,	
9			psychotherapists)	
10				
11				
12	Interventions	11a	Interventions for each group	10, 11
13			with sufficient detail to allow	
14			replication, including how and	
15			when they will be administered	
16				
17		11b	Criteria for discontinuing or	10
18			modifying allocated	
19			interventions for a given trial	
20			participant (eg, drug dose	
21			change in response to harms,	
22			participant request, or	
23			improving/worsening disease)	
24				
25				
26		11c	Strategies to improve	17, 18
27			adherence to intervention	
28			protocols, and any procedures	
29			for monitoring adherence (eg,	
30			drug tablet return, laboratory	
31			tests)	
32				
33				
34		11d	Relevant concomitant care	10
35			and interventions that are	
36			permitted or prohibited during	
37			the trial	
38				
39	Outcomes	12	Primary, secondary, and other	11, 12
40			outcomes, including the	
41			specific measurement variable	
42			(eg, systolic blood pressure),	
43			analysis metric (eg, change	
44			from baseline, final value, time	
45			to event), method of	
46			aggregation (eg, median,	
47			proportion), and time point for	
48			each outcome. Explanation of	
49			the clinical relevance of	
50			chosen efficacy and harm	
51			outcomes is strongly	
52			recommended	
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Participant	13	Time schedule of enrolment,	15, 16, 17, 18, Figure 1
4	timeline		interventions (including any	
5			run-ins and washouts),	
6			assessments, and visits for	
7			participants. A schematic	
8			diagram is highly	
9			recommended (see Figure)	
10				
11				
12	Sample size	14	Estimated number of	20-21
13			participants needed to achieve	
14			study objectives and how it	
15			was determined, including	
16			clinical and statistical	
17			assumptions supporting any	
18			sample size calculations	
19				
20				
21	Recruitment	15	Strategies for achieving	12,13
22			adequate participant	
23			enrolment to reach target	
24			sample size	
25				

Methods: Assignment of interventions (for controlled trials)

Allocation:

31	Sequence	16a	Method of generating the	11
32	generation		allocation sequence (eg,	
33			computer-generated random	
34			numbers), and list of any	
35			factors for stratification. To	
36			reduce predictability of a	
37			random sequence, details of	
38			any planned restriction (eg,	
39			blocking) should be provided	
40			in a separate document that is	
41			unavailable to those who enrol	
42			participants or assign	
43			interventions	
44				
45				
46				
47	Allocation	16b	Mechanism of implementing	11
48	concealment		the allocation sequence (eg,	
49	mechanism		central telephone; sequentially	
50			numbered, opaque, sealed	
51			envelopes), describing any	
52			steps to conceal the sequence	
53			until interventions are	
54			assigned	
55				
56				
57				
58				
59				
60				

1				
2				
3	Implementation	16c	Who will generate the	11
4			allocation sequence, who will	
5			enrol participants, and who will	
6			assign participants to	
7			interventions	
8				
9				
10	Blinding	17a	Who will be blinded after	5, 19
11	(masking)		assignment to interventions	
12			(eg, trial participants, care	
13			providers, outcome assessors,	
14			data analysts), and how	
15				
16		17b	If blinded, circumstances	19
17			under which unblinding is	
18			permissible, and procedure for	
19			revealing a participant's	
20			allocated intervention during	
21			the trial	
22				
23				
24	Methods: Data collection, management, and analysis			
25				
26	Data collection	18a	Plans for assessment and	12 to 19
27	methods		collection of outcome,	
28			baseline, and other trial data,	
29			including any related	
30			processes to promote data	
31			quality (eg, duplicate	
32			measurements, training of	
33			assessors) and a description	
34			of study instruments (eg,	
35			questionnaires, laboratory	
36			tests) along with their reliability	
37			and validity, if known.	
38			Reference to where data	
39			collection forms can be found,	
40			if not in the protocol	
41				
42				
43				
44		18b	Plans to promote participant	12 to 19
45			retention and complete follow-	
46			up, including list of any	
47			outcome data to be collected	
48			for participants who	
49			discontinue or deviate from	
50			intervention protocols	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Data	19	Plans for data entry, coding,	20
4	management		security, and storage,	
5			including any related	
6			processes to promote data	
7			quality (eg, double data entry;	
8			range checks for data values).	
9			Reference to where details of	
10			data management procedures	
11			can be found, if not in the	
12			protocol	
13				
14				
15	Statistical	20a	Statistical methods for	21, 22
16	methods		analysing primary and	
17			secondary outcomes.	
18			Reference to where other	
19			details of the statistical	
20			analysis plan can be found, if	
21			not in the protocol	
22				
23				
24		20b	Methods for any additional	22
25			analyses (eg, subgroup and	
26			adjusted analyses)	
27				
28				
29		20c	Definition of analysis	21-22
30			population relating to protocol	
31			non-adherence (eg, as	
32			randomised analysis), and any	
33			statistical methods to handle	
34			missing data (eg, multiple	
35			imputation)	
36				

Methods: Monitoring

37				
38				
39	Data monitoring	21a	Composition of data	20,
40			monitoring committee (DMC);	
41			summary of its role and	
42			reporting structure; statement	
43			of whether it is independent	
44			from the sponsor and	
45			competing interests; and	
46			reference to where further	
47			details about its charter can be	
48			found, if not in the protocol.	
49			Alternatively, an explanation of	
50			why a DMC is not needed	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	20
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17, 19, 20, 23
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20

Ethics and dissemination

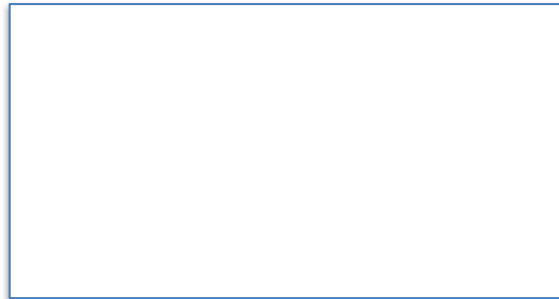
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A

1				
2				
3	A	27	How personal information	14-15, 25
4			about potential and enrolled	
5			participants will be collected,	
6			shared, and maintained in	
7			order to protect confidentiality	
8			before, during, and after the	
9			trial	
10				
11				
12	Declaration of	28	Financial and other competing	27
13	interests		interests for principal	
14			investigators for the overall	
15			trial and each study site	
16				
17	Access to data	29	Statement of who will have	27
18			access to the final trial dataset,	
19			and disclosure of contractual	
20			agreements that limit such	
21			access for investigators	
22				
23				
24	Ancillary and	30	Provisions, if any, for ancillary	25
25	post-trial care		and post-trial care, and for	
26			compensation to those who	
27			suffer harm from trial	
28			participation	
29				
30	Dissemination	31a	Plans for investigators and	23
31	policy		sponsor to communicate trial	
32			results to participants,	
33			healthcare professionals, the	
34			public, and other relevant	
35			groups (eg, via publication,	
36			reporting in results databases,	
37			or other data sharing	
38			arrangements), including any	
39			publication restrictions	
40				
41				
42				
43		31b	Authorship eligibility guidelines	25
44			and any intended use of	
45			professional writers	
46				
47		31c	Plans, if any, for granting	27
48			public access to the full	
49			protocol, participant-level	
50			dataset, and statistical code	
51				
52				

Appendices

1			
2			
3	Informed consent	32	Model consent form and other
4	materials		related documentation given to
5			participants and authorised
6			surrogates
7			
8	Biological	33	Plans for collection, laboratory
9	specimens		evaluation, and storage of
10			biological specimens for
11			genetic or molecular analysis
12			in the current trial and for
13			future use in ancillary studies,
14			if applicable
15			
16			

17 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013
18 Explanation & Elaboration for important clarification on the items. Amendments to the
19 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT
20 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)"
21 license.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



INFORMATION AND CONSENT FORM

Project Title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

Protocol Number: PROTECT 2020

- **Principal investigators at CHUSJ:** Dr. Francine M. Ducharme, MD, FRCPC, Paediatrician, Centre hospitalier universitaire Sainte-Justine (CHUSJ)
- **Principal investigator at the CHUM:** Dr. Cecile Tremblay, MD, FRCPC, Microbiologist/Infectiologist, Centre hospitalier universitaire de Montréal (CHUM)

Co-investigators:

- Decio Coviello, health economist, Hautes-Études Commerciales, University of Montreal
- Shirin Golchi, biostatistician, McGill university
- Cristina Longo, epidemiologist, University of Montreal
- Robert Platt, biostatistician, McGill University
- Caroline Quach, MD, Paediatrician microbiologist, CHUSJ
- Christian Renaud, pediatric microbiologist, CHUSJ
- John White, biochemist, McGill University

Co-investigators at the CHUM:

- Dr. Louis-Georges Sainte-Marie, MD, endocrinologist, CHUM
- Dr. Emil Toma, MD, Dsc, FRCPC, CHUM

Industrial Collaborators: Laboratoires Riva, Blainville, Quebec

Funding source: Canadian Institutes of Health Research (CIHR), in the context of the COVID-19 Rapid Research Funding Opportunity

Multicenter identifier: MP-21-2021-3044

CHUM project number : 20.319

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

Today, we are inviting you to participate in this research study because you are a healthcare worker who is working in a high COVID-19 incidence area and in a setting with a high risk of contact with COVID-19 infected cases. Please read this information to help you decide if you want to participate in this research project. It is important that you understand this information. We encourage you to ask questions. Please take all the time you need to make your decision. You may also want to discuss this study with your family doctor, a family member or a close friend.

WHY IS THIS STUDY BEING DONE?

During the current COVID-19 pandemic, many healthcare workers are working in an environment which increases their probability of contracting this viral infection. Healthcare workers are more frequently infected than the rest of the population. Infected healthcare workers can infect their family, their patients, and their contacts. In addition to being withdrawn from work, they could have transmitted the disease to other colleagues, which further impedes our ability to deliver care to the population.

Vitamin D supplementation can decrease the risk of having the common cold, but it is not known if it could have an effect on the COVID-19 infection. Vitamin D is produced in our bodies from exposure to the sun and can be obtained from supplements and certain foods. However, many Canadians do not have an adequate intake of vitamin D throughout the year.

However, studies testing supplementation with other seemingly harmless vitamins, such as beta carotene and vitamin E, have shown unexpected important adverse reactions. Therefore, it is necessary to properly assess the benefits and the potential unexpected adverse reactions in the context of a clinical study.

This study will investigate whether a high-dose vitamin D supplementation could reduce the risk and severity of COVID-19 infection and work absence in healthcare workers.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

We will be recruiting 2414 graduate healthcare workers, men and women, aged 18 to 69 years old, actively working and scheduled to continue working in a setting at high-risk of contact with people infected with COVID-19 and are working in high COVID incidence areas.

WHAT DOES THE STUDY INVOLVE?

If you agree to participate in this study, you will be assigned by chance to one of two groups. One group will receive one dose of 100,000 IU of vitamin D by mouth at the first visit and then take at home 1 pill of 10,000 IU of vitamin D once a week. The other group will receive a placebo dose at the first visit and then a placebo pill once a week. The vitamin D supplement is the active substance, meaning it could have an effect in the body. A placebo is an inactive substance, meaning it has no effect in the body. A placebo is used in clinical studies, such as this one, to ensure that observed changes are due to the active treatment and not to chance. You will have an equal chance of being assigned to each

group. The placebo and the vitamin D supplement look and taste exactly the same, so no one will know which treatment you are given, including the people involved in the study. In this informed consent form, we will use “Study supplement” to refer either to the vitamin D or the placebo.

This study should last 16 weeks and involves two visits. But prior to the first visit, we will conduct a screening/enrolment visit remotely by videoconferencing (or phone). If eligible and consenting, there will be a randomisation visit and an end-of-study visit, the latter two could be conducted in-person or remotely by videoconferencing, at your preference. Because of the pandemic, we wish to reduce the need and/or length of any in-person visit by doing as much as possible remotely.

Note that the study could finish earlier or be prolonged to 24 weeks, depending on the evolution of the pandemic. We ask that you don't change your usual diet or intake of vitamin supplements (if any) during the study.

Screening/Enrolment (pre-visit: about 45-60 minutes)

- ❖ We will review the eligibility questionnaire you have completed online, complete it with additional questions, explain the study in detail, and answer your questions.
- ❖ If eligible and consenting, you will be asked to sign the consent form, complete a few short study questionnaires and provide your contact information.
- ❖ We will ask you questions about your demographics (household, ethnicity), work-related activities and personal health (weight, height, skin color, smoking, medication, vitamins, supplements, health problems).
- ❖ To enable the creation of a medical and research pharmacy records, obtain information on COVID test, and ensure optimal contact with you throughout the study, we will ask personal information namely your RAMQ number, names (yours, your parents, your spouse), any drug allergy, your employee number (or practice number for physicians), your postal and email addresses, phone numbers and that of a next of kin and your preferred means to reach you.
- ❖ We could show you videos of key procedures (e.g. home blood collection) to help you chose your preferred type of randomisation visit.
- ❖ We will schedule the randomisation visit at a mutually convenient time and place given your choice of in-person or remote visit by videoconferencing. However, in case of a suspected prior COVID-19 infection, we would prefer you do an in-person visit to perform a rapid screening test for COVID-19 antibodies.

Randomisation visit (First visit: Week 0)

During the visit, which will last approximately an hour,

- ❖ If not already done, we will ask you to sign the consent forms, complete missing study questionnaires and your contact information.
- ❖ We will take a venous blood sample of about 15 mL (3 teaspoons) to measure the level of vitamin D, look for COVID-19 antibodies and to do an optional genetic analysis to examine a possible genetic predisposition to respond to vitamin D and to severity of COVID-19 symptoms.

- ❖ We would like to obtain a small drop of blood either from the venous puncture or via a finger-prick to look for COVID-19 antibodies in your blood using NADAL® COVID-19 IgG/IgM Rapid Test: if positive, you would not be eligible for this study. This test is not yet licensed for use in Canada and its use in this study is investigational. It has been selected for use prior to enrolment because it provides antibody results in 15 minutes. The results of this investigational test will be shared with you, acknowledging the risk of false positive or negative results. They will also be subsequently compared to the approved (Liaison IgG COVID-19) serology test.
- ❖ We will show you how a video on the TASSO home blood sampling kit at home for the last visit; if interested, we will show you how to use it, identify your sample, package it, and send it back to us (see below under First Remote visit). This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling.
- ❖ We will show you how to take a saliva sample by spitting into a tube. You will receive a pamphlet with instructions and could watch a video. You should not brush your teeth, eat, drink, smoke or chew gum for 30 minutes before spitting a small volume of saliva (2 mL). If you prefer to do an oro-nasopharyngeal sample, you would need to insert a swab (a small tube with a cotton tip) into the back of your mouth, then in one of your nostrils gently rotating the swab for about 5 seconds. We will ask you to take the saliva (or oro-nasopharyngeal) sample under our guidance. We will then show you how to identify it with our prepared labels, record the date and time, package it, and sent it back for analysis for COVID-19, and possibly other viruses and cells.
- ❖ You will be asked to take ten (10) pills of the Study supplement at this first visit only in front of the research personnel (in person or by videoconference). You will take home the bottle of Study supplement and be asked to take one (1) pill once a week until the end of the study.
- ❖ We will send you by text message or email as per your preference, a first reminder with a link to a questionnaire to confirm that you have received it and are able to complete and submit the brief questionnaire. The same approach will be used every week.
- ❖ We will give you all the other study materials including the saliva collection tube pre-printed labels, biohazard bags, insulated envelopes or boxes for shipment, prepaid courier waybills, and if you are interested in a **Remote end-of-study visit**, the TASSO home blood collection kit. It is possible that we ask you to use the NADAL® COVID-19 IgG/IgM Rapid Test at the last visit for validation purposes.

For participants choosing to have a **Remote First Visit**, in whom there is a suspicion that you may have had a prior undiagnosed COVID-19 infection in the past, we would prefer that you come for an in-person visit. Alternatively, we may send you first a finger-prick test kit to look for COVID-19 antibodies in your blood; if so, we would ask that you use it on your fingertip in front of us by videoconference. If positive, you would not be eligible for this study. If negative, the Study supplement and required materials would then be sent to the participant's home prior to the randomisation visit. We will ask you to take in front of us by videoconference, the Study supplement, the saliva sample, as well as the blood test and 2nd optional saliva sample (for genetic analysis).

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
- ❖ We will show you how to take a small sample (<1 mL) of capillary blood, using a blood collection kit specifically conceived for home collection, called TASSO-SST OnDemand. This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling. We will ask you to watch a short video and read the brochure explaining the procedure, then ask you to use it under our guidance. Briefly, you will need to warm the skin of your upper arm by rubbing it for about 45 seconds, disinfecting it, applying the little device on your arm, pressing on a button that will puncture a very small hole in the skin, then leave the device in place for about 5 minutes while blood flows slowly in a small tube. As only a small sample of blood can be obtained, it is very likely that we ask you to repeat this with a second kit. We will show you how to remove the small tube, close it with a small cap, identify the sample with our prepared labels, record the sampling date and time, package it, and prepare it to be sent for analysis for vitamin D and COVID-19 antibodies. We will ask your feedback on this type of blood collection method.
 - ❖ If you wish to participate in the optional genetic analysis, we will ask you to collect 2 mL of saliva in another small tube (as the blood sample made by TASSO is not enough for this analysis), identify and date the sample with our prepared labels, and send it to us.

28 **Between visits**

- 29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
- ❖ For the following weeks 0 to 16 (or later, should the study be extended up to 24 weeks) participants will take every week one (1) Study pill.
 - ❖ You will receive a message via email or text message, according to your indicated preference, to remind you
 - Once a week to take the Study supplement
 - Once every two weeks to take the Study supplement, and fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose.
 - ❖ If there is no response from you within a few days of sending the electronic questionnaire, we will contact you; if there is still no response from you within 7 days of us sending the electronic questionnaire, we will contact your next-of-kin indicated by you.

50 **If you are infected during the study**

51
52
53
54
55
56
57
58

If we obtain a positive COVID result from one of your saliva (or oro-nasopharyngeal) samples, you will be notified by a Study team member, by the preferred communication means you have indicated (phone, text message or email). As all positive results will be reported to the public health authorities you will be contacted as soon as possible by them for an assessment and instructions.

If you receive a positive COVID result from a test done outside this study (i.e., for clinical reasons), we ask that you inform us immediately and to indicate it in the follow-up questionnaire.

At the reception of a positive COVID test result,

- ❖ We will ask you to complete the daily diary of symptoms (duration of 1-2 minutes)
 - Until 48 hours after resolution of symptoms
 - Or if you remain asymptomatic, for a minimum of 14 days;
- ❖ If symptoms reappear, we will ask you to restart documenting them in the daily diary of symptoms
 - Until 48 hours after resolution of symptoms;
- ❖ If your symptoms continue beyond 14 days, we will ask you to complete the weekly diary of symptoms (duration of 1-2 minutes), once a week, until resolution of symptoms
- ❖ We will ask you to continue taking your weekly supplement and completing the follow-up questionnaire once every two weeks.

In case of an imminent vaccination against COVID-19

- ❖ If you expect to receive a vaccine against COVID-19 in the next few weeks, we will ask you to notify us immediately or via the questionnaire every two weeks.
 - We will rapidly organise a visit in person or remotely, before the scheduled date of the vaccination, to obtain a saliva sample to test for COVID-19 infection and a blood sample either in your vein (9 mL) or with the TASSO device at home to look for COVID-19 antibodies and level of vitamin D prior to the vaccination.
 - Just before, and about 1 month after, your second vaccine dose against COVID-19, we will ask you for another blood sample either in your vein (4.5 mL) or with the TASSO device at home to look for COVID-19 antibodies.
- ❖ We will ask you to continue
 - Once a week to take the Study supplement
 - Once every two weeks to fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose, whether you have been vaccinated or not.

End-of-study – Week 16 (or later if the study is prolonged)

At the end of the study, you will be invited to a last in-person or remote visit. This research visit will take approximately 30 minutes and involve the following:

- ❖ You will be asked to return the Study supplement bottle containing the unused pills.

- ❖ A venous (or capillary blood if done remotely) sample and, if you have not tested positive at COVID-19 before, a saliva (or oro-nasopharyngeal) sample will be collected.
- ❖ A rapid COVID-19 antibody test (NADAL®) may also be done on a blood drop (finger-prick or from the venous puncture) for validation purposes. If done remotely, this test may be done on blood sampled by finger-prick under our guidance by videoconference.
- ❖ You will complete the last few short study questionnaires and any missing information in the previous ones, if applicable.
- ❖ If conducted remotely, the samples, Study supplement bottle and unused material should also be shipped to the Coordinating Center.

Collecting information on COVID-19 tests made for clinical reasons

The results for a COVID-19 test performed for clinical reasons outside this study will be documented by you in the follow-up questionnaire (*faster means to inform us*) as well as in your institution's (Pandemic) or provincial database of COVID-19 cases, namely Trajectoire Santé publique (TSP) including all individuals who tested positive and all healthcare workers who tested positive under the supervision of the Ministère de la santé et des services sociaux (MSSS). If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we have complete information on the primary outcome of the study and thus allow us to determine accurately the impact of the intervention on the risk of infection with COVID-19.

Collecting information on healthcare services

The date, diagnosis, type of professional and of health care services which you have received during medical visits and hospitalisations will be obtained from the administrative databases of the Régie de l'assurance maladie du Québec (RAMQ) and Quebec hospital discharges (MED-ECHO). This will allow us to accurately determine the impact of the intervention on the severity of COVID-19 infection and other concomitant illnesses.

Collecting information of work absence

The number of days of work absence, overall, by type (i.e., holiday, illness, etc.), and specifically due to COVID-19, including absences due to an infection acquired at work or outside of work, preventive withdrawal due to pregnancy or other health conditions, awaiting test results/investigation, or other reason for quarantine will be collected from you via the follow-up questionnaire (*faster and most detailed means*), as well as from your institution's *Direction of Health Resources* or, if you are an attending physician, from the *Direction of professional services*. If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we accurately ascertain the impact of the intervention on work absences.

BIOBANK

For the purposes of this study, we will keep the biological samples collected (blood, saliva and/or oro-nasopharyngeal) in a biobank as well as the clinical and administrative data collected during the course of this study in order to complete the study's objectives, and to

1
2
3 conduct research on vitamin D, COVID-19 and its treatments and other related diseases.
4 We would like to quantify specific cellular receptors which allow entry of COVID-19 into
5 cells (for example, the angiotensin converting enzyme-ACE2) and inflammatory markers
6 (such as the C-reactive protein). The collected samples will be kept in a biobank in the
7 Research Center of CHU Sainte-Justine under the supervision of Dr Francine M.
8 Ducharme. The samples will be kept as long as the research team can guarantee their proper
9 management. Confidentiality of the identity of the samples will be guaranteed by assigning
10 them a specific code. Your sample will not be identified by your name and cannot be used
11 to identify you directly. After 5 years, the code key will be destroyed, and the samples will
12 become completely anonymous. Your samples could possibly be shared with other
13 researchers in other institutions. However, the access to data will only be allowed for
14 approved projects by an independent research ethics board.
15
16
17
18

19 **GENETIC ANALYSIS (optional)**

20
21 Each person has their own set of unique genes or “genome”. Genetic research aims to
22 determine if there are genetic predispositions which make you more susceptible to a
23 COVID-19 infection, to respond to vitamin D, to modulate disease severity and the
24 interaction of these factors.
25
26

27 If you accept to participate in the genetic analysis, these analyses will be done on a small
28 part (4 mL) of the venous blood sample provided during the first visit. If you decide to
29 participate remotely, we will ask you to provide a saliva sample in a small tube.
30
31

32 We would like to sequence your entire genome and conduct gene expression analyses. We
33 would also like to share your genetic data as well as other collected clinical data during the
34 PROTECT study with the Canadian database HostSeq COVID-19 for use for COVID-19
35 related research and other aspects of human health. This biobank will serve as a centralized
36 resource in Canada for COVID-19 research and other health-related studies. The data in
37 the HostSeq database are under the supervision of CGen, a national Canadian platform
38 financed by the federal government for sequencing and genome analysis. The principal
39 investigators of the PROTECT study as well as the administrators of the HostSeq biobank
40 COVID-19 will share your genetic and clinical information with other Canadian and
41 international researchers whom are approved by CGen (the sponsor). The data could also
42 be used for commercial use. However, your data will not be shared with until after an
43 examination by a data access committee. This committee will verify that the use of the
44 proposed research is in line with the objectives of the database HostSeq and that the
45 research team which requests access has already been granted the required approval in
46 accordance in terms of research ethics requirements. Approved researchers will sign
47 agreements. These agreements will control how the data will be used. Individual results of
48 any research conducted using your samples or any individual incidental findings will not
49 be shared with you, as the research conducted on your data will have no individual
50 diagnostic or therapeutic significance to you.
51
52
53
54
55
56

57 **WHAT ARE THE BENEFITS AND RISKS OF THIS STUDY?**

Benefits:

You may not benefit directly from the study intervention if it is not efficacious or if you have been assigned in the placebo group. However, the screening may identify earlier an active or past COVID-19 infection that was not apparent. Your participation will help advance our knowledge on vitamin D and on the prevention of COVID-19 infection in healthcare workers and other individuals at risk of infection.

Each positive COVID-19 result from the saliva (or oro-nasopharyngeal) or blood sample will be shared with you according to your preferred way of communication: telephone, text message or email. All positive saliva (or oro-nasopharyngeal) results will also be shared by the Microbiology Laboratory of CHUM with the Public health authorities and will be added into your file at the CHUM and Dossier Santé Québec. No other research result will be provided to you. Research findings resulting from your participation to this study could potentially contribute to creating commercial products from which you would not be able to claim any financial benefit.

Risks:**• Related to study medication:**

The vitamin D dose used in this study has been shown to be safe in adults. This dose is approved by Health Canada for the purpose of this study only, but not for clinical use yet. It is unlikely that you will have any side effects because of the amount of vitamin D used in this study as when combining the first and weekly doses, the total remains below the maximum amount allowed.

However, we will ask you to notify us immediately if you have any of the following, as they could be signs of an acute excess intake of vitamin D: mainly, a marked increase in thirst or an increase in the volume and frequency of urination (with or without fatigue, loss of appetite, nausea or vomiting, headaches, drowsiness, cardiac arrhythmias, constipation, muscle or bone or chest pain, mouth dryness or a metallic taste).

Later signs and symptoms that may indicate a chronic excess intake of vitamin D are: a marked increase in thirst, an increase in the volume and frequency of urination including during the night, loss of appetite, weight loss, red eye or conjunctivitis, inflammation of the pancreas, light sensitivity, runny nose, itching, fever, reduced libido, kidney stones, increased concentration of some analytes in the blood (BUN, AST, ALT, cholesterol), or in urine (albumin), ectopic calcification, hypertension, cardiac arrhythmias and rarely, a psychosis.

It's possible that other currently unknown risks are associated with Vitamin D intake.

One of the reasons we collect a blood sample is to measure the concentration of vitamin D in the blood at the start and end of the study. this will allow us to see if the vitamin D blood level is linked to the number and severity of COVID-19 confirmed cases.

• Related to study procedures:

1
2
3 The salivary collection sample is painless. If done, an oro-nasopharyngeal swab may cause
4 slight discomfort during collection that will subside after its removal. The side effects of
5 having blood collected by venous puncture or TASSO can include bleeding, bruising,
6 discomfort and pain at the sample site. It is possible that the NADAL COVID-19 IgG/IgM
7 Test may give false positive or false negative results. In case of divergence of results, we
8 will communicate to you the results of the approved IgG test when available.
9
10

11
12
13 **• Related to confidentiality:**

14 There is always a small risk that your data could one day be re-identified. The genetic
15 information is unique to each person, just as your fingerprint. This means that theoretically,
16 you could be identified using your genetic code; however, this is not easy to do.
17 Considering the advances in technology, there could be new ways to link you to data that
18 we have not foreseen today, despite the strict confidentiality measures in place. Possible
19 re-identification or unintentional disclosure of your genetic and clinical research data could
20 lead to a loss in confidentiality and a possible future discrimination against yourself or your
21 biological parents. But all security measures will be put in place to protect your privacy.
22
23

24 **WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?**

25
26 The Study supplements are provided free of charge by the manufacturer, Laboratoire
27 RIVA.
28

29
30 **WHAT ARE THE OTHER FINANCIAL ASPECTS?**

31 For each completed visit (0 and 16 weeks), you will receive a \$25 check by mail to
32 compensate for your time. The check may arrive at your home between 4 and 8 weeks after
33 the visit.
34
35

36 **HOW IS PRIVACY INSURED?**

37
38 During your participation in this research study, the investigators responsible for this study
39 as well as the members of their research team will collect, in a research file, the required
40 personal information to answer the scientific objectives of this research project.
41

42 These information could include your demographic data (name, sex, date of birth, ethnic
43 origin, weight and height), your past and present health status, your health-related habits,
44 medication you take, your work absences, and the results of all tests, exams, and procedures
45 which you will participate in. Your personal file will include your address, email, telephone
46 numbers, RAMQ number, and employee or practice number be kept in a separate file with
47 restricted access; this information is required to create a medical and pharmacy file at the
48 CHUM and for communication purposes during the study.
49

50
51 The coded blood, saliva (and/or oro-nasopharyngeal) samples will be sent to the biobank
52 located at the Research Center of CHU Sainte-Justine under the supervision of Dr Francine
53 M. Ducharme. The coded results of completed analyses will be kept on a protected server
54 with restricted access at DACIMA company during the study, and thereby transferred to a
55 secure server with restricted access in the Research Center of CHU Sainte-Justine under
56 the supervision of Dr Francine M. Ducharme. During the study, the personal information
57
58

1
2
3 used to arrange virtual and in-person study visit appointments will be kept on a protected
4 server with restricted access at the company providing the appointment-making software.
5 Following the conclusion of the study, this information of yours will be transferred to
6 a secure server with restricted access in the Research Center of CHU Sainte-Justine under
7 the supervision of Dr Francine M. Ducharme. The database of HostSeq will be kept on
8 secure cloud servers (online) that are based in Canada and will be indefinitely kept or until
9 they are not useful for research.
10

11
12 To ensure your privacy, a copy of the consent form as well as the results to the diagnostic
13 tests required for conducting the research project, will be copied in the research and
14 medical file of the CHUM. Therefore, each person or company which you authorize to
15 consult your medical file, will have access to this information.
16

17 The research data will be kept for at least 25 years by the principle investigator. The data
18 collected could be published or discussed during scientific meetings, but it would not be
19 possible to identify you.
20

21 All collected information will remain confidential within the limits provided by law. You
22 will only be identified by a code number. The key to the code linking your name to your
23 research file will be kept by the investigator responsible for this research project.
24

25
26 To ensure your safety, a copy of the consent form as well as the results of the diagnostic
27 tests required for research purposes will be placed in the research file and the medical file
28 of the CHUM. Consequently, any person or company to whom you give access to your
29 medical file will have access to this information.
30

31 Research data will be kept for at least 25 years by the investigator responsible for this
32 research project. Research data may be published or be the subject of scientific discussion,
33 but it will not be possible to identify you.
34
35

36
37 For the purposes of surveillance, control, safety and marketing of the Study drug, your
38 research as well as your medical files could be consulted by a person mandated by a
39 regulatory organization, in Canada or elsewhere, such as Health Canada, as well as sponsor
40 representatives of the company manufacturing the vitamin D pills for this project
41 (Laboratoire RIVA), the institution or research ethics committee. These people and
42 organizations adhere to a strict confidentiality agreement.
43

44 You have the right to consult your research file to verify the collected data and to correct
45 them, if needed. Moreover, access to certain information before the end of the study could
46 mean your removal from this study in order to maintain the study's integrity.
47
48
49

50 **IS YOUR PARTICIPATION VOLUNTARY?**

51
52 Yes. Taking part in this study is voluntary. You may choose not to be in this study. You
53 can decide to stop being in the study at any time, without needing to provide any reason,
54 but simply informing the research team.
55
56
57
58

1
2
3 Your decision to refuse participation or to stop participating in the study at a later time,
4 will have no effect on the quality of care or services to which you are entitled or on your
5 relationship with the people that provide them.
6

7
8 The principal investigators of this study, the research ethics board, the funding agency or
9 the sponsor could decide to end your participation in the study without your consent. This
10 could happen if there are new information or findings that indicate your participation is no
11 longer in the best of your interests, or if you have not been following the study instructions
12 as explained, or if there are other administrative-related reasons to stop the project.
13

14 If you stop participating in the study or if you have been removed from it, the collected
15 information and material already received will be kept (as well as the data pertaining to
16 healthcare services and work absences will continue to be collected) and analysed to ensure
17 the validity of this project, unless you specifically ask for them to be destroyed. If this is
18 the case, these data and/or material will be removed from the biobank provided that the
19 code key (linking between nominal data and the study code) is still available, that is, up to
20 5 years after the end of the study.
21
22

23 If you decide to drop out of the HostSeq database, your data will no longer be shared, and
24 no new data will be collected. The data already in the HostSeq database will be destroyed
25 once informed about this decision. However, it could be impossible to remove the results
26 once they have been compiled with the results of other participants or if they have been
27 published. Moreover, if certain data have been shared with other researchers, it could be
28 possible not to be able to remove this part of the data. In such a case of unsuccessful
29 withdrawal from the study, your identity will always be protected.
30
31

32 All new information acquired during the course of the study which could have an impact
33 on your decision to continue participation will be shared with you rapidly, which is the
34 reason why we would like to keep your personal information and have your approval to
35 communicate with you after the end of the study (optional).
36
37
38
39
40
41
42
43
44

45 **WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**

46
47 If you have any questions about the research project or if you have any problems that you
48 believe are related to your participation in the project, you can call the researchers
49 responsible for the project:
50

- 51 • Dr. Francine M. Ducharme at 514 345 4931, extension 4398
 - 52 • Dr. Cecile Tremblay at 514 890-8000, extension 14645
- 53
54
55
56
57
58

1
2
3 If you would like information about your rights related to your participation in the research,
4 you may contact the Ombudsman - complaints and quality services of the CHU Sainte-
5 Justine at 514 345-4749, of the CHUM at 514 890-8484 or your CIUSSS/CIUSSS:
6
7

- 8 • CIUSSS de l'Est-de-l'Île-de-Montréal : 514 252-3510
- 9 • CIUSSS de l'Ouest-de-l'Île-de-Montréal : 514-989-1885, extension: 1010
- 10 • CIUSSS du Centre-Sud-de-l'Île-de-Montréal : 514 593-3600
- 11 • CISSS de la Montérégie-Est : 450-468-8447
- 12 • CISSS de la Montérégie-Centre : 450-466-5434

13 14 15 **RESEARCH ETHICS COMMITTEE**

16 The Research Ethics Board of CHU Sainte-Justine has approved this study and will
17 continue to monitor it for all participating institutions of the Quebec Health and Social
18 Services network.
19

20 21 22 **LIABILITY**

23
24 This research is not funded by a private industry. In case of side effects resulting from the
25 study medication or from procedures required for this research project, you will receive all
26 necessary medical care covered by the Quebec's provincial health insurance plan (RAMQ)
27 or by your private drug insurance plan. You will be responsible for paying the portion of
28 any costs not covered.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CONSENT FORM

Research project title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

The nature and procedures of this research project were explained to me. I have read the information and consent forms and I kept a copy, or a copy has been provided to me. I was able to ask my questions and they were answered to my satisfaction. After consideration, I agree to participate in this research project.

I authorize the research team to consult the collected data about me in the COVID infection database (Pandemic) of my institution and/or the provincial TSP database, the medical and hospitalisation services database (RAMQ and MED-ECHO), and the workplace absenteeism database (Human Resources Directorate or Professional Services Directorate) to obtain information that is pertinent to this project.

By agreeing to participate in this study, you are not waiving any of my rights under the law. You are not releasing the investigators from their legal and professional liability.

Name of participant (Print)

Signature

Date

1. I consent to the analysis of gene expression and the sequencing of the whole genome of my coded biological material (blood, saliva, and/or oro-nasopharyngeal). The whole genome sequence could be hosted in the Canadian HostSeq COVID-19 biobank and linked to a database containing the viral genome. This would serve to explore any genetic predisposition to COVID-19, the severity of the disease and response to vaccine.

Yes _____ (Initials) No _____ (Initials)

2. I consent to prolonging the access to my coded data on healthcare use, COVID-19 infections and work absenteeism for 12 months following the study end date, to explore the long-term impact of COVID-19 infection and vaccination.

Yes _____ (Initials) No _____ (Initials)

3. I consent to being contacted to update my personal information, obtain additional information about my health or to be invited to participate in new research.

Yes _____ (Initials) No _____ (Initials)

4. In case I receive a vaccine against COVID-19 during the study, I agree to do the blood samples before the first and second vaccine dose as well as 1 month after the 2nd vaccine dose, even if these samples were to be done after the end-of-study's visit planned at week 16 (or 24).

Yes _____ (Initials) No _____ (Initials)

Participant's signature: _____

1
2 I have explained the research study and the terms of this information and consent form to the research
3 participant, and I answered all his/her questions. I explained that participation in a research project is
4 free and voluntary and could be stopped at any time they choose.
5
6
7

8
9 _____
10 Name of person obtaining consent (Print) Signature Date
11

12
13 **(FOR THE CHUM PARTICIPANTS ONLY)**
14

15 **COMMITMENT OF THE PRINCIPAL INVESTIGATOR AT THE CHUM**
16

17 I certify that this information and consent form was explained to the research participant, and that the
18 questions the participant had were answered.
19

20
21 I undertake, together with the research team, to respect what was agreed upon in the information and
22 consent form, and to give a signed and dated copy of this form to the research participant.}
23
24
25
26
27

28 _____
29 Name (Print) Signature of the principal
30 investigator at the CHUM Date
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

BMJ Open

Prevention of COVID-19 with oral vitamin D supplemental therapy in essential healthcare teams (PROTECT): protocol for a multicentre, triple-blind, randomized, placebo-controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-064058.R2
Article Type:	Protocol
Date Submitted by the Author:	20-Apr-2023
Complete List of Authors:	Ducharme, Francine; CHU Sainte-Justine, Departments of Pediatrics and of Social and preventive medicine Tremblay, Cécile; University of Montreal, Microbiologie Golchi, Shirin; McGill University Hosseini, Banafsheh ; University of Montreal, Longo, Cristina; University of Montreal White, John; McGill University, Physiology Coviello, Decio; HEC Montreal Quach, Caroline; University of Montreal Ste- Marie, Louis- Georges; University of Montreal Platt, Robert; McGill University
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Infectious diseases
Keywords:	COVID-19, PREVENTIVE MEDICINE, Clinical trials < THERAPEUTICS

SCHOLARONE™
Manuscripts

1
2
3 1 **Prevention of COVID-19 with oral vitamin D supplemental therapy in essential healthcare**
4
5 2 **teams (PROTECT): protocol for a multicentre, triple-blind, randomized, placebo-controlled**
6
7 3 **trial**
8
9

10 4
11
12 5 Ducharme FM^{1,2,3}, Tremblay CL⁴, Golchi S⁵, Hosseini B¹, Longo C^{3,6}, White JH⁷, Coviello D⁸,
13
14 6 Quach C⁹, Ste-Marie LG¹⁰, Platt RW^{5,11}
15
16
17 7

18
19 8 **Correspondence to:**
20

21 9 Dr. Francine M. Ducharme
22 10 Professor, Department of Pediatrics and of Social and Preventive Medicine
23 11 University of Montreal
24 12 Sainte-Justine University Hospital Centre
25 13 3175 Côte Ste-Catherine, room 17-B-000
26 14 Montreal, Quebec, H3T 1C5, Canada
27 15 francine.m.ducharme@umontreal.ca
28
29

30
31 16

32
33 17 Word count: 5513
34
35

36 18
37
38

39 ¹ Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada

40 ² Department of Social and Preventive Medicine, School of Public Health, University of Montreal,
41 Montreal, Quebec, Canada

42 ³ Clinical Research and Knowledge Transfer Unit on Childhood Asthma (CRUCA), Research Centre,
43 Sainte-Justine University Hospital Centre, Montreal, Quebec, Canada

44 ⁴ Department of Microbiology and Infectious disease, Centre Universitaire de santé de Montréal,
45 University of Montreal, Quebec, Canada

46 ⁵ Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Quebec,
47 Canada

48 ⁶ Faculty of Pharmacy, University of Montreal, Montréal, Quebec, Canada

49 ⁷ Department of Physiology, McGill University, Montreal, Quebec, Canada

50 ⁸ Department of Applied Economics, HEC Montreal, Montreal, Quebec, Canada

51 ⁹ Department of Microbiology, Infectious Diseases & Immunology, University of Montreal, Montreal,
52 Quebec, Canada

53 ¹⁰ Department of Medicine, University of Montreal, Montreal, Quebec, Canada

54 ¹¹ Department of Pediatrics, McGill University, Montreal, Quebec, Canada
55
56
57
58
59
60

1
2
3 19 **ABSTRACT**
4

5 20 **Introduction:** In the COVID-19 pandemic, health care workers (HCWs) were at high-risk of
6
7 21 infection due to their exposure to COVID infections. HCWs were the backbone of our health care
8
9 22 response to this pandemic; every health care worker withdrawn or lost due to infection had a
10
11 23 substantial impact on our capacity to deliver care. Primary prevention was a key approach to reduce
12
13 24 infection. Vitamin D insufficiency is highly prevalent in Canadians and worldwide. Vitamin D
14
15 25 supplementation has been shown to significantly decrease the risk of respiratory infections.
16
17 26 Whether this risk reduction would apply to COVID-19 infections remained to be determined. This
18
19 27 study aimed to determine the impact of high-dose vitamin D supplementation on incidence of
20
21 28 laboratory-confirmed COVID19 infection rate and severity in HCWs working in high COVID
22
23 29 incidence areas.

24
25 30 **Methods and analysis:** PROTECT was a triple-blind, placebo-controlled, parallel-group
26
27 31 multicentre trial of vitamin D supplementation in HCWs. Participants were randomly allocated in
28
29 32 a 1:1 ratio in variable block size to intervention (one oral loading dose of 100,000 IU vitamin D3 +
30
31 33 10000 IU weekly vitamin D3) or control (identical placebo loading dose + weekly placebo). The
32
33 34 primary outcome was the incidence of laboratory-confirmed COVID-19 infection, documented by
34
35 35 RT-qPCR on salivary (or nasopharyngeal) specimens obtained for screening or diagnostic
36
37 36 purposes, as well as self-obtained salivary specimens and COVID-19 seroconversion at endpoint.
38
39 37 Secondary outcomes included disease severity; duration of COVID-19 related symptoms; COVID-
40
41 38 19 seroconversion documented at endpoint; duration of work absenteeism; duration of
42
43 39 unemployment support; and adverse health events. The trial was terminated prematurely, due to
44
45 40 recruitment difficulty.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 42 **Ethics and dissemination:** This study was approved by the Research Ethics Board of the CHU
4
5 43 Sainte-Justine as the central committee for participating institutions ((MP-21-2021-3044).
6
7 44 Participants provided written informed consent. Results are being disseminated to the medical
8
9 45 community via national/international conferences and publications in peer-reviewed journals.
10
11

12 46 **Trial registration:** NCT04483635.
13
14
15 47

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

48 **Strengths and limitations of this study**

- 49 • This trial was designed as a hybrid study enabling partially or totally remote screening,
50 randomisation, follow-up, as well as outcome documentation by use of home capillary blood
51 and saliva sampling, visits conducted by videoconference, monitoring by electronic reminders
52 and questionnaires, and communication by phone, text messaging or emails.
- 53 • The trial used a pragmatic subject selection and easily applicable intervention to maximise
54 subsequent implementation in practice and selected a primary outcome, the risk of laboratory-
55 confirmed infection, that would likely change practice.
- 56 • A single loading dose followed by regular doses have been shown to lead to rapid and
57 sustained increase in serum level of 25-hydroxy-vitamin D and ensure adequate group
58 separation, both properties desired in the context of a rapidly expanding epidemic while
59 facilitating adherence in exhausted frontline health workers.
- 60 • Given the uncertainty in the progression of the infection rate, the use of a Bayesian adaptive
61 design allowed for adaptations (early stopping or prolongation of duration of follow-up) at
62 the interim analysis according to the projection of infection rates.
- 63 • Although the trial aimed for high-intensity recruitment, the delay in setting up a remote study
64 in the context of the pandemic, combined with high use of vitamin D and successful
65 vaccination program in health care workers, resulted in severe recruitment difficulty and
66 early stopping of the trial for futility.

67

68 Introduction

69 The Coronavirus 2 (SARS-CoV-2) disease (COVID-19) outbreak has rapidly expanded to a global
70 pandemic. Healthcare workers (HCWs) played a crucial role in the fight against the COVID-19
71 pandemic. It was therefore a public health priority to develop strategies to decrease the risk and
72 severity of COVID-19 in this vulnerable population. Indeed, there was rising concern as HCW
73 were overrepresented in terms of infections (3.8% of infected individuals in Wuhan, China,[1]
74 10% in Italy and 12% in Spain, 10-20% in US)[2] [3] and perhaps severity. Working in long-term
75 care facilities (LTCF) and with aerosol generating medical procedures (e.g., hospitals) further
76 increased the risk (Odds Ratio [OR]: 2.3).[4] The risk of reporting COVID-19 infection in front-
77 line HCWs, defined as those in direct contact with patients, was 10-fold greater than the general
78 population at the beginning of the pandemic (Hazard Ratio [HR]= 11.61). [5] Recent research also
79 indicated that HCWs who were Blacks, Asians, or other minority ethnic populations, had a higher
80 likelihood of contracting COVID-19.[5] Compared to those unexposed to COVID-19 patients, the
81 risk was two to five-fold higher in HCWs exposed to suspected (HR= 2.39) or confirmed (HR=
82 4.83) COVID-19 cases, even with adequate personal protection equipment (PPE).[5] Although
83 infections may have been due to contact with infected patients, community-, or family-acquired
84 disease, cases were rapidly emerging from cross-infection with asymptomatic infected HCW.

85 Vitamin D is an immunomodulatory micronutrient, and its levels in the body may vary due to diet
86 and environmental conditions. Vitamin D insufficiency had been associated with increased risk of
87 respiratory infections, and possibly COVID-19,[6] asthma exacerbations, and acute respiratory
88 distress syndrome (ARDS) among others.[7-9] Optimal pro-immune and anti-inflammatory
89 impacts likely occur at 25-hydroxyvitamin D (25OHD) levels above 75 nmol/L (30
90 ng/mL).[10,11] In a systematic review of 25 randomized controlled trials (RCT) of 11321
91 individuals, daily/weekly vitamin D supplementation decreased by 19% the rate of acute

1
2
3 92 respiratory infections (two-step analysis; OR 0.81, 95% CI 0.72 to 0.91),[12,13] with a stronger
4
5 93 effect in subjects with baseline 25OHD <25 nmol/L. Whereas subgroup analyses suggested a
6
7 94 protective effect, primary in individuals receiving daily or weekly vitamin D supplement, and not
8
9
10 95 in those with bolus,[14] other important differences in population (e.g., malnutrition),[15,16] age
11
12 96 (infant),[16] chronic disease (e.g. asthma, COPD)[17-21] and type of infection (e.g.
13
14 97 bacterial)[15,16] could have contributed to the apparent lesser effect. Of interest, Vitamin D
15
16 98 supplementation significantly reduced the rate of severe exacerbations (i.e., requiring rescue
17
18 99 systemic corticosteroids), a condition association with airway inflammation, with no impact
19
20
21 100 according to bolus use or not. [14] Vitamin D supplementation was also found to be associated
22
23 101 with a decreased load of rhinovirus (common cold), consistent with an increased antiviral immune
24
25 102 response.[22] A systematic review and several studies reported an inverse association between
26
27 103 serum vitamin D levels and COVID-19 severity, in-patient mortality, as well as serum levels of C-
28
29 104 reactive protein (CRP) and lymphocyte percentage.[23,24] These findings suggested that vitamin
30
31 105 D status was linked with the severity and mortality of the COVID-19 infection in the general
32
33 106 population, particularly in severe COVID-19 cases. Whether Vitamin D could have prevented or
34
35 107 lessened infection and/or the inflammatory response associated with the COVID-19 remained to
36
37 108 be explored.[25] At the time of funding (June 2020) and study initiation (February 2021), no other
38
39 109 primary prevention trials were published. Since then, one positive and two negative trials testing
40
41 110 different vitamin D intervention as primary prevention were published. [26-28]
42
43
44
45
46
47
48

49 112 The vitamin D receptor is expressed on innate and adaptive immune cells which also synthesize
50
51 113 the active metabolite 1,25-hydroxyvitamin D₃ (1,25(OH)₂D₃); thus, vitamin D could strengthen
52
53 114 innate and adaptive cellular immunity by increasing local production of antimicrobial peptides,
54
55
56
57
58
59
60

1
2
3 115 decreasing secretion of pro-inflammatory cytokines, inhibiting dendritic cell activation,
4
5 116 suppressing T helper cell type 1 response, and promoting T regulatory cells induction. These
6
7
8 117 cellular effects are crucial for host responses against infection and can reduce the survival and
9
10 118 replication of respiratory viruses.[13,24] 1,25(OH)₂D₃ is also produced locally in bronchial
11
12 119 epithelial cells and downregulates inflammatory cytokines (e.g. interleukin-8) and chemokines
13
14
15 120 (e.g. leucocyte attracting CXCL10) expression from stimulated cells.[29]
16
17 121

18
19 122 The protocol of a placebo-controlled parallel-group triple-blind RCT to explore the impact of
20
21 123 vitamin D₃ supplementation on reducing the risk and severity of laboratory-confirmed COVID19
22
23 124 infection in HCWs is described herein, as per Standard Protocol Items: Recommendation for
24
25 125 Intervention Trials guidelines (**Supplementary file 1**). After funding, but prior to the start of
26
27 126 recruitment, the protocol underwent four amendments (8 protocol versions) in view of the rapidly
28
29 127 evolving science, multiple challenges faced with conducting a large scale COVID-19 trial of high-
30
31 128 risk health-care workers during the pandemic, including difficulty in obtaining large-scale
32
33 129 supplies, as well as favorable pilot results of two novel technologies (**Table 1**). These original and
34
35 130 final (1.8, January 18, 2021) protocol versions are described below. The trial was initiated but
36
37 131 stopped prematurely due to recruitment difficulty.
38
39
40
41

42 132

43 44 133 **Objectives**

45
46 134 The primary research question was whether one oral dose of 100,000 IU vitamin D₃ (administered
47
48 135 at baseline) plus weekly supplement of 10,000 IU vitamin D₃ could decrease the risk of laboratory-
49
50 136 confirmed COVID-19 infection, versus placebo, in frontline HCWs in high COVID-19 incidence
51
52
53 137 areas.
54
55
56
57
58
59
60

1
2
3 138 Additionally, the study aimed to examine if, compared with placebo, the vitamin D intervention
4
5 139 reduced: (i) illness severity, (ii) symptom duration, (iii) work absenteeism, and (iv) unemployment
6
7
8 140 among frontline health care workers (HCW) in high COVID-19 incidence areas. This study was
9
10 141 to also assess various exploratory outcomes.
11

12 142

143 **Hypothesis**

14 143
15
16
17 144 We hypothesised that compared to placebo, vitamin D supplementation would decrease the
18
19 145 incidence of laboratory-confirmed symptomatic COVID-19 infection by 20% in frontline HCWs
20
21 146 working in high COVID-19 incidence area.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

147 **Table 1. Study amendments and notifications**

Version number	Clinical trial Application (CTA)				Investigational Testing Authorization (ITA)	
	Changes	Description	Submitted	Approved	Submitted	Approval
Version 0.0 11-05-2020						
Version 1.0 23-08-2020	<ul style="list-style-type: none"> Eligibility Outcomes & Covariates 	<ul style="list-style-type: none"> Strengthening of exclusion of 'suspected or previously undocumented COVID-19 infection' by adding: (i) a questionnaire of symptoms elaborated by Menni et al. and (ii) a rapid (15-minute) serology test, not yet approved nor tested in Canada, <u>to be pre-tested in a pilot study.</u> Addition of capillary blood self-collection with Tasso-SST device <u>(to be pre-tested in a pilot study).</u> 	23-08-2020	16-09-2020	N/A	N/A
Amendment 1 Version 1.1 23-10-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes Main outcome 	<ul style="list-style-type: none"> Clarification of the NADAL® COVID-19 IgG/IgM Rapid Test (Teracero Pharma Inc., Lachine Canada) on venous whole blood as the rapid serology test to exclude prior infection (<u>following pilot comparative study</u>) Validation of Nadal serology test compared to IgG SARS-CoV2 serology as endpoint exploratory outcome Salivary COVID-19 RT-PCR test method prioritized over nasopharyngeal samples for twice-monthly self-collection or accepted for clinical diagnostic by qPCR 	23-10-2020 (CTA-A) †	02-11-2020	23-10-2020 (NADAL)	14-11-2020
Amendment 2 Versions 1.2-1.4 Version 1.5 27-11-2020	<ul style="list-style-type: none"> Primary Outcome Outcomes 	<ul style="list-style-type: none"> Removal of q2 weeks saliva sample for COVID-19 rt-PCR analysis due to supply problem with 50 mL Falcon centrifuge tube caused by a global plastics shortage combined with un-acceptable delay for public tender for a contract with courier service for q2weeks biological samples Addition of questions and procedures to account for the possible effect of the Vitamin D supplementation on immune response to COVID-19 vaccine, including a saliva sampling for COVID-19 by RT-PCR and a blood test for serology (and vitamin D) prior to vaccination 	27-11-2020 (CTA-A) †	30-11-2020	23-11-2020 (TASSO & NADAL)	2-12-2020

Clinical trial Application (CTA)					Investigational Testing Authorization (ITA)	
Version number	Changes	Description	Submitted	Approved	Submitted	Approval
	<ul style="list-style-type: none"> Covariates & Outcome (Device) 	<ul style="list-style-type: none"> Specification of the TASSO SST on Demand (Tasso Inc, Seattle, USA) as choice for capillary blood self-sampling (following pilot comparative study) 				
Amendment 3 versions 1.6 & Version 1.7 12-12-2020	<ul style="list-style-type: none"> Eligibility Exploratory Outcomes 	<ul style="list-style-type: none"> Exclusion of health care workers who have received the COVID-19 vaccine prior to enrolment Addition of (i) effect of high-dose vitamin D on SARS-CoV-2 IgG titers before & after 2nd dose of COVID-19 vaccine and (ii) the long-term infection rate up to 12 months after end-of-study Modifying exploratory outcome to allow exploration of modulating effect of vitamin D, not only on the risk of COVID-19 infection but also on response to vaccine 	12-12-2020 (CTA-A) †	16-12-2020	12-12-2020 (TASSO & NADAL)	23-12-2020
Amendment 4 Version 1.8 18-01-2021	<ul style="list-style-type: none"> Exploratory Outcomes Eligibility 	<ul style="list-style-type: none"> Clarification that the serology to be done just prior the second dose of a COVID-19 vaccine may not always be at 3 or 4 weeks (as recommended by vaccine manufacturer) to reflect the recent governmental decision to delay the timing of the 2nd vaccine dose to 12 to 16 weeks Slightly modifying wording to target healthcare workers at risk of contact with infected individuals that were not suspected of being infected (e.g., patients, colleagues, students, etc.) 	18-01-2021 (CTA-N) ‡	N/A	18-01-2020 ¶	01-02-2021

† CTA-A, Clinical Trial Application -Amendment

‡ CTA-N, Clinical Trial Application -Notification

¶ As there is no ‘notification’ category for the Investigational Testing Authorization (ITA), each amendment or notification to the Clinical Trial Application (CTA) must be submitted as a new amendment for the devices to be reviewed by ITA.

153 **Methods and analysis**

154 **Study design**

155 PROTECT was a pragmatic 16-week, triple-blind, placebo-controlled, parallel-group, randomized
156 trial comparing supplemental vitamin D3 and placebo in HCWs with the possibility of extending
157 the study follow-up up to 24 weeks, depending on infection rate progression during an interim
158 analysis (**Figure 1**).

160 **Participants**

161 HCWs (i.e., physicians, allied health care workers, orderlies, etc.) were eligible if they: (i) were
162 aged ≥ 18 and < 70 years old; (ii) were authorized to practice in Quebec; (iii) were working or
163 scheduled to work over the next 16 weeks in a setting at high-risk of contact with COVID-19
164 infected individuals, particularly (but not only) those involved with aerosol generating medical
165 procedures in hospitals and/or caring for patients in long-term care facilities; (iv) were working in
166 high COVID incidence areas in the greater Montreal area and surroundings; (v) were covered by
167 the provincial universal public health insurance (*Régie de l'assurance-maladie du Québec*
168 [RAMQ] for medical services and hospitalisations; (vi) had a personal email or phone (to which
169 to send reminders and questionnaire by email or text messages); (vii) had a fixed address (to which
170 to send the material) in the greater Montreal or surrounding areas.

171 HCWs were excluded if they met any of the following criteria: vitamin D supplementation
172 (cholecalciferol or calcitriol) intake > 400 IU/day (or $> 12,000$ IU/month) in past 3 months;
173 intention to take > 400 IU per day during the study period; suspected or previously documented
174 COVID-19 infection; history of nephrolithiasis, hypercalcemia, hyperphosphatemia,
175 hyperparathyroidism, granulomatous disease (e.g., tuberculosis, sarcoidosis), renal failure, or

1
2
3 176 active cancer; current intake of medications that may cause hypercalcemia such as lithium,
4
5 177 teriparatide, or digoxin; anticipated prolonged absence from work during the study period (i.e.,
6
7 178 pregnancy); anticipated difficult follow-up; enrolment in a concurrent interventional randomized
8
9 179 trial; have already received the vaccine against COVID-19. Participation in this trial did not
10
11 180 preclude subsequent enrolment in a COVID-19 therapeutic (but not preventive) trial, which would
12
13
14 181 be documented.
15
16

17 182

19 183 **Study intervention**

20
21 184 Participants in the intervention group received 100,000 IU vitamin D₃ (cholecalciferol) at
22
23 185 randomization followed by a weekly dose of 10,000 IU vitamin D₃ for 16 weeks. Participants in
24
25 186 the control groups received an identical placebo bolus followed by placebo weekly supplement for
26
27 187 16 weeks. Sufficient supply was provided for 24 weeks, in case of prolongation study based on the
28
29 188 interim analysis. Participants in both groups were asked to take the study intervention with their
30
31 189 most copious meal. Treatment of co-morbidities were permitted. Vitamin D intake up to 400 IU
32
33 190 per day was allowed.
34
35
36
37

38 191

40 192 **Randomization**

41
42 193 Randomization was implemented using a computer-generated random list stratified by one of 11
43
44 194 workplaces (*Centre Hospitalier Universitaire* [CHU]) or health region (*Centre Intégré*
45
46 195 *Universitaire de Santé et Services Sociaux* [CIUSSS] or *Centre Intégré de Santé et Services*
47
48 196 *Sociaux* [CISSS]). HCWs were allocated (1:1) using permuted block randomization to enhance
49
50 197 concealment. Group allocation codes for each stratum was held in a secure location with restricted
51
52 198 access by the Central Pharmacy and Data Management.
53
54
55
56
57
58
59
60

199

200 Patient and public involvement

201 Participant burden of research measures was assessed using feedback from patients participating
202 in one pilot round. Patients were not involved in study design, recruitment of participants or
203 conduct of the study.

204

205 Outcomes*206 Primary outcome*

207 The original primary outcome, incidence of laboratory-confirmed COVID-19 infection, was
208 originally based on (i) bi-monthly self-obtained mid-turbinate nasopharyngeal (NP) swabs,
209 complemented by (ii) NP swabs obtained clinically for screening or diagnostic purposes
210 throughout the study, both analysed by RT-qPCR approved by Health Canada. Faced with the
211 unexpected cancellation of our large order of Falcon tubes to collect saliva sample for qPCR,
212 combined with the unacceptable additional delay for a public tender to securing a contract with a
213 private courier service, and in view of the uniform protocol for screening symptomatic or COVID-
214 19 exposed health care workers throughout the Province of Quebec and the reliability of IgG
215 serology, we decided to forgo the twice-monthly saliva sampling for qPCR analysis. The revised
216 definition of the primary outcome became the incidence of laboratory-confirmed COVID-19
217 infection, documented by RT-qPCR based on salivary (or nasopharyngeal) specimens (i) obtained
218 for screening or diagnostic purposes throughout the study and (ii) self-obtained salivary specimens
219 obtained at endpoint as well as (iii) COVID-19 IgG seroconversion at endpoint (in COVID-
220 unvaccinated individuals: ≥ 15 UA on the anti-S SARS-CoV-2 IgG Diasorin on Liaison XL

221 platform; in COVID-vaccinated individuals : ≥ 1.40 index (S/C) on the anti-N SARS-CoV-2 IgG
222 on ARCHITECT platform)

223
224 *Secondary outcomes*
225 (i) *Distribution of disease severity* on a 5-category ordinal scale [asymptomatic; mild (managed at
226 home); moderate (hospitalisation without supplemental oxygen); severe (oxygen
227 supplementation); critical (mechanical ventilation/death)], (self-reported, RAMQ); (ii) *Duration*
228 *of COVID-19 positivity* between 1st COVID+ to first COVID- test) revised to *Duration of COVID-*
229 *19 related symptoms* in individuals with laboratory confirmation of COVID infection, (self-
230 reported on diary); (iii) COVID-19 IgG seroconversion documented at endpoint (see above); (iv)
231 duration of work absenteeism (self-reported, medical records or human resources databases); (iv)
232 duration of unemployment support (human resource databases); (v) Adverse health events (self-
233 reported). Several *exploratory outcomes* pertained to the: incidence of post-acute and chronic
234 symptoms; long-term (1-year) morbidity and work absence related to COVID-19; change in gene
235 expression of ACE2 and TMPRSS2 in saliva cells; change in inflammatory markers (i.e., CRP),
236 immune response post vaccination; other viral infections; and genetic markers (including changes
237 in gene expression).

238
239 **Study procedures**
240 To facilitate the recruitment of participants, this study was conceived as hybrid trial enabling
241 partially or totally remote trial participation including screening, randomization, follow-up, and
242 end-of-study visit.

243

1
2
3 244 *Pre-screening*
4

5 245 Advertisements were placed in health institutions, newspapers, social media and online, where
6
7 246 participants were invited to complete an online pre-screening form, read and download the consent
8
9 247 forms; and if eligible and interested, to book a virtual screening appointment (via a secured
10
11 248 videoconferencing platform) with research team who would confirm eligibility, explain the study,
12
13 249 obtain informed consent, and schedule a virtual or in-person randomization visit.
14
15
16

17 250

18
19 251 *Screening*
20

21 252 At the virtual screening visit by videoconferencing, research coordinators completed with the
22
23 253 individual a more extensive eligibility questionnaire, which included additional questions about:
24
25 254 anticipated work exposure over the next 16 weeks to COVID-infected or suspected individuals
26
27 255 and to high-risk medical procedures; work place (*Centre Hospitalier Universitaire [CHU]*) or
28
29 256 *Centre Hospitalier Universitaire Sainte-Justine*) or health region (*CIUSSS* or *CISSS*), serving as
30
31 257 randomization stratum; prior laboratory-confirmed or physician-suspected COVID-19 infection;
32
33 258 assessment of the likelihood of prior/current, yet undocumented, COVID-19 infection using the 5-
34
35 259 item questionnaire developed by Menni et al[30] (score >0.50 interpreted as high likelihood of
36
37 260 prior infection); and finally, the comfort level with the study design and procedures, including
38
39 261 saliva and capillary blood sampling self-collection demonstrated by instructional videos. Eligible
40
41 262 and consenting individuals electronically signed an online consent form (with the signed PDF
42
43 263 consent form automatically emailed to participants). Then, two additional questionnaires were
44
45 264 completed on line with the research coordinators namely: (i) the baseline questionnaire collecting
46
47 265 information about household, ethnicity, part- vs. full-time work, personal health, skin color
48
49 266 (measured with the Fitzpatrick scale),[31] concomitant medications or supplements, and (ii) the
50
51
52
53
54
55
56
57
58
59
60

1
2
3 267 nominative CRF collecting demographic information essential to opening a medical and
4
5 268 pharmaceutical research record (i.e., public health insurance number, allergies) and maintaining
6
7 269 contact with the research team throughout the study (preferred means to receive electronic
8
9 270 reminders/questionnaires and to be notified of positive test results; address to receive study
10
11 271 material or for biological sample pick-up; and next-of-kin contact in case of inability to respond
12
13 272 to questionnaire due to illness), and to document work absence (employee number).
14
15
16
17 273

18
19 274 Finally, at the screening visit, the participant was asked to choose an appointment for a *virtual* (via
20
21 275 a secured videoconferencing platform) or *in-person* randomization visit at one of several locations.
22
23 276 To help select their preferred visit format, videos of key procedures (such as home blood
24
25 277 collection) were shown. Only in participants with a significant likelihood of a current or past
26
27 278 undocumented (Menni score > 0.5) was an *in-person* randomization visit mandatory to receive the
28
29 279 rapid COVID-19 serology test, prior to randomisation.
30
31
32

33 280

34
35 281 *Preparation and shipment of study drug by research pharmacy*

36
37 282 The list of new participants approved by one of the PIs was sent daily by email to the CHUM
38
39 283 research team to be open a medical chart and send an electronically signed prescription for the
40
41 284 Study medication, to the Research Pharmacy for preparation of study drug.
42
43
44

45 285

46
47 286 Prior to randomization, a list of all consenting and eligible participants was automatically sent
48
49 287 every night to the one of the co-principal investigators (FMD or CT) who reviewed screening and
50
51 288 baseline questionnaires to approve or refuse study entry and electronically signed their decision.
52
53 289 The daily list of new approved participants was sent electronically daily to the CHUM research
54
55
56
57
58
59
60

1
2
3 290 team. Medical and pharmaceutical records were opened and an electronically signed prescription
4
5 291 for the Study medication sent to the Research Pharmacy for preparation of study drug for a given
6
7
8 292 target date.

9
10 293 To enable remote randomization, the randomization took place about one week prior to the
11
12 294 randomization visit to allow enough time for the preparation and shipment of patient-specific study
13
14 295 supplement to the research team and, in turn, the shipment of the Study supplement and all
15
16
17 296 materials required for the randomization visit by the research team to the participant.

18
19 297

20
21 298 *Randomization visit*

22
23
24 299 Seventy-two and 24 hours prior to, and at, the randomisation visit, participants were screened by
25
26 300 questionnaire for recent travel, symptoms suggestive of SARS-Cov2 infection, or exposure to
27
28 301 COVID-19 infected individuals. Those who responded positively were asked to get tested, notify
29
30
31 302 their institutional health service, and await end of quarantine and/or confirmed negative test to
32
33 303 reschedule the randomisation visit.

34
35 304

36
37
38 305 Randomization visit (week 0) was performed *in person* (60 minutes) or *remotely* (90 minutes),
39
40 306 depending on the availability and preference of participants as well as their likelihood of a past
41
42 307 COVID-19 infection.

43
44
45 308

46
47 309 *In-person* visits were conducted—by appointment only—in designated rooms with restricted
48
49 310 access. The research coordinators wore personal protection equipment (PPE); all procedures, from
50
51 311 participant arrival to departure, were approved by the institutional Infection Control and Safety
52
53 312 committee. The *in-person* visit entailed (i) ascertainment of the signed consent form, (ii) capillary

1
2
3 313 blood sample collection to perform NADAL® COVID-19 IgG/IgM Rapid Test (Teracero Pharma
4
5 314 Inc., Lachine Canada), (iii) venous blood sample collection for baseline serum 25(OH)D and
6
7 315 SARS-CoV-2 IgG serology analyses and if genetic consent, DNA; (iv) viewing of the saliva
8
9 316 collection video and instruction pamphlet, (v) collection of the first specimen under supervision,
10
11 317 (vi) a final verification of the eligibility and exclusion criteria; (vii) randomization; (viii) oral
12
13 318 administration of 100,000 IU vitamin D₃ or an identical placebo, and (ix) distribution of the study
14
15 319 material including study supplement, saliva sampling kit for end-of-study, biohazard and sampling
16
17 320 bag, and, if a remote visit was anticipated at endpoint, capillary blood collection kits (TASSO
18
19 321 OnDemand SST device). Any patient with a positive NADAL COVID-19 IgM/IgG Rapid Test
20
21 322 serology test were excluded prior to randomisation.
22
23
24
25

26 323
27
28 324 The *remote* randomization visit, conducted by video-conference, was similar to the *in-person*
29
30 325 randomization visit with the following additions: (a) viewing of the capillary blood collection
31
32 326 video and instructional pamphlet; (b) remote capillary sampling under guidance using the TASSO-
33
34 327 SST device (TASSO Inc., Seattle, USA); (c) viewing of the saliva collection video and
35
36 328 instructional pamphlet (OG-600 Oragene DNA Collection Kit, DNA Genotek Inc., Ottawa,
37
38 329 Canada); (d) remote DNA salivary sampling under guidance; (e) preparation of biological samples
39
40 330 for shipment with phase change and insulated envelopes under guidance and (f) organising
41
42 331 collection of biological specimens by approved courier service to respective laboratories. Note that
43
44 332 a Nadal serology test was not conducted remotely.
45
46
47
48

49 333

50
51 334 *Follow-up*
52
53
54
55
56
57
58
59
60

1
2
3 335 Participants received *weekly electronic (SMS or email) reminders* to take their weekly Study
4
5 336 Supplement (10,000 IU vitamin D or an identical placebo) and to start completing an *online daily*
6
7 337 *diary* if they tested positive to SARS-CoV2 or they developed symptoms suggestive of COVID-
8
9 338 19 infection.

10
11
12 339 Every two weeks, participants received a link to complete a *brief online questionnaire* asking to
13
14 340 report: their adherence to weekly Study Supplement intake; health status including recent COVID-
15
16 341 19 related exposure, symptoms, or testing; adverse health events or new co-morbidities; change in
17
18 342 concomitant medications or supplement intake; work status (active duty, quarantined, holiday,
19
20 343 sick) and work setting (ED, ICU, etc.); as well as expected/recent COVID-19 vaccination (date
21
22 344 and vaccine name) if any; the latter question served to enable timely shipment of materials for
23
24 345 additional sampling prior to vaccination, as COVID-19 vaccination was permitted during the
25
26 346 study. In participants who planned to get vaccinated during the study, three additional blood, and
27
28 347 one additional saliva, samplings, either *on-site* or *remotely*, were planned including: saliva (for
29
30 348 COVID-19 qPCR analysis) and blood (for SARS-CoV-2 anti-S IgG serology) sampled prior to
31
32 349 vaccination, a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology) collected just prior
33
34 350 to the second vaccine dose, and a blood sample (for SARS-CoV-2 anti-S and anti-N IgG serology)
35
36 351 collected one month after second vaccine dose and endpoint. Regardless of their vaccination status,
37
38 352 participants were asked to continue taking the weekly Study Supplement and complete the bi-
39
40 353 monthly questionnaire until the end of the study. If questionnaires were not completed within 2
41
42 354 days of the target date, the research coordinator reached out the participant to complete the
43
44 355 information.

45
46
47 356
48
49 357 *End-of-study visit*
50
51
52
53
54
55
56
57
58
59
60

1
2
3 358 An end-of-study visit was conducted either in-person (60 minutes) or remotely (90 minutes),
4
5 359 depending on the availability and preference of participants and likelihood of a current COVID-
6
7 360 19 infection. The *in-person end-of-study visit* entailed the collection of a (i) venous blood sample
8
9 361 for serum 25(OH)D and SARS-CoV-2 anti-S IgG serological results and in vaccinated participants
10
11 362 a SARS-CoV-2 anti-N IgG serology, (ii) capillary blood sample to perform the NADAL®
12
13 363 COVID-19 IgG/IgM Rapid Test, (iii) a saliva sample for SARS-CoV-2 qPCR analysis as well as
14
15 364 guessing of allocation and return of the study supplement bottle to assess adherence and any
16
17 365 unused material.
18
19
20
21
22

23
24 367 The *remote end-of-study visit* conducted via videoconference entailed the same procedures as the
25
26 368 in-person end-of-study visit with one exception: the self-collection of a capillary (instead of
27
28 369 venous) blood using TASSO-SST devices (for the serum 25(OH)D and SARS-CoV-2 anti-S
29
30 370 with/without anti-N serology). Individuals were guided into self-performing the pinprick capillary
31
32 371 method to perform the NADAL® COVID-19 IgG/IgM Rapid Test and return of biological samples
33
34 372 and materials by pre-paid approved courier.
35
36
37

38 373 39 40 374 *Covariates*

41
42 375 Several covariates that could act as confounders or interaction variables in the magnitude of effect
43
44 376 associated with the intervention were documented, namely: baseline serum 25OHD level;
45
46 377 smoking; concomitant supplements or drug(s) that can alter calcium or vitamin D absorption or
47
48 378 metabolism such as diuretics and anti-epileptics (reported at baseline and every 2 weeks); skin
49
50 379 color (documented at baseline); obesity (documented by height & weight [BMI] at baseline); other
51
52 380 comorbidities (i.e., diabetes, hypertension, etc.) that may affect the severity of COVID-19
53
54
55
56
57
58
59

1
2
3 381 infection and receipt of a COVID-19 vaccine (documented by verbal report bi-monthly). All
4
5 382 external (governmental and institutional) databases were to be obtained 3 months before, and up
6
7 383 to 16 months following, randomization (as well as 12 months after then study endpoint).
8
9

10 384

11
12 385 *During an event*
13

14 386 During COVID-19 related symptoms or documented SARS-CoV infection, participants were
15
16 387 instructed to complete a daily symptom diary from date of onset of symptoms or positive test, until
17
18 388 two days with no symptoms or 14 days if asymptomatic,
19
20

21 389

22
23
24 390 *Risk management*
25

26 391 Clinical and biochemical adverse health events (AHEs) were monitored throughout the study and
27
28 392 reported for all patients at the end of the study. No specific laboratory safety monitoring was
29
30 393 planned given the established safety of the loading dose of 100,000 IU and weekly dose of 10,000
31
32 394 IU.[32,33] Adverse Health Events (AHE) were recorded via electronic questionnaires throughout
33
34 395 the study. Participant who reported symptoms suggestive of vitamin D intoxication had a venous
35
36 396 blood sampling (total and ionised calcium, phosphorus, alkaline phosphatase, albumin, and
37
38 397 creatinine). Any abnormal laboratory value was interpreted as ‘clinically significant’ or ‘not
39
40 398 clinically significant’ by the Site endocrinologist blinded to study allocation. Further investigation
41
42 399 or action for individual participants (including interruption, cessation, or unblinding of the study
43
44 400 drug via pharmacy or by analysis of serum 25OHD) was determined by the Site endocrinologist,
45
46 401 if indicated to ensure participant safety. The AHE’s occurrence was reviewed periodically by the
47
48 402 Data and Safety Monitoring Board (DSMB). Code breaking was allowed only if deemed essential
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 403 for participant management. If relevant, summary reports aggregating (or not if requested) both
4
5 404 groups were to be provided to the DSMB.
6
7

8 405

9
10 406 **Data management and monitoring**

11
12 407 The principal investigator (FMD) and statistical group (SG, RP) oversaw randomization, data
13
14 408 management, progress monitoring, and all analyses, including those for Data Monitoring Safety
15
16 409 Board (DMSB). The DSMB membership included: Lehana Thabane, biostatistician (Chair), Gary
17
18 410 Kobinger, infectious disease specialist, Kevin Thorpe, biostatistician, and Edgar Delvin,
19
20 411 biochemist & expert in Vitamin D. DACIMA was used for online data entry and management.
21
22
23

24 412

25
26 413 A combination of *remote* monitoring activities and *in-person* routine monitoring visits were
27
28 414 conducted by an independent Study Monitor with the first randomised participants at each site and
29
30 415 on a bi-monthly basis, to ensure that each Site adhered to the study protocol, Good Clinical Practice
31
32 416 guidelines, and data collection completeness.
33
34

35 417

36
37
38 418 **Sample size calculation**

39
40 419 Given uncertainties in infection progression, a Bayesian adaptive design was used where the
41
42 420 posterior probability of effectiveness, i.e., $P(OR < 1 | \text{data})$ was the basis of inference and decision
43
44 421 making.[34] Assuming an expected OR of 0.80 and 1:1 treatment allocation, a total net sample
45
46 422 size of 2100 was required to identify a 20% reduction in the risk of COVID-19 in the vitamin D
47
48 423 vs. control group, with 80% power with the design described above. Considering a drop-out rate
49
50 424 of 15%, 2414 participants were targeted. An interim analysis was planned when 75% of
51
52
53 425 participants would have reached week 12, at which time the following assessments were to be
54
55
56
57
58
59

1
2
3 426 made: the *progression over time in the incidence of infection* (slope of the curve of infection) was
4
5 427 to be updated and if the probability of effectiveness exceeded 0.95 [$p(\text{OR}<1)>0.95$], the trial would
6
7 428 have been terminated for efficacy at the interim point (12 weeks); otherwise, the study would have
8
9 429 continued to 16-week follow-up. Simulation results showed that, with the net sample size of 2100
10
11 430 (assuming an expected OR of 0.80 and 1:1 treatment allocation), there was about a 55% chance
12
13 431 that the trial would be terminated for efficacy at the interim analysis.[35] The overall infection rate
14
15 432 was monitored on a monthly basis: note that the study could have been extended to 24 weeks based
16
17 433 on the progress of the infection rate, if required.
18
19
20
21
22

434

435 **Statistical analysis**

436 *Primary outcome*

27
28 437 An intention-to-treat (ITT) analysis was to be carried out with all randomized participants. For the
29
30 438 primary outcome, the posterior distribution of the odds ratio of COVID-19 infection (OR) was the
31
32 439 basis of inference in interim and final analyses. The posterior distribution of the OR was to be
33
34 440 estimated by drawing samples from the posterior risks under each arm, which could be obtained
35
36 441 analytically in a Beta-binomial model. Flat prior distributions were assumed for the risks
37
38 442 (Beta(1,1)). Posterior 95% credible intervals were to be reported as interval estimates for the OR.
39
40 443 Crude analyses as well as analyses adjusted for important covariates (i.e., potential confounders,
41
42 444 effect modification, and baseline group imbalances) were to be conducted. Subgroup analyses
43
44 445 would be conducted on baseline 25OHD, age, sex, BMI, occupational risk, and COVID-19
45
46 446 vaccination. A stratified analysis on geographical infection rate would be explored; sensitivity
47
48 447 analysis censoring to date of COVID-19 vaccination, would be conducted if applicable.
49
50
51
52
53

448

1
2
3 449 *Secondary outcomes*
4

5 450 Distribution of disease severity defined as a 5-level ordinal outcome would have been examined
6
7
8 451 with a Bayesian analysis using a proportional odds (PO) model; the posterior probability of OR
9
10 452 would have been obtained by Markov chain Monte Carlo sampling implemented in Stan.[34]
11
12 453 Duration of symptoms, duration of workday absences and of unemployment would have been
13
14 454 examined by a zero-inflated Poisson distribution.
15
16

17 455
18
19 456 **Ethics and dissemination**
20

21 457 This study was reviewed and approved by the research ethics board (REB) of the CHU Sainte-
22
23 458 Justine, serving as the central REB of all participating institutions (MP-21-2021-3044). A non-
24
25 459 objection letter (NOL) from Health Canada had been obtained to use high-dose Vitamin D loading
26
27 460 dose as well as the Tasso OnDemand device for home blood sampling and the NADAL COVID-
28
29 461 19 IgM/IgG Rapid serology test. Written informed consent for study participation, for biobanking
30
31 462 specimens for ancillary studies, and for subsequent publication of results was obtained from all
32
33 463 participants, with the knowledge that participation was voluntary and could be withdrawn at any
34
35 464 time with no effect on their current/future medical care. As part of the informed consent, enrolees
36
37 465 had the option to participate in the HostSeq COVID-19 Canadian biobank conducted under the
38
39 466 supervision of CGen, a national Canadian platform for sequencing and genome analysis
40
41 467 (**Supplementary file 2**). In Canada, health care is provided to those who suffer harm from trial
42
43 468 participation.
44
45
46

47
48
49 469 All protocol amendments were submitted to Health Canada, investigators, and REB; if these
50
51 470 changes implied a revision of consent forms, ongoing trial participants were informed of new
52
53 471 modifications to provide informed consent. All information obtained during the study were and
54
55
56
57
58
59

1
2
3 472 would continue to be kept confidential as per the law. Data was collected directly by electronic
4
5 473 data capture on Dacima Clinical Suite (DACIMA Software Inc., Montreal, Canada). Data safety
6
7 474 and confidentiality was upheld at all data collection stages by assigning a unique subject ID to
8
9 475 each participant, with data and samples kept under lock and key, electronic password protection
10
11 476 and access restricted to study personnel. Samples collected during the study were labelled with the
12
13 477 unique research code, prior to transfer and storage at the CHUSJ biobank, with access restricted to
14
15 478 authorised personnel.
16
17
18

19 479 This trial used pragmatic patient (irrespective of baseline 25OHD level) and intervention to attempt
20
21 480 to maximise subsequent implementation into practice. If the intervention had been shown to be
22
23 481 effective in reducing infection and morbidity, this approach would have been readily
24
25 482 implementable and could have markedly influenced practice during the COVID-19 pandemic. No
26
27 483 participant identifiers were used in the dissemination of this research. Health care professionals
28
29 484 serving as partners were informed the study design and pre-tested all questionnaires.
30
31
32

33 485 Results are being disseminated to the medical community via national/international conferences
34
35 486 and publications in peer-reviewed journals.
36
37
38

39 487

40 41 488 **Trial status, challenges, and discussion**

42
43 489 The study was conducted as per version 1.8 (January 18, 2021). The recruitment started on
44
45 490 February 9, 2021. Upon the DSMB recommendation, recruitment was stopped prematurely on
46
47 491 March 18, 2021, after 34 participants were enrolled, due to the inability to recruit approximately
48
49 492 200 participants/week required to meet the target sample size of 2415 participants. The DSMB
50
51 493 advised that the continuation of the trial, as originally designed, would not be able to answer the
52
53 494 research question and recommended that recruitment be stopped for futility. Recruitment
54
55
56
57
58
59
60

1
2
3 495 difficulties were attributed in part to the high use of vitamin D and high concurrent vaccination rate
4
5 496 among our target population, healthcare workers, the first targeted to be vaccinated from January
6
7 497 2021 onwards. Based on the recommendations of the study's endocrinologist, a premature end of
8
9 498 follow-up after a minimum of 4 weeks from randomization was deemed sufficient to monitor the
10
11 499 safety of the intervention in all participants. The timeframe was deemed sufficient to ensure
12
13 500 participant safety while learning for the study, that is, transforming the PROTECT study into a
14
15 501 pilot study to document the impact of the Study intervention on the rise in Vitamin D serum level,
16
17 502 participants' adherence the Study intervention and procedures in the context of a hybrid study, etc.
18
19 503 The last end-of-visit was conducted on May 4, 2022.
20
21
22
23
24 504

25
26 505 Potential redirections of the study were discussed. The first option was to change the main outcome
27
28 506 for an immunogenicity study in the general adult population. However, after strong consideration
29
30 507 of the amount of changes to be made to the protocol and related documents (standards of
31
32 508 procedures, case report forms, participant' instructions and notification, etc.), the expected delay
33
34 509 in obtaining approval by all regulatory and ethical authorities, the impossible logistic of recruiting
35
36 510 participants after the same duration of exposure to the Study intervention prior to their vaccination,
37
38 511 combined with the government of Quebec announcement that all willing adults would be
39
40 512 vaccinated by June 24, 2021, the research team judged that it would unfeasible to perform a
41
42 513 scientific solid and feasible trial on immunogenicity if one could not control the timing of
43
44 514 immunization, combined with the expected very short recruitment timeframe.
45
46
47
48
49

50 515
51
52 516 A second option that received very strong consideration was to replicate the PROTECT trial in
53
54 517 children aged 9 years and over. Again, after considering changes to be made to the protocol and
55
56
57
58
59
60

1
2
3 518 related documents, the expected delay for obtaining approval by all regulatory and ethical
4
5 519 authorities including school boards, combined with the Pfizer-BioNtech announcement that their
6
7 520 vaccine was not only 100% successful for preventing COVID-19 infection in adolescents aged 12
8
9 521 to 15 years, but that they forecast vaccinating teenagers in time for the September 2021 school
10
11 522 entry, the PI judge that it was unrealistic to aim for the large recruitment target within such a short
12
13
14 523 timeframe.

15
16
17 524
18
19 525 The protocol was submitted for publication after the last patient end-of-study visit, due to the
20
21 526 incredible amount of work done to set-up and initiate this large hybrid trial. Of note, the latter
22
23 527 included two pilot studies testing two experimental devices to enable partially or totally remote
24
25 528 participation, in the context of the pandemic which also imposed large protocol and space
26
27 529 restrictions for recruiting on-site potentially COVID-19 infected health care workers, several
28
29 530 protocol amendments to facilitate and adjust the trial in the context of emerging science and
30
31 531 anticipated vaccination campaign and their impact of all electronic documents, manual of
32
33 532 procedures, and regulatory approvals, coupled with the premature end-of-follow-up in enrolled
34
35 533 participants.

36
37
38 534
39
40 535 With the gained experience and knowledge, it is crucial that a future trial must begin fast prior to
41
42 536 widespread vaccination and in populations where infection rate is high.[28] Permitting study entry
43
44 537 to individuals with prior infection and prior vaccination (given common reinfections and
45
46 538 temporary vaccine protection) [36] could have been considered, but it would have significantly
47
48 539 reduced the event rate, required prolongation beyond 24 weeks (and additional funding), and
49
50 540 compromised study power as was noted in other primary prevention trials.[26,27] Restricting
51
52
53
54
55
56
57
58
59
60

1
2
3 541 eligibility to patients with vitamin D deficiency (<25 nmol/l) would have severely interfered with
4
5 542 recruitment ability in population-based or health care workers studies. [26-28] Use of calcifediol
6
7 543 (25-hydroxy-vitamine D or (25OHD) may have been associated with more potency and rapid rise
8
9 544 in serum 25OHD than expected for cholecalciferol[37] (Vitamin D3) although the choice is
10
11 545 debated[38] and rapid access to study drug and matching placebo remain a crucial challenge at the
12
13 546 onset of a pandemic. Revisiting the intervention dose and frequency of administration in light of
14
15 547 the latest literature on SARS-CoV2 and related virus could be considered, although current
16
17 548 evidence suggest that, with similar doses, high-incidence population may be more important than
18
19 549 dosing in primary prevention [26,28] and high doses are effective in tertiary prevention.[35] Of
20
21 550 interest, we have demonstrated that the intervention significantly rose 25OHD levels well above
22
23 551 75 nmol/L, that is, in the hypothesised range for optimal pro-immune and anti-inflammatory
24
25 552 impact.[39] A pragmatic design with fewer outcomes and monitoring via administrative databases
26
27 553 appeared theoretically more efficient, but required rapid access to data when interim analyses are
28
29 554 planned to monitor event rate; any delayed in data access could raise serious challenges and
30
31 555 hamper trial decisions. Pursuing a hybrid approach to facilitate enrolment in the context of a
32
33 556 pandemic was feasible, although electronic self-screening and outcome monitoring required a lot
34
35 557 of programming that have contributed to implementation delays.
36
37
38
39
40
41
42
43

44 559 The publication of this protocol is meant to share our experience, including conducting a hybrid
45
46 560 (virtual and/or in-person) trial and lessons learned, to enable serve as template to accelerate
47
48 561 protocol writing and its improvement in the context of another epidemic/pandemic, and to serve
49
50 562 as reference for the publication of our pilot studies that enabled this trial, and lessons learned from
51
52 563 this experience. As Vitamin D supplementation has shown a benefit as tertiary prevention in severe
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

564 COVID-19 cases, with insufficient data to conclude its impact as primary and secondary
565 prevention, testing this approach remains worthy to test. [40]

566

For peer review only

567 **Contributors**

568 FMD designed the study protocol, secured funding, and oversaw the overall conduct of the project.

569 CLT contributed to the protocol and amendments, directed the study implementation at the

570 CHUM, and coordinated the prescription of study drug, pharmacy dispensation, as well as salivary

571 sample reception and interpretation. SG conceived the statistical approach and sample size

572 calculation and along with RWP, oversaw randomization and statistical analysis. CL, JHW and

573 CQ contributed to the study design and amendments. BH wrote the first manuscript draft. LGSM

574 oversaw the safety assessment. DC is responsible for the work absenteeism analysis. All co-

575 authors approved the manuscript. Authorship eligibility on resulting manuscripts will follow

576 standard guidelines.

577

578 **Competing interests**

579 The authors have no competing interests.

580

581 **Funding**

582 This study is funded by a grant awarded through a peer-reviewed process of the COVID-19 May

583 2020 Rapid Response Funding Opportunity by the Canadian Institute of Health Research, 160

584 Elgin Street, Ottawa, ON K1A 0W9, Canada (grant number #447317).

585

586 **Data availability statement**

587 After publication of the primary results, datasets used and analyzed during the current study will

588 be made available by the corresponding author on reasonable request.

1
2
3 5894
5 590 **Acknowledgements**

6
7
8 591 We acknowledge the precious collaboration of Danny Germain from Quebec Riva Laboratories
9
10 592 who agree to provide free of charge Study Preparations (vitamin D and matching placebo),
11
12 593 available in bottles of 60 tablets, allowing for study prolongation. We sincerely thank Benoit
13
14 594 Hebert of Teracero Pharma Inc, for providing free-of-charge the NADAL COVID-19 IgM/IgG
15
16 595 Rapid serology test kits. We are indebted to Martin Sauvageau for implementing and coordinating
17
18 596 the RT-qPCR analysis of saliva samples at the Montreal Clinical Research Institute, Christian
19
20 597 Renaud for coordinating the COVID-19 serology analysis, and Claude Bourassa for coordinating
21
22 598 all other blood analyses at the Sainte-Justine University Health Centre. We acknowledge the
23
24 599 precious collaboration of Raymond Loyer from EFS Solution Santé who adapted their appointment
25
26 600 software for our needs as well as John Padoba, Rabie Razgallah, and Mustapha Gharb who
27
28 601 programmed and revised the eCRF to our needs. We sincerely thank Anna Smyrnova for
29
30 602 coordinating the development of the eCRF and data management. We are indebted to Catherine
31
32 603 Lamontague from Orokom Communication Marketing who developed the communication
33
34 604 strategy and tools and oversaw the publicity campaign with Marie-Line B nard-Cyr of the CHUSJ
35
36 605 who also developed the PROTECT website and Laureanne Marceau of the CHUM. We sincerely
37
38 606 thank the members of the Data Monitoring Safety Board namely Lehana Thabane (Chair), Gary
39
40 607 Kobinger, Kevin Thorpe and Edgar Delvin.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

608 **References**

- 609 1. Zhang Y. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-
610 19) in China. *CCDC weekly* 2020;41(2):145-51.
- 611 2. Flaxman SM, Swapnil; Gandy, Alex: et al. Estimating the number of infections and the impact of non-
612 pharmaceutical interventions on COVID-19 in 11 European countries, 2020.
- 613 3. CDC COVID-19 Response Team. Characteristics of Health Care Personnel with COVID-19 — United
614 States, February 12–April 9, 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:477-81. doi:
615 <http://dx.doi.org/10.15585/mmwr.mm6915e6>
- 616 4. Ran L, Chen X, Wang Y, et al. Risk Factors of Healthcare Workers with Corona Virus Disease 2019: A
617 Retrospective Cohort Study in a Designated Hospital of Wuhan in China. *Clin Infect Dis* 2020 doi:
618 10.1093/cid/ciaa287 [published Online First: 2020/03/18]
- 619 5. Nguyen LH, Drew DA, Graham MS, et al. Risk of COVID-19 among front-line health-care workers and
620 the general community: a prospective cohort study. *The Lancet Public Health* doi: 10.1016/S2468-
621 2667(20)30164-X
- 622 6. Ilie PCS, Simina; Smith, Lee The role of Vitamin D in the prevention of Coronavirus Disease 2019 infection
623 and mortality (pre-print). *Research Square* 2020 doi: 10.21203/rs.3.rs-21211/v1 [published Online
624 First: 08 April 2020,]
- 625 7. Hughes DA, Norton R. Vitamin D and respiratory health. *Clin Exp Immunol* 2009;158(1):20-5. doi:
626 10.1111/j.1365-2249.2009.04001.x
- 627 8. Herr C, Greulich T, Koczulla RA, et al. The role of vitamin D in pulmonary disease: COPD, asthma,
628 infection, and cancer. *Respir Res* 2011;12:31. doi: 10.1186/1465-9921-12-31
- 629 9. Zosky GR, Berry LJ, Elliot JG, et al. Vitamin D deficiency causes deficits in lung function and alters lung
630 structure. *Am J Respir Crit Care Med* 2011;183(10):1336-43. doi: 10.1164/rccm.201010-1596OC
- 631 10. Hewison M. An update on vitamin D and human immunity. *Clinical Endocrinology* 2012;76(3):315-25.
632 doi: 10.1111/j.1365-2265.2011.04261.x
- 633 11. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system
634 and the clinical implications of vitamin D deficiency. *Mol Nutr Food Res* 2011;55(1):96-108. doi:
635 10.1002/mnfr.201000174 [published Online First: 2010/09/09]
- 636 12. Martineau A. Vitamin D supplementation to prevent asthma exacerbations; Authors' reply. *The Lancet*
637 *Respiratory Medicine* 2018;6(6):e26-e27. doi: 10.1016/S2213-2600(18)30199-1
- 638 13. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory
639 tract infections: systematic review and meta-analysis of individual participant data. *Bmj*
640 2017;356:i6583. doi: 10.1136/bmj.i6583 [published Online First: 2017/02/17]
- 641 14. Martineau AR, Jolliffe DA, Greenberg L, et al. Vitamin D supplementation to prevent acute respiratory
642 infections: individual participant data meta-analysis. *Health Technol Assess* 2019;23(2):1-44. doi:
643 10.3310/hta23020 [published Online First: 2019/01/25]
- 644 15. Manaseki-Holland S, Qader G, Isaq Masher M, et al. Effects of vitamin D supplementation to children
645 diagnosed with pneumonia in Kabul: a randomised controlled trial. *Trop Med Int Health*
646 2010;15(10):1148-55. doi: 10.1111/j.1365-3156.2010.02578.x
- 647 16. Manaseki-Holland S, Maroof Z, Bruce J, et al. Effect on the incidence of pneumonia of vitamin D
648 supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority
649 trial. *Lancet* 2012;379(9824):1419-27. doi: 10.1016/S0140-6736(11)61650-4
- 650 17. Jensen ME, Mailhot G, Alos N, et al. Vitamin D intervention in preschoolers with viral-induced asthma
651 (DIVA): a pilot randomised controlled trial. *Trials* 2016;17(1):353. doi: 10.1186/s13063-016-1483-
652 1

- 1
2
3 653 18. Castro M, King TS, Kunselman SJ, et al. Effect of vitamin D3 on asthma treatment failures in adults with
4 654 symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *Jama*
5 655 2014;311(20):2083-91. doi: <http://dx.doi.org/10.1001/jama.2014.5052>
6 656
7 657 19. Denlinger LC, King TS, Cardet JC, et al. Vitamin D Supplementation and the Risk of Colds in Patients
8 658 with Asthma. *Am J Respir Crit Care Med* 2016;193(6):634-41. doi: 10.1164/rccm.201506-1169OC
9 659 [published Online First: 2015/11/06]
10 659 20. Martineau AR, MacLaughlin BD, Hooper RL, et al. Double-blind randomised placebo-controlled trial of
11 660 bolus-dose vitamin D₃ supplementation in adults with asthma (ViDiAs). *Thorax*
12 661 2015;70(5):451-57. doi: 10.1136/thoraxjnl-2014-206449
13 662 21. Martineau AR, James WY, Hooper RL, et al. Vitamin D3 supplementation in patients with chronic
14 663 obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial.
15 664 *Lancet Respir Med* 2015;3(2):120-30. doi: 10.1016/s2213-2600(14)70255-3 [published Online
16 665 First: 2014/12/06]
17 666 22. Goodall EC, Granados AC, Luinstra K, et al. Vitamin D3 and gargling for the prevention of upper
18 667 respiratory tract infections: a randomized controlled trial. *BMC Infect Dis* 2014;14:273. doi:
19 668 10.1186/1471-2334-14-273 [published Online First: 2014/06/03]
20 669 23. Maghbooli Z, Sahraian MA, Ebrahimi M, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at
21 670 least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection.
22 671 *PLoS One* 2020;15(9):e0239799. doi: 10.1371/journal.pone.0239799 [published Online First:
23 672 2020/09/26]
24 673 24. Ismailova A, White JH. Vitamin D, infections and immunity. *Reviews in Endocrine and Metabolic*
25 674 *Disorders* 2021 doi: 10.1007/s11154-021-09679-5
26 675 25. Jafarzadeh A, Chauhan P, Saha B, et al. Contribution of monocytes and macrophages to the local tissue
27 676 inflammation and cytokine storm in COVID-19: Lessons from SARS and MERS, and potential
28 677 therapeutic interventions. *Life Sci* 2020;257:118102. doi: 10.1016/j.lfs.2020.118102 [published
29 678 Online First: 2020/07/21]
30 679 26. Jolliffe DA, Holt H, Greenig M, et al. Effect of a test-and-treat approach to vitamin D supplementation
31 680 on risk of all cause acute respiratory tract infection and covid-19: phase 3 randomised controlled
32 681 trial (CORONAVIT). *BMJ* 2022;378:e071230. doi: 10.1136/bmj-2022-071230
33 682 27. Brunvoll SH, Nygaard AB, Ellingjord-Dale M, et al. Prevention of covid-19 and other acute respiratory
34 683 infections with cod liver oil supplementation, a low dose vitamin D supplement: quadruple
35 684 blinded, randomised placebo controlled trial. *BMJ* 2022;378:e071245. doi: 10.1136/bmj-2022-
36 685 071245
37 686 28. Villasis-Keever MA, López-Alarcón MG, Miranda-Novales G, et al. Efficacy and Safety of Vitamin D
38 687 Supplementation to Prevent COVID-19 in Frontline Healthcare Workers. A Randomized Clinical
39 688 Trial. *Arch Med Res* 2022;53(4):423-30. doi: 10.1016/j.arcmed.2022.04.003 [published Online
40 689 First: 2022/04/30]
41 690 29. Pfeiffer PE, Hawrylowicz CM. Vitamin D and lung disease. *Thorax* 2012;67(11):1018. doi:
42 691 10.1136/thoraxjnl-2012-202139
43 692 30. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict
44 693 potential COVID-19. *Nature medicine* 2020;26(7):1037-40. doi: 10.1038/s41591-020-0916-2
45 694 31. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol*
46 695 1988;124(6):869-71.
47 696 32. Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin d supplementation in adult
48 697 populations: a systematic review. *Endocrine practice : official journal of the American College of*
49 698 *Endocrinology and the American Association of Clinical Endocrinologists* 2014;20(4):341-51. doi:
50 699 10.4158/EP13265.RA [published Online First: 2013/11/20]
51
52
53
54
55
56
57
58
59
60

- 1
2
3 700 33. Vieth R, Holick MF. Chapter 57B - The IOM—Endocrine Society Controversy on Recommended Vitamin
4 701 D Targets: In Support of the Endocrine Society Position. In: Feldman D, ed. Vitamin D (Fourth
5 702 Edition): Academic Press 2018:1091-107.
6 703
7 704 34. Harrell FL, Chris. Statistical Design and Analysis Plan for Randomized Trial of Hydroxychloroquine for
8 705 Treatment of COVID-19: ORCHID. 2020. <http://hbiostat.org/proj/covid19/bayesplan.html>.
9 706
10 707 35. Golchi S. Estimating design operating characteristics in Bayesian adaptive clinical trials. *Can J Stat*
11 708 2022;50(2):417-36. doi: 10.1002/cjs.11699 [published Online First: 2022/05/17]
12 709
13 710 36. Eythorsson E, Runolfsson HL, Ingvarsson RF, et al. Rate of SARS-CoV-2 Reinfection During an Omicron
14 711 Wave in Iceland. *JAMA Network Open* 2022;5(8):e2225320-e20. doi:
15 712 10.1001/jamanetworkopen.2022.25320
16 713
17 714 37. Pérez-Castrillón JL, Dueñas-Laita A, Brandi ML, et al. Calcifediol is superior to cholecalciferol in
18 715 improving vitamin D status in postmenopausal women: a randomized trial. *Journal of Bone and*
19 716 *Mineral Research* 2021;36(10):1967-78. doi: <https://doi.org/10.1002/jbmr.4387>
20 717
21 718 38. Sosa Henríquez M, Gómez de Tejada Romero MJ. Cholecalciferol or Calcifediol in the Management of
22 719 Vitamin D Deficiency. *Nutrients* 2020;12(6) doi: 10.3390/nu12061617 [published Online First:
23 720 2020/06/04]
24 721
25 722 39. Hosseini B, Tremblay CL, Longo C, et al. Oral vitamin D supplemental therapy to attain a desired serum
26 723 25-hydroxyvitamin D concentration in essential healthcare teams. *Trials* 2022;23(1):1019. doi:
27 724 10.1186/s13063-022-06944-z
28 725
29 726 40. Hosseini B, El Abd A, Ducharme FM. Effects of Vitamin D Supplementation on COVID-19 Related
30 727 Outcomes: A Systematic Review and Meta-Analysis. *Nutrients* 2022;14(10):2134. doi:
31 728 10.3390/nu14102134 [published Online First: 2022/05/29]
32 729
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 724 **Figure legend**
4
5 725 **Figure 1. Study outline**
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

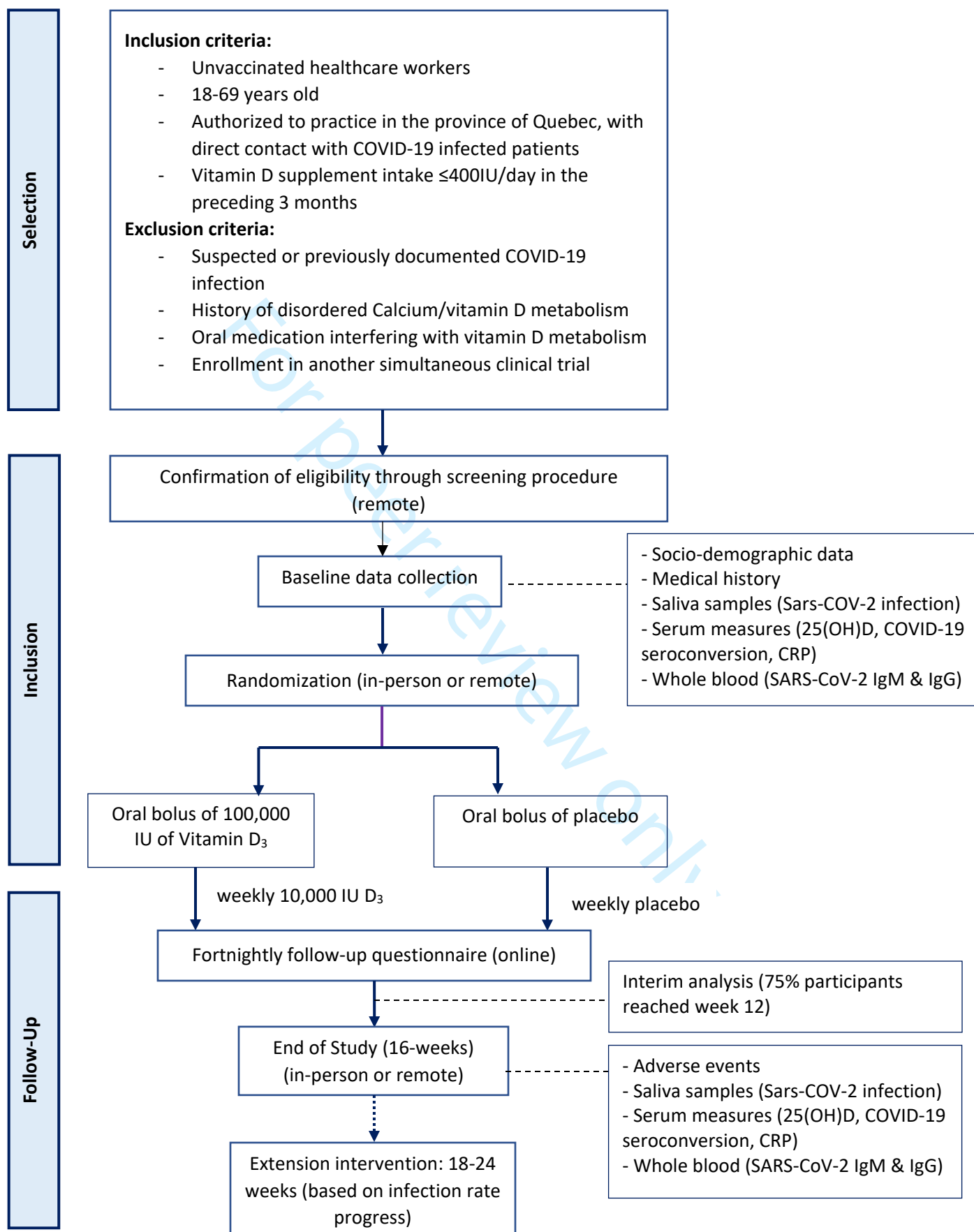


Figure 1



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	8 (line 138)
Funding	4	Sources and types of financial, material, and other support	1, 25, supplemental funding file
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 26-27
	5b	Name and contact information for the trial sponsor	1, 26
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	26

5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19, 20, 26,27
----	--	---------------

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
	6b	Explanation for choice of comparators	6, 7
Objectives	7	Specific objectives or hypotheses	8, 9
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	9, 10

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	13
---------------	---	--	----

1				
2				
3	Eligibility criteria	10	Inclusion and exclusion criteria	9, 10
4			for participants. If applicable,	
5			eligibility criteria for study	
6			centres and individuals who	
7			will perform the interventions	
8			(eg, surgeons,	
9			psychotherapists)	
10				
11				
12	Interventions	11a	Interventions for each group	10, 11
13			with sufficient detail to allow	
14			replication, including how and	
15			when they will be administered	
16				
17		11b	Criteria for discontinuing or	10
18			modifying allocated	
19			interventions for a given trial	
20			participant (eg, drug dose	
21			change in response to harms,	
22			participant request, or	
23			improving/worsening disease)	
24				
25				
26		11c	Strategies to improve	17, 18
27			adherence to intervention	
28			protocols, and any procedures	
29			for monitoring adherence (eg,	
30			drug tablet return, laboratory	
31			tests)	
32				
33				
34		11d	Relevant concomitant care	10
35			and interventions that are	
36			permitted or prohibited during	
37			the trial	
38				
39	Outcomes	12	Primary, secondary, and other	11, 12
40			outcomes, including the	
41			specific measurement variable	
42			(eg, systolic blood pressure),	
43			analysis metric (eg, change	
44			from baseline, final value, time	
45			to event), method of	
46			aggregation (eg, median,	
47			proportion), and time point for	
48			each outcome. Explanation of	
49			the clinical relevance of	
50			chosen efficacy and harm	
51			outcomes is strongly	
52			recommended	
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Participant	13	Time schedule of enrolment,	15, 16, 17, 18, Figure 1
4	timeline		interventions (including any	
5			run-ins and washouts),	
6			assessments, and visits for	
7			participants. A schematic	
8			diagram is highly	
9			recommended (see Figure)	
10				
11				
12	Sample size	14	Estimated number of	20-21
13			participants needed to achieve	
14			study objectives and how it	
15			was determined, including	
16			clinical and statistical	
17			assumptions supporting any	
18			sample size calculations	
19				
20				
21	Recruitment	15	Strategies for achieving	12,13
22			adequate participant	
23			enrolment to reach target	
24			sample size	
25				

Methods: Assignment of interventions (for controlled trials)

Allocation:

31	Sequence	16a	Method of generating the	11
32	generation		allocation sequence (eg,	
33			computer-generated random	
34			numbers), and list of any	
35			factors for stratification. To	
36			reduce predictability of a	
37			random sequence, details of	
38			any planned restriction (eg,	
39			blocking) should be provided	
40			in a separate document that is	
41			unavailable to those who enrol	
42			participants or assign	
43			interventions	
44				
45				
46				
47	Allocation	16b	Mechanism of implementing	11
48	concealment		the allocation sequence (eg,	
49	mechanism		central telephone; sequentially	
50			numbered, opaque, sealed	
51			envelopes), describing any	
52			steps to conceal the sequence	
53			until interventions are	
54			assigned	
55				
56				
57				
58				
59				
60				

1				
2				
3	Implementation	16c	Who will generate the	11
4			allocation sequence, who will	
5			enrol participants, and who will	
6			assign participants to	
7			interventions	
8				
9				
10	Blinding	17a	Who will be blinded after	5, 19
11	(masking)		assignment to interventions	
12			(eg, trial participants, care	
13			providers, outcome assessors,	
14			data analysts), and how	
15				
16		17b	If blinded, circumstances	19
17			under which unblinding is	
18			permissible, and procedure for	
19			revealing a participant's	
20			allocated intervention during	
21			the trial	
22				
23				
24	Methods: Data collection, management, and analysis			
25				
26	Data collection	18a	Plans for assessment and	12 to 19
27	methods		collection of outcome,	
28			baseline, and other trial data,	
29			including any related	
30			processes to promote data	
31			quality (eg, duplicate	
32			measurements, training of	
33			assessors) and a description	
34			of study instruments (eg,	
35			questionnaires, laboratory	
36			tests) along with their reliability	
37			and validity, if known.	
38			Reference to where data	
39			collection forms can be found,	
40			if not in the protocol	
41				
42				
43				
44		18b	Plans to promote participant	12 to 19
45			retention and complete follow-	
46			up, including list of any	
47			outcome data to be collected	
48			for participants who	
49			discontinue or deviate from	
50			intervention protocols	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3	Data	19	Plans for data entry, coding,	20
4	management		security, and storage,	
5			including any related	
6			processes to promote data	
7			quality (eg, double data entry;	
8			range checks for data values).	
9			Reference to where details of	
10			data management procedures	
11			can be found, if not in the	
12			protocol	
13				
14				
15	Statistical	20a	Statistical methods for	21, 22
16	methods		analysing primary and	
17			secondary outcomes.	
18			Reference to where other	
19			details of the statistical	
20			analysis plan can be found, if	
21			not in the protocol	
22				
23				
24		20b	Methods for any additional	22
25			analyses (eg, subgroup and	
26			adjusted analyses)	
27				
28				
29		20c	Definition of analysis	21-22
30			population relating to protocol	
31			non-adherence (eg, as	
32			randomised analysis), and any	
33			statistical methods to handle	
34			missing data (eg, multiple	
35			imputation)	
36				

Methods: Monitoring

37				
38				
39	Data monitoring	21a	Composition of data	20,
40			monitoring committee (DMC);	
41			summary of its role and	
42			reporting structure; statement	
43			of whether it is independent	
44			from the sponsor and	
45			competing interests; and	
46			reference to where further	
47			details about its charter can be	
48			found, if not in the protocol.	
49			Alternatively, an explanation of	
50			why a DMC is not needed	
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	20
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17, 19, 20, 23
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20

Ethics and dissemination

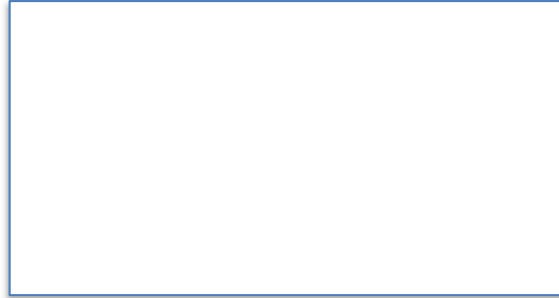
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A

1				
2				
3	A	27	How personal information	14-15, 25
4			about potential and enrolled	
5			participants will be collected,	
6			shared, and maintained in	
7			order to protect confidentiality	
8			before, during, and after the	
9			trial	
10				
11				
12	Declaration of	28	Financial and other competing	27
13	interests		interests for principal	
14			investigators for the overall	
15			trial and each study site	
16				
17	Access to data	29	Statement of who will have	27
18			access to the final trial dataset,	
19			and disclosure of contractual	
20			agreements that limit such	
21			access for investigators	
22				
23				
24	Ancillary and	30	Provisions, if any, for ancillary	25
25	post-trial care		and post-trial care, and for	
26			compensation to those who	
27			suffer harm from trial	
28			participation	
29				
30	Dissemination	31a	Plans for investigators and	23
31	policy		sponsor to communicate trial	
32			results to participants,	
33			healthcare professionals, the	
34			public, and other relevant	
35			groups (eg, via publication,	
36			reporting in results databases,	
37			or other data sharing	
38			arrangements), including any	
39			publication restrictions	
40				
41				
42				
43		31b	Authorship eligibility guidelines	25
44			and any intended use of	
45			professional writers	
46				
47		31c	Plans, if any, for granting	27
48			public access to the full	
49			protocol, participant-level	
50			dataset, and statistical code	
51				
52				

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary file 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supplementary file 2

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.



INFORMATION AND CONSENT FORM

Project Title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

Protocol Number: PROTECT 2020

- **Principal investigators at CHUSJ:** Dr. Francine M. Ducharme, MD, FRCPC, Paediatrician, Centre hospitalier universitaire Sainte-Justine (CHUSJ)
- **Principal investigator at the CHUM:** Dr. Cecile Tremblay, MD, FRCPC, Microbiologist/Infectiologist, Centre hospitalier universitaire de Montréal (CHUM)

Co-investigators:

- Decio Coviello, health economist, Hautes-Études Commerciales, University of Montreal
- Shirin Golchi, biostatistician, McGill university
- Cristina Longo, epidemiologist, University of Montreal
- Robert Platt, biostatistician, McGill University
- Caroline Quach, MD, Paediatrician microbiologist, CHUSJ
- Christian Renaud, pediatric microbiologist, CHUSJ
- John White, biochemist, McGill University

Co-investigators at the CHUM:

- Dr. Louis-Georges Sainte-Marie, MD, endocrinologist, CHUM
- Dr. Emil Toma, MD, Dsc, FRCPC, CHUM

Industrial Collaborators: Laboratoires Riva, Blainville, Quebec

Funding source: Canadian Institutes of Health Research (CIHR), in the context of the COVID-19 Rapid Research Funding Opportunity

Multicenter identifier: MP-21-2021-3044

CHUM project number : 20.319

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

Today, we are inviting you to participate in this research study because you are a healthcare worker who is working in a high COVID-19 incidence area and in a setting with a high risk of contact with COVID-19 infected cases. Please read this information to help you decide if you want to participate in this research project. It is important that you understand this information. We encourage you to ask questions. Please take all the time you need to make your decision. You may also want to discuss this study with your family doctor, a family member or a close friend.

WHY IS THIS STUDY BEING DONE?

During the current COVID-19 pandemic, many healthcare workers are working in an environment which increases their probability of contracting this viral infection. Healthcare workers are more frequently infected than the rest of the population. Infected healthcare workers can infect their family, their patients, and their contacts. In addition to being withdrawn from work, they could have transmitted the disease to other colleagues, which further impedes our ability to deliver care to the population.

Vitamin D supplementation can decrease the risk of having the common cold, but it is not known if it could have an effect on the COVID-19 infection. Vitamin D is produced in our bodies from exposure to the sun and can be obtained from supplements and certain foods. However, many Canadians do not have an adequate intake of vitamin D throughout the year.

However, studies testing supplementation with other seemingly harmless vitamins, such as beta carotene and vitamin E, have shown unexpected important adverse reactions. Therefore, it is necessary to properly assess the benefits and the potential unexpected adverse reactions in the context of a clinical study.

This study will investigate whether a high-dose vitamin D supplementation could reduce the risk and severity of COVID-19 infection and work absence in healthcare workers.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

We will be recruiting 2414 graduate healthcare workers, men and women, aged 18 to 69 years old, actively working and scheduled to continue working in a setting at high-risk of contact with people infected with COVID-19 and are working in high COVID incidence areas.

WHAT DOES THE STUDY INVOLVE?

If you agree to participate in this study, you will be assigned by chance to one of two groups. One group will receive one dose of 100,000 IU of vitamin D by mouth at the first visit and then take at home 1 pill of 10,000 IU of vitamin D once a week. The other group will receive a placebo dose at the first visit and then a placebo pill once a week. The vitamin D supplement is the active substance, meaning it could have an effect in the body. A placebo is an inactive substance, meaning it has no effect in the body. A placebo is used in clinical studies, such as this one, to ensure that observed changes are due to the active treatment and not to chance. You will have an equal chance of being assigned to each

1
2
3 group. The placebo and the vitamin D supplement look and taste exactly the same, so no
4 one will know which treatment you are given, including the people involved in the study.
5 In this informed consent form, we will use “Study supplement” to refer either to the vitamin
6 D or the placebo.
7

8 This study should last 16 weeks and involves two visits. But prior to the first visit, we will
9 conduct a screening/enrolment visit remotely by videoconferencing (or phone). If eligible
10 and consenting, there will be a randomisation visit and an end-of-study visit, the latter two
11 could be conducted in-person or remotely by videoconferencing, at your preference.
12 Because of the pandemic, we wish to reduce the need and/or length of any in-person visit
13 by doing as much as possible remotely.
14
15

16 Note that the study could finish earlier or be prolonged to 24 weeks, depending on the
17 evolution of the pandemic. We ask that you don't change your usual diet or intake of
18 vitamin supplements (if any) during the study.
19

20 **Screening/Enrolment** (pre-visit: about 45-60 minutes)

- 21 ❖ We will review the eligibility questionnaire you have completed online, complete
22 it with additional questions, explain the study in detail, and answer your questions.
- 23 ❖ If eligible and consenting, you will be asked to sign the consent form, complete a
24 few short study questionnaires and provide your contact information.
- 25 ❖ We will ask you questions about your demographics (household, ethnicity), work-
26 related activities and personal health (weight, height, skin color, smoking,
27 medication, vitamins, supplements, health problems).
- 28 ❖ To enable the creation of a medical and research pharmacy records, obtain
29 information on COVID test, and ensure optimal contact with you throughout the
30 study, we will ask personal information namely your RAMQ number, names
31 (yours, your parents, your spouse), any drug allergy, your employee number (or
32 practice number for physicians), your postal and email addresses, phone numbers
33 and that of a next of kin and your preferred means to reach you.
- 34 ❖ We could show you videos of key procedures (e.g. home blood collection) to
35 help you chose your preferred type of randomisation visit.
- 36 ❖ We will schedule the randomisation visit at a mutually convenient time and place
37 given your choice of in-person or remote visit by videoconferencing. However, in
38 case of a suspected prior COVID-19 infection, we would prefer you do an in-person
39 visit to perform a rapid screening test for COVID-19 antibodies.
40
41
42
43
44

45 **Randomisation visit (First visit: Week 0)**

46 During the visit, which will last approximately an hour,
47
48

- 49 ❖ If not already done, we will ask you to sign the consent forms, complete missing
50 study questionnaires and your contact information.
- 51 ❖ We will take a venous blood sample of about 15 mL (3 teaspoons) to measure the
52 level of vitamin D, look for COVID-19 antibodies and to do an optional genetic
53 analysis to examine a possible genetic predisposition to respond to vitamin D and
54 to severity of COVID-19 symptoms.
55
56
57
58

- ❖ We would like to obtain a small drop of blood either from the venous puncture or via a finger-prick to look for COVID-19 antibodies in your blood using NADAL® COVID-19 IgG/IgM Rapid Test: if positive, you would not be eligible for this study. This test is not yet licensed for use in Canada and its use in this study is investigational. It has been selected for use prior to enrolment because it provides antibody results in 15 minutes. The results of this investigational test will be shared with you, acknowledging the risk of false positive or negative results. They will also be subsequently compared to the approved (Liaison IgG COVID-19) serology test.
- ❖ We will show you how a video on the TASSO home blood sampling kit at home for the last visit; if interested, we will show you how to use it, identify your sample, package it, and send it back to us (see below under First Remote visit). This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling.
- ❖ We will show you how to take a saliva sample by spitting into a tube. You will receive a pamphlet with instructions and could watch a video. You should not brush your teeth, eat, drink, smoke or chew gum for 30 minutes before spitting a small volume of saliva (2 mL). If you prefer to do an oro-nasopharyngeal sample, you would need to insert a swab (a small tube with a cotton tip) into the back of your mouth, then in one of your nostrils gently rotating the swab for about 5 seconds. We will ask you to take the saliva (or oro-nasopharyngeal) sample under our guidance. We will then show you how to identify it with our prepared labels, record the date and time, package it, and sent it back for analysis for COVID-19, and possibly other viruses and cells.
- ❖ You will be asked to take ten (10) pills of the Study supplement at this first visit only in front of the research personnel (in person or by videoconference). You will take home the bottle of Study supplement and be asked to take one (1) pill once a week until the end of the study.
- ❖ We will send you by text message or email as per your preference, a first reminder with a link to a questionnaire to confirm that you have received it and are able to complete and submit the brief questionnaire. The same approach will be used every week.
- ❖ We will give you all the other study materials including the saliva collection tube pre-printed labels, biohazard bags, insulated envelopes or boxes for shipment, prepaid courier waybills, and if you are interested in a **Remote end-of-study visit**, the TASSO home blood collection kit. It is possible that we ask you to use the NADAL® COVID-19 IgG/IgM Rapid Test at the last visit for validation purposes.

For participants choosing to have a **Remote First Visit**, in whom there is a suspicion that you may have had a prior undiagnosed COVID-19 infection in the past, we would prefer that you come for an in-person visit. Alternatively, we may send you first a finger-prick test kit to look for COVID-19 antibodies in your blood; if so, we would ask that you use it on your fingertip in front of us by videoconference. If positive, you would not be eligible for this study. If negative, the Study supplement and required materials would then be sent to the participant's home prior to the randomisation visit. We will ask you to take in front of us by videoconference, the Study supplement, the saliva sample, as well as the blood test and 2nd optional saliva sample (for genetic analysis).

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
- ❖ We will show you how to take a small sample (<1 mL) of capillary blood, using a blood collection kit specifically conceived for home collection, called TASSO-SST OnDemand. This device is not yet licensed for use in Canada and its use in this study is investigational. However, we have successfully pre-tested it and have validated the concordance between test results obtained with the TASSO and venous sampling. We will ask you to watch a short video and read the brochure explaining the procedure, then ask you to use it under our guidance. Briefly, you will need to warm the skin of your upper arm by rubbing it for about 45 seconds, disinfecting it, applying the little device on your arm, pressing on a button that will puncture a very small hole in the skin, then leave the device in place for about 5 minutes while blood flows slowly in a small tube. As only a small sample of blood can be obtained, it is very likely that we ask you to repeat this with a second kit. We will show you how to remove the small tube, close it with a small cap, identify the sample with our prepared labels, record the sampling date and time, package it, and prepare it to be sent for analysis for vitamin D and COVID-19 antibodies. We will ask your feedback on this type of blood collection method.
 - ❖ If you wish to participate in the optional genetic analysis, we will ask you to collect 2 mL of saliva in another small tube (as the blood sample made by TASSO is not enough for this analysis), identify and date the sample with our prepared labels, and send it to us.

28 **Between visits**

- 29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
- ❖ For the following weeks 0 to 16 (or later, should the study be extended up to 24 weeks) participants will take every week one (1) Study pill.
 - ❖ You will receive a message via email or text message, according to your indicated preference, to remind you
 - Once a week to take the Study supplement
 - Once every two weeks to take the Study supplement, and fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose.
 - ❖ If there is no response from you within a few days of sending the electronic questionnaire, we will contact you; if there is still no response from you within 7 days of us sending the electronic questionnaire, we will contact your next-of-kin indicated by you.

50 **If you are infected during the study**

51
52
53
54
55
56
57
58

If we obtain a positive COVID result from one of your saliva (or oro-nasopharyngeal) samples, you will be notified by a Study team member, by the preferred communication means you have indicated (phone, text message or email). As all positive results will be reported to the public health authorities you will be contacted as soon as possible by them for an assessment and instructions.

If you receive a positive COVID result from a test done outside this study (i.e., for clinical reasons), we ask that you inform us immediately and to indicate it in the follow-up questionnaire.

At the reception of a positive COVID test result,

- ❖ We will ask you to complete the daily diary of symptoms (duration of 1-2 minutes)
 - Until 48 hours after resolution of symptoms
 - Or if you remain asymptomatic, for a minimum of 14 days;
- ❖ If symptoms reappear, we will ask you to restart documenting them in the daily diary of symptoms
 - Until 48 hours after resolution of symptoms;
- ❖ If your symptoms continue beyond 14 days, we will ask you to complete the weekly diary of symptoms (duration of 1-2 minutes), once a week, until resolution of symptoms
- ❖ We will ask you to continue taking your weekly supplement and completing the follow-up questionnaire once every two weeks.

In case of an imminent vaccination against COVID-19

- ❖ If you expect to receive a vaccine against COVID-19 in the next few weeks, we will ask you to notify us immediately or via the questionnaire every two weeks.
 - We will rapidly organise a visit in person or remotely, before the scheduled date of the vaccination, to obtain a saliva sample to test for COVID-19 infection and a blood sample either in your vein (9 mL) or with the TASSO device at home to look for COVID-19 antibodies and level of vitamin D prior to the vaccination.
 - Just before, and about 1 month after, your second vaccine dose against COVID-19, we will ask you for another blood sample either in your vein (4.5 mL) or with the TASSO device at home to look for COVID-19 antibodies.
- ❖ We will ask you to continue
 - Once a week to take the Study supplement
 - Once every two weeks to fill out the brief electronic questionnaire (duration of 3-5 minutes) regarding your health and work status in the previous 2 weeks.
 - At any point in time if you have symptoms, to complete the daily symptoms diary (duration of 1-2 minutes) until 48 hours after the resolution of symptoms. If you have symptoms that should prompt testing for COVID-19, as listed on the cv19quebec.ca website, we will ask you to contact your health office for this purpose, whether you have been vaccinated or not.

End-of-study – Week 16 (or later if the study is prolonged)

At the end of the study, you will be invited to a last in-person or remote visit. This research visit will take approximately 30 minutes and involve the following:

- ❖ You will be asked to return the Study supplement bottle containing the unused pills.

- ❖ A venous (or capillary blood if done remotely) sample and, if you have not tested positive at COVID-19 before, a saliva (or oro-nasopharyngeal) sample will be collected.
- ❖ A rapid COVID-19 antibody test (NADAL®) may also be done on a blood drop (finger-prick or from the venous puncture) for validation purposes. If done remotely, this test may be done on blood sampled by finger-prick under our guidance by videoconference.
- ❖ You will complete the last few short study questionnaires and any missing information in the previous ones, if applicable.
- ❖ If conducted remotely, the samples, Study supplement bottle and unused material should also be shipped to the Coordinating Center.

Collecting information on COVID-19 tests made for clinical reasons

The results for a COVID-19 test performed for clinical reasons outside this study will be documented by you in the follow-up questionnaire (*faster means to inform us*) as well as in your institution's (Pandemic) or provincial database of COVID-19 cases, namely Trajectoire Santé publique (TSP) including all individuals who tested positive and all healthcare workers who tested positive under the supervision of the Ministère de la santé et des services sociaux (MSSS). If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we have complete information on the primary outcome of the study and thus allow us to determine accurately the impact of the intervention on the risk of infection with COVID-19.

Collecting information on healthcare services

The date, diagnosis, type of professional and of health care services which you have received during medical visits and hospitalisations will be obtained from the administrative databases of the Régie de l'assurance maladie du Québec (RAMQ) and Quebec hospital discharges (MED-ECHO). This will allow us to accurately determine the impact of the intervention on the severity of COVID-19 infection and other concomitant illnesses.

Collecting information of work absence

The number of days of work absence, overall, by type (i.e., holiday, illness, etc.), and specifically due to COVID-19, including absences due to an infection acquired at work or outside of work, preventive withdrawal due to pregnancy or other health conditions, awaiting test results/investigation, or other reason for quarantine will be collected from you via the follow-up questionnaire (*faster and most detailed means*), as well as from your institution's *Direction of Health Resources* or, if you are an attending physician, from the *Direction of professional services*. If you are unable to answer the follow-up questionnaires, the information documented in these databases would ensure that we accurately ascertain the impact of the intervention on work absences.

BIOBANK

For the purposes of this study, we will keep the biological samples collected (blood, saliva and/or oro-nasopharyngeal) in a biobank as well as the clinical and administrative data collected during the course of this study in order to complete the study's objectives, and to

1
2
3 conduct research on vitamin D, COVID-19 and its treatments and other related diseases.
4 We would like to quantify specific cellular receptors which allow entry of COVID-19 into
5 cells (for example, the angiotensin converting enzyme-ACE2) and inflammatory markers
6 (such as the C-reactive protein). The collected samples will be kept in a biobank in the
7 Research Center of CHU Sainte-Justine under the supervision of Dr Francine M.
8 Ducharme. The samples will be kept as long as the research team can guarantee their proper
9 management. Confidentiality of the identity of the samples will be guaranteed by assigning
10 them a specific code. Your sample will not be identified by your name and cannot be used
11 to identify you directly. After 5 years, the code key will be destroyed, and the samples will
12 become completely anonymous. Your samples could possibly be shared with other
13 researchers in other institutions. However, the access to data will only be allowed for
14 approved projects by an independent research ethics board.
15
16
17
18

19 **GENETIC ANALYSIS (optional)**

20
21 Each person has their own set of unique genes or “genome”. Genetic research aims to
22 determine if there are genetic predispositions which make you more susceptible to a
23 COVID-19 infection, to respond to vitamin D, to modulate disease severity and the
24 interaction of these factors.
25
26

27 If you accept to participate in the genetic analysis, these analyses will be done on a small
28 part (4 mL) of the venous blood sample provided during the first visit. If you decide to
29 participate remotely, we will ask you to provide a saliva sample in a small tube.
30
31

32 We would like to sequence your entire genome and conduct gene expression analyses. We
33 would also like to share your genetic data as well as other collected clinical data during the
34 PROTECT study with the Canadian database HostSeq COVID-19 for use for COVID-19
35 related research and other aspects of human health. This biobank will serve as a centralized
36 resource in Canada for COVID-19 research and other health-related studies. The data in
37 the HostSeq database are under the supervision of CGen, a national Canadian platform
38 financed by the federal government for sequencing and genome analysis. The principal
39 investigators of the PROTECT study as well as the administrators of the HostSeq biobank
40 COVID-19 will share your genetic and clinical information with other Canadian and
41 international researchers whom are approved by CGen (the sponsor). The data could also
42 be used for commercial use. However, your data will not be shared with until after an
43 examination by a data access committee. This committee will verify that the use of the
44 proposed research is in line with the objectives of the database HostSeq and that the
45 research team which requests access has already been granted the required approval in
46 accordance in terms of research ethics requirements. Approved researchers will sign
47 agreements. These agreements will control how the data will be used. Individual results of
48 any research conducted using your samples or any individual incidental findings will not
49 be shared with you, as the research conducted on your data will have no individual
50 diagnostic or therapeutic significance to you.
51
52
53
54
55
56

57 **WHAT ARE THE BENEFITS AND RISKS OF THIS STUDY?**

Benefits:

You may not benefit directly from the study intervention if it is not efficacious or if you have been assigned in the placebo group. However, the screening may identify earlier an active or past COVID-19 infection that was not apparent. Your participation will help advance our knowledge on vitamin D and on the prevention of COVID-19 infection in healthcare workers and other individuals at risk of infection.

Each positive COVID-19 result from the saliva (or oro-nasopharyngeal) or blood sample will be shared with you according to your preferred way of communication: telephone, text message or email. All positive saliva (or oro-nasopharyngeal) results will also be shared by the Microbiology Laboratory of CHUM with the Public health authorities and will be added into your file at the CHUM and Dossier Santé Québec. No other research result will be provided to you. Research findings resulting from your participation to this study could potentially contribute to creating commercial products from which you would not be able to claim any financial benefit.

Risks:**• Related to study medication:**

The vitamin D dose used in this study has been shown to be safe in adults. This dose is approved by Health Canada for the purpose of this study only, but not for clinical use yet. It is unlikely that you will have any side effects because of the amount of vitamin D used in this study as when combining the first and weekly doses, the total remains below the maximum amount allowed.

However, we will ask you to notify us immediately if you have any of the following, as they could be signs of an acute excess intake of vitamin D: mainly, a marked increase in thirst or an increase in the volume and frequency of urination (with or without fatigue, loss of appetite, nausea or vomiting, headaches, drowsiness, cardiac arrhythmias, constipation, muscle or bone or chest pain, mouth dryness or a metallic taste).

Later signs and symptoms that may indicate a chronic excess intake of vitamin D are: a marked increase in thirst, an increase in the volume and frequency of urination including during the night, loss of appetite, weight loss, red eye or conjunctivitis, inflammation of the pancreas, light sensitivity, runny nose, itching, fever, reduced libido, kidney stones, increased concentration of some analytes in the blood (BUN, AST, ALT, cholesterol), or in urine (albumin), ectopic calcification, hypertension, cardiac arrhythmias and rarely, a psychosis.

It's possible that other currently unknown risks are associated with Vitamin D intake.

One of the reasons we collect a blood sample is to measure the concentration of vitamin D in the blood at the start and end of the study. this will allow us to see if the vitamin D blood level is linked to the number and severity of COVID-19 confirmed cases.

• Related to study procedures:

1
2
3 The salivary collection sample is painless. If done, an oro-nasopharyngeal swab may cause
4 slight discomfort during collection that will subside after its removal. The side effects of
5 having blood collected by venous puncture or TASSO can include bleeding, bruising,
6 discomfort and pain at the sample site. It is possible that the NADAL COVID-19 IgG/IgM
7 Test may give false positive or false negative results. In case of divergence of results, we
8 will communicate to you the results of the approved IgG test when available.
9
10

11
12
13 **• Related to confidentiality:**

14 There is always a small risk that your data could one day be re-identified. The genetic
15 information is unique to each person, just as your fingerprint. This means that theoretically,
16 you could be identified using your genetic code; however, this is not easy to do.
17 Considering the advances in technology, there could be new ways to link you to data that
18 we have not foreseen today, despite the strict confidentiality measures in place. Possible
19 re-identification or unintentional disclosure of your genetic and clinical research data could
20 lead to a loss in confidentiality and a possible future discrimination against yourself or your
21 biological parents. But all security measures will be put in place to protect your privacy.
22
23

24 **WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?**

25
26 The Study supplements are provided free of charge by the manufacturer, Laboratoire
27 RIVA.
28

29
30 **WHAT ARE THE OTHER FINANCIAL ASPECTS?**

31 For each completed visit (0 and 16 weeks), you will receive a \$25 check by mail to
32 compensate for your time. The check may arrive at your home between 4 and 8 weeks after
33 the visit.
34
35

36 **HOW IS PRIVACY INSURED?**

37
38 During your participation in this research study, the investigators responsible for this study
39 as well as the members of their research team will collect, in a research file, the required
40 personal information to answer the scientific objectives of this research project.
41

42 These information could include your demographic data (name, sex, date of birth, ethnic
43 origin, weight and height), your past and present health status, your health-related habits,
44 medication you take, your work absences, and the results of all tests, exams, and procedures
45 which you will participate in. Your personal file will include your address, email, telephone
46 numbers, RAMQ number, and employee or practice number be kept in a separate file with
47 restricted access; this information is required to create a medical and pharmacy file at the
48 CHUM and for communication purposes during the study.
49

50
51 The coded blood, saliva (and/or oro-nasopharyngeal) samples will be sent to the biobank
52 located at the Research Center of CHU Sainte-Justine under the supervision of Dr Francine
53 M. Ducharme. The coded results of completed analyses will be kept on a protected server
54 with restricted access at DACIMA company during the study, and thereby transferred to a
55 secure server with restricted access in the Research Center of CHU Sainte-Justine under
56 the supervision of Dr Francine M. Ducharme. During the study, the personal information
57
58

1
2
3 used to arrange virtual and in-person study visit appointments will be kept on a protected
4 server with restricted access at the company providing the appointment-making software.
5 Following the conclusion of the study, this information of yours will be transferred to
6 a secure server with restricted access in the Research Center of CHU Sainte-Justine under
7 the supervision of Dr Francine M. Ducharme. The database of HostSeq will be kept on
8 secure cloud servers (online) that are based in Canada and will be indefinitely kept or until
9 they are not useful for research.
10

11
12 To ensure your privacy, a copy of the consent form as well as the results to the diagnostic
13 tests required for conducting the research project, will be copied in the research and
14 medical file of the CHUM. Therefore, each person or company which you authorize to
15 consult your medical file, will have access to this information.
16

17 The research data will be kept for at least 25 years by the principle investigator. The data
18 collected could be published or discussed during scientific meetings, but it would not be
19 possible to identify you.
20

21 All collected information will remain confidential within the limits provided by law. You
22 will only be identified by a code number. The key to the code linking your name to your
23 research file will be kept by the investigator responsible for this research project.
24

25
26 To ensure your safety, a copy of the consent form as well as the results of the diagnostic
27 tests required for research purposes will be placed in the research file and the medical file
28 of the CHUM. Consequently, any person or company to whom you give access to your
29 medical file will have access to this information.
30

31 Research data will be kept for at least 25 years by the investigator responsible for this
32 research project. Research data may be published or be the subject of scientific discussion,
33 but it will not be possible to identify you.
34
35

36
37 For the purposes of surveillance, control, safety and marketing of the Study drug, your
38 research as well as your medical files could be consulted by a person mandated by a
39 regulatory organization, in Canada or elsewhere, such as Health Canada, as well as sponsor
40 representatives of the company manufacturing the vitamin D pills for this project
41 (Laboratoire RIVA), the institution or research ethics committee. These people and
42 organizations adhere to a strict confidentiality agreement.
43

44 You have the right to consult your research file to verify the collected data and to correct
45 them, if needed. Moreover, access to certain information before the end of the study could
46 mean your removal from this study in order to maintain the study's integrity.
47
48
49

50 **IS YOUR PARTICIPATION VOLUNTARY?**

51
52 Yes. Taking part in this study is voluntary. You may choose not to be in this study. You
53 can decide to stop being in the study at any time, without needing to provide any reason,
54 but simply informing the research team.
55
56
57
58

1
2
3 Your decision to refuse participation or to stop participating in the study at a later time,
4 will have no effect on the quality of care or services to which you are entitled or on your
5 relationship with the people that provide them.
6

7
8 The principal investigators of this study, the research ethics board, the funding agency or
9 the sponsor could decide to end your participation in the study without your consent. This
10 could happen if there are new information or findings that indicate your participation is no
11 longer in the best of your interests, or if you have not been following the study instructions
12 as explained, or if there are other administrative-related reasons to stop the project.
13

14 If you stop participating in the study or if you have been removed from it, the collected
15 information and material already received will be kept (as well as the data pertaining to
16 healthcare services and work absences will continue to be collected) and analysed to ensure
17 the validity of this project, unless you specifically ask for them to be destroyed. If this is
18 the case, these data and/or material will be removed from the biobank provided that the
19 code key (linking between nominal data and the study code) is still available, that is, up to
20 5 years after the end of the study.
21
22

23 If you decide to drop out of the HostSeq database, your data will no longer be shared, and
24 no new data will be collected. The data already in the HostSeq database will be destroyed
25 once informed about this decision. However, it could be impossible to remove the results
26 once they have been compiled with the results of other participants or if they have been
27 published. Moreover, if certain data have been shared with other researchers, it could be
28 possible not to be able to remove this part of the data. In such a case of unsuccessful
29 withdrawal from the study, your identity will always be protected.
30
31

32 All new information acquired during the course of the study which could have an impact
33 on your decision to continue participation will be shared with you rapidly, which is the
34 reason why we would like to keep your personal information and have your approval to
35 communicate with you after the end of the study (optional).
36
37
38
39
40
41
42
43
44

45 **WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**

46
47 If you have any questions about the research project or if you have any problems that you
48 believe are related to your participation in the project, you can call the researchers
49 responsible for the project:
50

- 51 • Dr. Francine M. Ducharme at 514 345 4931, extension 4398
 - 52 • Dr. Cecile Tremblay at 514 890-8000, extension 14645
- 53
54
55
56
57
58

1
2
3 If you would like information about your rights related to your participation in the research,
4 you may contact the Ombudsman - complaints and quality services of the CHU Sainte-
5 Justine at 514 345-4749, of the CHUM at 514 890-8484 or your CIUSSS/CIUSSS:
6
7

- 8 • CIUSSS de l'Est-de-l'Île-de-Montréal : 514 252-3510
- 9 • CIUSSS de l'Ouest-de-l'Île-de-Montréal : 514-989-1885, extension: 1010
- 10 • CIUSSS du Centre-Sud-de-l'Île-de-Montréal : 514 593-3600
- 11 • CISSS de la Montérégie-Est : 450-468-8447
- 12 • CISSS de la Montérégie-Centre : 450-466-5434

13 14 15 **RESEARCH ETHICS COMMITTEE**

16 The Research Ethics Board of CHU Sainte-Justine has approved this study and will
17 continue to monitor it for all participating institutions of the Quebec Health and Social
18 Services network.
19

20 21 22 **LIABILITY**

23
24 This research is not funded by a private industry. In case of side effects resulting from the
25 study medication or from procedures required for this research project, you will receive all
26 necessary medical care covered by the Quebec's provincial health insurance plan (RAMQ)
27 or by your private drug insurance plan. You will be responsible for paying the portion of
28 any costs not covered.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CONSENT FORM

Research project title: PRevention of COVID-19 with Oral Vitamin D supplemental Therapy in Essential healthCare Teams (PROTECT)

The nature and procedures of this research project were explained to me. I have read the information and consent forms and I kept a copy, or a copy has been provided to me. I was able to ask my questions and they were answered to my satisfaction. After consideration, I agree to participate in this research project.

I authorize the research team to consult the collected data about me in the COVID infection database (Pandemic) of my institution and/or the provincial TSP database, the medical and hospitalisation services database (RAMQ and MED-ECHO), and the workplace absenteeism database (Human Resources Directorate or Professional Services Directorate) to obtain information that is pertinent to this project.

By agreeing to participate in this study, you are not waiving any of my rights under the law. You are not releasing the investigators from their legal and professional liability.

Name of participant (Print)

Signature

Date

1. I consent to the analysis of gene expression and the sequencing of the whole genome of my coded biological material (blood, saliva, and/or oro-nasopharyngeal). The whole genome sequence could be hosted in the Canadian HostSeq COVID-19 biobank and linked to a database containing the viral genome. This would serve to explore any genetic predisposition to COVID-19, the severity of the disease and response to vaccine.

Yes _____ (Initials)

No _____ (Initials)

2. I consent to prolonging the access to my coded data on healthcare use, COVID-19 infections and work absenteeism for 12 months following the study end date, to explore the long-term impact of COVID-19 infection and vaccination.

Yes _____ (Initials)

No _____ (Initials)

3. I consent to being contacted to update my personal information, obtain additional information about my health or to be invited to participate in new research.

Yes _____ (Initials)

No _____ (Initials)

4. In case I receive a vaccine against COVID-19 during the study, I agree to do the blood samples before the first and second vaccine dose as well as 1 month after the 2nd vaccine dose, even if these samples were to be done after the end-of-study's visit planned at week 16 (or 24).

Yes _____ (Initials)

No _____ (Initials)

Participant's signature: _____

1
2 I have explained the research study and the terms of this information and consent form to the research
3 participant, and I answered all his/her questions. I explained that participation in a research project is
4 free and voluntary and could be stopped at any time they choose.
5
6
7

8
9

Name of person obtaining consent (Print) Signature Date

10
11
12
13 **(FOR THE CHUM PARTICIPANTS ONLY)**

14
15 **COMMITMENT OF THE PRINCIPAL INVESTIGATOR AT THE CHUM**

16
17 I certify that this information and consent form was explained to the research participant, and that the
18 questions the participant had were answered.
19

20
21 I undertake, together with the research team, to respect what was agreed upon in the information and
22 consent form, and to give a signed and dated copy of this form to the research participant.}
23
24
25
26
27

28

Name (Print) Signature of the principal
29 investigator at the CHUM Date
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58