PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prevalence of asymptomatic SARS-CoV-2 positive individuals in the general population of northern Italy and evaluation of a diagnostic serological ELISA test: a cross sectional study protocol.
AUTHORS	Guerriero, Massimo; Bisoffi, Zeno; Poli, Albino; Micheletto, Claudio; Pomari, Carlo

VERSION 1 – REVIEW

REVIEWER	Matthew Krasowski University of Iowa Hospitals, USA
REVIEW RETURNED	19-Jun-2020

GENERAL COMMENTS	This study proposes to analyze the prevalence of asymptomatic SARS-CoV-2 positive individuals in the population of Verona in northern Italy. This is of interest as Italy has had one of the higher disease burdens in the world. The study is also evaluating a diagnostic ELISA test for SARS-CoV-2 antibodies. The strengths of the study are to capture data on vital parameters and clinical history related to COVID-19 symptoms and combine that with SARS-CoV-2 RT-PCR and serology testing. The main limitation is that a number of studies have been published recently on prevalence of SARS-CoV-2 antibodies, including some studies that have compared multiple SARS-CoV-2 serology tests. These type of studies have been especially facilitated by the availability of high-throughput serology assays from the major diagnostic vendors such as Abbott Diagnostics and Roche Diagnostics. It is likely that more of these type of studies will be published in the near future. The following are my specific critiques/suggestions: (1) The authors should review the more recent literature on prevalence and serology studies for SARS-CoV-2. This is a very fast-moving area, and the authors should verify that the proposed study will be novel. It may be given the serious impact of COVID-19 on Italy, but it is a limitation to consider. (2) One concern with serology assays is false positives. What is the specificity of the ELISA assay used? The authors should discuss the challenge of false positives. Is there a possibility to test positive samples by a second serology method targeting another protein of SARS-CoV-2? Of note, a number of more recent studies incorporating serology have used multiple assays to better assess potential for false positives and false negatives. (3) The clinical value of measuring IgA antibodies specifically for SARS-CoV-2 is controversial. Many of the assays that are now marketed are either total antibodies (e.g., Roche) or IgG only. (4) Check reference citations throughout. Some of the citations appear to be off. An example is that reference 13 (a general article on the STARD approach) is used of p.5 of the protocol to reference study of IgA antibodies.

REVIEWER	Florian Krammer Icahn School of Medicine at Mount Sinai, USA
REVIEW RETURNED	01-Jul-2020

GENERAL COMMENTS	In this manuscript Guerriero and colleagues present a study protocol for serology testing of asymptomatic SARS-CoV-2 cases in Verona. There are several points that need the author's attention. Major points 1) So, the authors don't indicate which test they will use. They still need to evaluate tests, which is part of the protocol. In order to get specificity and sensitivity for asymptomatic cases, the initial performance testing needs to be done on asymptomatic, confirmed cases. How will this be done efficiently? 2) What is the current case number in Verona? What proportion of the population was 'officially' infected? I guess stating that and including those numbers would be important. 3) Samples will be taken – if possible – within the car a person arrives. I have a hard time imagining how this would work out with blood samples. 4) Samples are collected and stored at 4C until their transfer within 24 hours. And then they will be stored until they are process. So, how long will it take until serum/plasma is processed and safely frozen down? Minor points 1) Page 1, line 52: Define 'SARS-CoV-2'. 2) Page 2, line 54-55: Please update the numbers. 3) Page 3, line 15-16: This sentence is weird and needs to be rephrased. 4) Page 3, line 20: Please define 'some' 5) Page 5, line 45: Should be 'SARS-CoV-2', not 'SARS-Cov-2'

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Matthew Krasowski
Institution and Country: University of Iowa Hospitals, USA
Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below
This study proposes to analyze the prevalence of asymptomatic SARS-CoV-2 positive individuals in the population of Verona in northern Italy. This is of interest as Italy has had one of the higher disease burdens in the world. The study is also evaluating a diagnostic ELISA test for SARS-CoV-2 antibodies. The strengths of the study are to capture data on vital parameters and clinical history

related to COVID-19 symptoms and combine that with SARS-CoV-2 RT-PCR and serology testing. The main limitation is that a number of studies have been published recently on prevalence of SARS-CoV-2 antibodies, including some studies that have compared multiple SARS-CoV-2 serology tests. These type of studies have been especially facilitated by the availability of high-throughput serology assays from the major diagnostic vendors such as Abbott Diagnostics and Roche Diagnostics. It is likely that more of these type of studies will be published in the near future.
The following are my specific critiques/suggestions:
(1) The authors should review the more recent literature on prevalence and serology studies for SARS-CoV-2. This is a very fast-moving area, and the authors should verify that the proposed study will be novel. It may be given the serious impact of COVID-19 on Italy, but it is a limitation to consider.
R. We thank the Reviewer for considering our protocol of potential interest. We appreciate that this will not be the first study of SARS-CoV2 prevalence based on a random sample of the general population. However, we would like him to consider that over 3 months ago, when this paper was submitted, there was no such study published (only a few on selected populations). Anyway, at the moment and at our knowledge, no such study has yet been published from Italy. We think that the detailed study protocol, including all the practical and logistical procedures, can be useful to those planning similar studies on this virus epidemiology.
(2) One concern with serology assays is false positives. What is the specificity of the ELISA assay used? The authors should discuss the challenge of false positives. Is there a possibility to test positive samples by a second serology method targeting another protein of SARS-CoV-2? Of note, a number of more recent studies incorporating serology have used multiple assays to better assess potential for false positives and false negatives.
R. The Reviewer is right, and we have added to the paper the reference to the test methods used and currently available literature data on their sensitivity and specificity.
(3) The clinical value of measuring IgA antibodies specifically for SARS-CoV-2 is controversial. Many of the assays that are now marketed are either total antibodies (e.g., Roche) or IgG only.
R. Again, the Reviewer is right, and the interpretation of IgA results will have to be taken with caution and in light of the other test results.

(4) Check reference citations throughout. Some of the citations appear to be off. An example is that reference 13 (a general article on the STARD approach) is used of p.5 of the protocol to reference study of IgA antibodies.
R. Thank you very much for pointing out, the reference list has been carefully revised and amended.

Reviewer: 2
Reviewer Name: Florian Krammer
Institution and Country: Icahn School of Medicine at Mount Sinai, USA
Please state any competing interests or state ‘None declared’: None.

Please leave your comments for the authors below
In this manuscript Guerriero and colleagues present a study protocol for serology testing of asymptomatic SARS-CoV-2 cases in Verona. There are several points that need the author’s attention.

Major points

1) So, the authors don’t indicate which test they will use. They still need to evaluate tests, which is part of the protocol. In order to get specificity and sensitivity for asymptomatic cases, the initial performance testing needs to be done on asymptomatic, confirmed cases. How will this be done efficiently?
R. The test evaluation is a complement of the population prevalence study. As the Reviewer correctly states, there is no gold standard, so the evaluation will be based on probabilistic techniques ((Latent

Class Analysis), with models based on combination of the actual test results with those of clinical co-variates. We explain this better in the revised version.

2) What is the current case number in Verona? What proportion of the population was 'officially' infected? I guess stating that and including those numbers would be important.
R. Thank you, we have added the figures available at the time of the actual field study.

3) Samples will be taken – if possible – within the car a person arrives. I have a hard time imagining how this would work out with blood samples.
R. Absolutely right. This only concerns the swabs. We explain this better in the current version.

4) Samples are collected and stored at 4C until their transfer within 24 hours. And then they will be stored until they are process. So, how long will it take until serum/plasma is processed and safely frozen down?

R. This detail has also been added to the text.

Minor points

1) Page 1, line 52: Define 'SARS-CoV-2'.
R. Done

2) Page 2, line 54-55: Please update the numbers.
R. We think that the figures should remain those available at the time we wrote and submitted the study protocol.

3) Page 3, line 15-16: This sentence is weird and needs to be rephrased.
R. We preferred to delete the sentence as in fact out of context

4) Page 3, line 20: Please define 'some'
R. Done

5) Page 5, line 45: Should be 'SARS-CoV-2', not 'SARS-Cov-2'
R. Done, thank you

VERSION 2 – REVIEW

REVIEWER	Matthew D. Krasowski University of Iowa Hospitals and Clinics Iowa City, IA USA
REVIEW RETURNED	17-Aug-2020

GENERAL COMMENTS	The authors have revised the manuscript and adequately addressed my crit.