Supplementary Material*

Caulley L, Corsten M, Eapen L, et al. Salivary detection of COVID-19. Ann Intern Med. 2020. [Epub ahead of print]. doi:10.7326/M20-4738

Supplement. **Supplemental Methods**

* This supplementary material was provided by the authors to give readers further details on their article. The material was reviewed but not copyedited.

Supplemental Methods

1. Screening Criteria at an official COVID-19 Assessment Center

Reported symptoms of COVID-19 warranting testing at an official COVID-19 Assessment Center

- Common symptoms such as fever, cough, or difficulty breathing.
- Less common symptoms such as unexplained fatigue, delirium (a serious medical condition that involves confusion, changes to memory, and odd behaviors), falls, acute functional decline, worsening of chronic conditions, nausea, vomiting, diarrhea, abdominal pain, chills, headaches, croup, or loss of taste/smell.
- New or worsening respiratory symptoms such as: sore throat, runny nose, sneezing, nasal congestion, hoarse voice, or difficulty swallowing

Asymptomatic individuals deemed high-risk of exposure warranting testing at an official COVID-19 Assessment Center:

- Healthcare workers or staff who work in health care facilities and members of their households
- Residents and staff in long-term care homes, retirement homes, correctional facilities, homeless shelters, mental health institution, hospice, and other congregate living settings
- Hospitalized individuals and those who likely will be hospitalized
- Members of remote, isolated, rural, and/or indigenous communities
- Caregivers and care providers
- First responders such as firefighters, police, and paramedics
- Individuals with frequent healthcare contact such as patients with cancer or undergoing chemotherapy, dialysis therapy, pre- or post-transplant, pregnancy, and newborns
- Returning international travellers who seek medical attention
- Critical infrastructure workers – this includes grocery stores, food services, maintenance and transportation workers, and utilities.
- Close contacts of confirmed or probable cases

2. Study Methods

Study Population

From April 16 through May 19, 2020, sample collection was conducted at one COVID-19 Assessment Center in Ontario, Canada. Standardized testing, in the form of a nasopharyngeal or oropharyngeal swab, was performed in individuals that qualified for testing based on public health recommendations. We enrolled consecutive adults (over 18 years of age) for saliva testing who were asymptomatic high-risk persons or who demonstrated mild symptoms suggestive of COVID-19, and were able to provide a saliva sample of at least 1 mL. Demographic data, including patient age and gender was collected for descriptive statistics. All participants provided informed consent. Participants were provided with a printed description of the study. Study participation did not alter the standard of care. Only the results from the standard swab were made available to participants.

Laboratory Methods

Standard swabs and saliva samples were collected from participants meeting the study eligibility criteria during a single visit to the testing center. Standardized tests were collected by a certified healthcare provider. Standard swabs were transported in universal transport medium to ensure RNA stability until testing was performed. Use of nasopharyngeal or oropharyngeal swabs was based on resource availability at the testing center. Swab collection and testing was completed as per the government of Ontario protocol and no member of the research team was involved.

Saliva samples of at least 1 mL were collected using OMNIgene•ORAL, OM-505 by DNA Genotek, Inc. according to standard technique. OMNIgene•ORAL, OM-505 kits are designed for saliva self-collection without expert assistance and can be stored at room temperature with no degradation of viral material over 21 days (1,2). Participants were instructed how to collect saliva samples and

were guided through each step to ensure samples were collected using clean techniques. Specifically, participants were instructed how to open the cap on the test kit, expectorate saliva into the tube, and close the cap. A preservative/viricidal fluid mixture was automatically released into the sealed saliva sample and mixed vigorously with the sample such that RNA stability was maintained until testing was performed. Each saliva sample was coded with a patient-specific study number and stored at the Ottawa Hospital Research Institute (Ottawa, Canada). The individuals collecting the standard swabs and saliva samples were blinded to the test results.

Testing was split between two laboratories to accommodate the demand for testing resources within the pandemic context. Standard swabs were analyzed at the Eastern Ontario Regional Laboratory (Ottawa, Canada). Batched saliva samples were sent to the National Microbiology Laboratory (Winnipeg, Canada) for analysis. Both participating laboratories are reference laboratories that follow quality assurance guidelines. Bias was minimized through blinding, since technicians were not aware of the results of the complementary test.

The collected saliva specimens were subjected to total nucleic acid (TNA) extraction and real-time reverse transcription–polymerase chain reaction (RT-PCR). Swab samples were tested using the Allplex 2019-nCoV assay (Seegene) to detect the presence of nucleoprotein (N), envelope (E) and ribonucleic acid (RNA)-dependent RNA polymerase (RdRp) gene targets of SARS-CoV-2. Samples were extracted using the STARMag Universal cartridge kit (Seegene) on a Nimbus (Seegene) or Starlet (Seegene) extractor. RNA amplification was performed on the CFX96 thermal cycler (Biorad). Any SARS-CoV-2 target gene with a positive cycle threshold value in standard swabs was reported as positive for SARS-CoV-2. Saliva samples were tested using an RT-PCR assay targeting the E gene only and classified as positive based on the cycle threshold value, as previously described (3). The E gene screening assay is a widely accepted and highly sensitive target gene for SARS-CoV-2 (4). Saliva samples with an E assay cycle threshold value less than 37, were repeated

and followed by confirmation with an assay to detect RdRp gene according to the National Microbiology Laboratory of Canada testing guidelines.

Since the performance of multi-target and single-target PCR assays adds some complexity and testing methods differed across laboratories, a direct comparison was performed of the target E gene with a cycle threshold value less than 37 in the saliva and standard swab samples.

Study Design, Equipment and Oversight

The study was reviewed and approved by the Ottawa Hospital Research Ethics Board (CRRF ID: 2102) as well as the Health Canada-Public Health Agency of Canada REB (2020-005P). The study was supported by The Ottawa Hospital Academic Medical Organization COVID-19 Innovation Project. The granting agency was not involved in the design, conduct, or analysis of this study, or in the decision to submit the manuscript for publication. OMNIgene•ORAL, OM-505, saliva collection kits were donated by DNA Genotek, Inc. DNA Genotek, Inc was not involved in the design, conduct, or analysis of this study, or in the decision to submit the manuscript for publication.

3. Statistical Analysis

Continuous variables were displayed as median and interquartile range and categorical variables were reported as counts and percentages. The proportion of positive cases in the population was defined as any positive RT-PCR results from either standard swab or saliva sample. That is, a positive saliva test was assumed to be a true positive that was missed by swab testing. Data was analyzed using R Studio version 1.2.5042 (R Studio Team, Boston, USA).

References

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