Evaluation of six commercial mid to high volume antibody and six point of care lateral flow assays for detection of SARS-CoV-2 antibodies

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Abstract

Background: COVID serological tests are essential to determine the overall seroprevalence of a population, and to facilitate exposure estimates within that population.

Methods: We performed a head-to-head assessment of enzyme immunoassays (EIA) and point of care lateral flow assays (POCT) to detect SARS-CoV-2 antibodies. Demographics, symptoms, co-morbidities, treatment, and mortality of patients whose sera was used were also reviewed.

Results: Six EIAs (Abbott, Affinity, BioRad, DiaSorin, Euroimmun, and Roche), and six POCTs (BTNX, Biolidics, Deep Blue, Genrui, Getein BioTech, and Innovita) were evaluated for the detection of SARS-CoV-2 antibodies in known COVID-19 infected individuals. Sensitivity of EIAs ranged from 50-100%, with only four assays having overall sensitivities >95% after 21 days post symptom onset. Notably, cross-reactivity with other respiratory viruses (PIV-4 (n=5), hMPV (n=3), rhinovirus/enterovirus (n=1), CoV-229E (n=2), CoV-NL63 (n=2), and CoV-OC43 (n=2) was observed; however, overall specificity for EIAs was good (92-100%; where all but one assay had specificity above 95%). POCTs were 0-100% sensitive >21 days post onset, with specificity ranging from 96-100%. However, many POCTs had faint banding and were often difficult to interpret.

Conclusions: Serology assays can detect SARS-CoV-2 antibodies as early as 10 days post onset. Serology assays vary in their sensitivity based on the marker (IgA/M vs. IgG vs. total) and by manufacturer; however, overall only 4 EIA and 4 POCT assays had sensitivities >95% >21 days post symptom onset. Cross-reactivity with other seasonal
coronaviruses is of concern. The use of serology assays should not be used for the diagnosis of acute infection, but rather for use in carefully designed serosurveys to facilitate understanding of seroprevalence in a population and to identify previous exposure to SARS-CoV-2.
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected as an unknown cause of pneumonia in December 2019\(^1\). By January 8, 2020, the Chinese Center for Disease Control and Prevention officially announced a novel coronavirus to be the cause of the outbreak seen in Hubei province\(^2\). The disease spread quickly; by the writing of this paper (July 1, 2020), there were 10,357,662 confirmed cases and 508,055 deaths associated with SARS-CoV-2 globally, which has affected nearly every country in the world\(^3\).

The ability to rapidly diagnose disease, isolate infected patients, and employ contact tracing strategies to mitigate spread of the virus is paramount to slowing the spread. Public health laboratories and acute diagnostic laboratories globally have rapidly developed and implemented diagnostic tests to identify COVID-19 disease. In the acute phases of illness, molecular detection of the virus is the primary tool for early and accurate diagnosis of disease\(^4,5\), as antibody production is usually delayed or absent in the acute phase. Serological assays are now being developed as an epidemiological tool for population based serosurveys and identification of remote infection\(^6\). However, the full extent of SARS-CoV-2 infection in large populations has yet to be determined due to limited testing\(^5,7\) and the presence of asymptomatic infection. Therefore, serosurveys must be well-designed to best represent the population of interest. To this end, accurate and high-throughput serology assays that can be integrated into laboratory information systems are key to facilitating these large-scale studies, and
improving the understanding of the true proportion of the population that has recovered from COVID-19.

The primary objective of this study was to conduct a direct comparison of six high- to mid-volume commercial enzyme immunoassays (IgG only or IgG with IgM or IgA or total antibody) and six lateral flow point of care assays (IgG/IgM) for detection of SARS-CoV-2 antibodies. All assays were tested against the same panel of serology samples from patients with confirmed COVID-19 and a group of negative controls. High- to mid-volume enzyme immunoassays were also evaluated with a separate panel of convalescent sera to evaluate cross-reactivity to common respiratory viruses and non-SARS-CoV-2 coronaviruses. This performance data, coupled with clinical data from SARS-CoV-2 positive patients, makes our evaluation panel particularly robust, and significantly adds to the current understanding of serology assays for SARS-CoV-2.

Materials and Methods

Assay evaluation:

We evaluated six commercial high- to mid-volume kits: two chemiluminescence immunoassays (CMIA/CLIA; SARS-CoV-2 IgG assay (Abbott Laboratories, Abbott Park, IL, USA); and SARS-CoV-2 S1/S2 IgG (DiaSorin, Stillwater, MN, USA)), three enzyme-linked immunosorbent assays (ELISA; EDI Novel coronavirus COVID-19 IgM and IgG ELISA (Epitope Diagnostics Inc., supplied by AFFINITY Diagnostics Corp, Toronto, ON, Canada); Novel Coronavirus COVID-19 IgM and IgG (DRG International Inc., supplied
by BioRad, Hercules, CA, USA); and Anti-SARS-CoV-2 ELISA IgA and IgG assay (Euroimmun, Mississaug, ON, Canada)) and one electrochemiluminescence immunoassay (ECLIA; Anti-SARS-CoV-2 (Roche Diagnostics, Indianapolis, IN, USA)) for detection of SARS-CoV-2 antibodies. All assays were assessed for detection of SARS-CoV-2 IgG antibodies. Additionally, Affinity and BioRad were assessed for detection of IgM, and Euroimmun was assessed for detection of IgA antibodies. Testing was performed as per manufacturer specifications and cut-offs were determined as described in the package insert. All values greater than the published cut-off were considered positive. Importantly, all kits were assessed using the same patient samples from single-use aliquots, which negated analyte degradation due to increased freeze/thaw cycles, and allowed for a direct comparison amongst a large number of commercially available serology assays. For the purposes of this study, CLIA/ECLIA/ELISA assays will be referred to as enzyme immunoassays (EIA). Where specified, targets of EIAs are listed in Table 1.

Additionally, we assessed six point of care lateral flow tests (POCT): Rapid Response (BTN; BTN Markham, Ont, Canada), 2019 nCoV IgM/IgG Detection Kit (Biolidics; Biolidics Limited, Mapex, Singapore), SARS-CoV-2 IgG/IgM Ab Test Kit (Deep Blue; Anhui Deep Blue Medical Technology Co., Ltd., Anhui, China), Novel Coronavirus IgG/IgM Test Kit (Genrui; Genrui Biotech Inc., Shenzhen, China), One Step Test for Novel Coronavirus (Geitein; Geitein Biotech Inc. Nanjing, China), and 2019-nCoV Ab test (Innovita; Innovita Biological Technology Co. Ltd., Qian'an, Hebei, China). All assays were assessed for the detection of IgM and IgG antibodies. A positive result...
was determined by any banding detected for either IgM, IgG. Faint banding was considered positive. Assays where the control line was absent were considered invalid.

Testing was performed as per manufacturer specifications. Results were read independently by two laboratorians, and when there was a discrepancy, a third laboratorian reading was used as an arbitrator (+/- was considered equivocal, +/- was considered positive). Sensitivities at various time intervals and specificities, as well as binomial exact 95% confidence intervals and Fisher’s exact tests were calculated (Microsoft Excel and STATA v.15 (StataCorp. 2017. College Station, TX, USA).

**Precision and reproducibility studies**

Patient sera from 4 patients positive for SARS-CoV-2 by reverse transcriptase real-time polymerase chain reaction (rRT-PCR) were pooled and used as the positive control, while patient sera from 4 patients with sera collected prior to November 1, 2019 (from otherwise healthy individuals with specimens sent for immunity screening) were pooled to create the negative control. Reproducibility was assessed by running replicates of 3 in triplicate for all EIAs. Precision was determined with quality control (QC) material provided by the manufacturer (positive and negative controls), which was run singly each day of testing (Abbott, DiaSorin), or in triplicate for each day of testing (Affinity, BioRad and Euroimmun). As no QC material was provided for the Roche assay, the pooled positive and pooled negative controls were used as positive and negative controls.

**Sample collection:**
Negative samples were retrieved from bio-banked sera stored at the Public Health Laboratory (Alberta Precision Laboratories) in Alberta collected before November 1, 2019. To develop a panel of positive sera from patients with COVID-19, serum samples were collected from hospitalized patients confirmed to be positive for SARS-CoV-2 on nasopharyngeal swab or endotracheal aspirate testing by rRT-PCR. Samples were collected, spun down (3000 RPM for 10 min), aliquoted into single-use aliquots, and frozen at -80 °C until the time of testing.

Eleven COVID-19 positive patients had serum collected at multiple time periods, however, only one sample per patient was used per time interval to calculate assay sensitivity. When more than one serum sample from the same individual was within the same time interval, only the most recently collected serum sample was included.

To evaluate cross-reactivity of the EIA serology tests with other respiratory viruses, convalescent sera (either retrieved from stored sera or prospectively collected) were used (note: cross-reactivity panel was not assessed on the POCTs). The sera were from patients who had tested negative for COVID-19 by in-house rRT-PCR, but positive for other viruses as follows (with number of sera used indicated): influenza A (n=5), influenza B (n=5), respiratory syncytial virus (RSVA, n=6; RSVB, n=1), rhinovirus/enterovirus (n=6), human metapneumovirus (hMPV; n=5), parainfluenza virus (PIV-1 and PIV-4; n=4), coronavirus (CoV)-229E (n=6), CoV-NL63 (n=11), CoV-OC43 (n=7), or CoV-HKU1 (n=7). One patient was positive for multiple viruses (RSVA and enterovirus/rhinovirus). All non-COVID-19 respiratory virus testing was done using the
Luminex Respiratory Pathogen Panel (RPP; NxTAG® Respiratory Pathogen Panel, Luminex, Austin, TX, USA).

**Chart review**

To obtain baseline demographic variables and outcomes for the patients with confirmed SARS-CoV-2 infection, a retrospective chart review of each patient’s electronic medical record was performed by two study team members using the provincial electronic medical record (Epic Systems Corporation, Verona, WI, USA). All symptoms listed were at presentation to hospital, and death was attributed to COVID-19 if within 30 days of symptom onset. Basic summary statistics (proportions, median, range) were calculated using Microsoft Excel.

**Determining the date of symptom onset**

The date of symptom onset for each case of laboratory confirmed COVID-19 was determined via history taking using a standardized history intake form by a member from the Alberta Health Services Communicable Diseases Team (Public Health). All serum samples were stratified by this date to determine the number of days between collection of serum and time of symptom onset.

**Ethics**

This study received ethics approval from both the University of Calgary and University of Alberta Health Research Ethics Boards. Certification approval numbers are REB20-0516 and Pro00099818, respectively.
Results

Population demographics

Serum from twenty-eight patients who tested positive for SARS-CoV-2 by rRT-PCR were used in this study. The mean age of patients was 70.1 (range 34-102 years), with a majority being male (57%; Table 2). Seven percent of patients were ambulatory, while most were hospitalized (93%), and 35% were admitted to the intensive care unit (ICU). Of those who were hospitalized, 27% required mechanical ventilation and 92% developed COVID-19-associated pneumonia. The most common co-morbidities in the cohort were: hypertension (64%), dyslipidemia (57%), and hypothyroidism (36%). All dates of symptom onset were reported earlier than the date of diagnostic sample collection (mean 16 days (range 2 to 48 days)). The date of symptom onset to date of hospitalization ranged from 0-19 days with a mean of 5 days post symptom onset. Recent travel was reported in 14% of all cases, with the United States being the most frequent location of travel (Table 2).

Performance characteristics of EIA assays

In total, 46 samples from 28 different patients testing positive for SARS-CoV-2 by rRT-PCR and 50 negative samples from serum samples stored prior to November 1, 2019, were run on each assay. Overall, the positivity rate for each assay increased over time (Table 3). With the exception of the Affinity assay (100% detection), all assays
performed poorly prior to seven days post symptom onset (range 40-60%; data not shown). However, this improved over time, and all assays had at least 80% sensitivity (range 80-100%) after 21 days (Table 3). The earliest time to detection was seen when assays had a combination of IgG with either IgM or IgA. For example, Affinity IgM and IgG assays had sensitivities of 76% and 62% respectively when considered individually at 0-14 days post symptom onset, however when combined (with either IgM or IgG positive in a sample), the overall sensitivity increased to 90% (Table 3). Notably, sera collected 46 and 48 days after symptom onset were still detectable for IgM with both BioRad and Affinity Assays, and IgA with the Euroimmun assay.

Only the Affinity IgM assay was able to detect antibodies with >95% sensitivity before 21 days post symptom onset. After 21 days post symptom onset, four assays (Abbott, Affinity, BioRad, and Euroimmun) achieved >95% sensitivity – all four had 100% overall sensitivity (Table 3). However, due to the relatively small sample size, confidence intervals show a substantial overlap between time periods. All assays, with the exception of the Euroimmun IgA assay had specificities >95% for samples collected from patients pre-November 1, 2019 (Table 3).

**Precision and reproducibility studies**

Reproducibility for the EIAs was excellent; all assays showed 100% concordance for all samples. Likewise, precision of the assays was high, with all assays having 100% qualitative agreement for positive and negative controls.
Performance characteristics of POCTs

The same validation panel used for the EIA assays was used for all POCTs, with the exception of one specimen (collected day 13 post symptom onset), as the volume of serum was exhausted following evaluation of the EIA assays. As with the EIA assays, performance of the POCTs was poor <7 days post onset with sensitivities ranging from 40-60% (data not shown). The performance of the assays increased over time, and all POCTs had >75% sensitivity after 14 days post onset and >90% sensitivity after 21 days (Table 4). However, only 4 assays had overall sensitivities that would be acceptable for use in a clinical laboratory (>95%) after 21 days post symptom onset (Table 4). Of note, IgM was poorly detected by a number of POCT assays; Getein detected IgM in only 1 of 42 positive specimens, while Biolidics detected IgM in 10-12% of positive samples. The highest sensitivities for IgM detection among the POCT assays were consistently reported for BTNX, Deep Blue and Genrui; these kits had statistically significantly higher overall (i.e. all-time points calculation) IgM sensitivities as compared to Getein (p<0.001 for all), Biolidics (P≤0.004 for all) and Innovita (p≤0.009 for all) (Table 4).

Reading the lateral flow assays was often challenging; many kits frequently produced only very faint lines. Because package inserts did not indicate appropriate density of bands to call a positive, for this study, any visualization of a band for either IgM or IgG was considered positive. This approach may have over-called the sensitivity of the lateral flow assays. Of the total reported positives, equivocal bands were observed in 9% (3 of 32) of positive samples for Biolidics, 9% (3 of 32) for BTXN, 15% (5 of 33) for
Deep Blue, 0% (0 of 33) for Genrui, 4% (1 of 26) for Getein GP BioTech, and 37% (11 of 29) for Innovita.

Cross-reactivity studies with EIA assays

An additional 62 serum samples were used to assess cross-reactivity with other respiratory viruses on EIA assays. Of those, 15 sera were collected prior to the first case of SARS-CoV-2 diagnosis in Alberta, and 47 were collected after the first case of SARS-CoV-2 was detected in Alberta. All samples collected after the first case were confirmed to be from patients who tested negative for SARS-CoV-2 by rRT-PCR on nasopharyngeal swab testing. Time of RPP positive to serum collection ranged from 11 to 135 days (mean 45 days) from the date of the original RPP result.

Overall, all assays performed well, with only a few samples showing cross-reactivity.

Most notably, the BioRad IgM assay showed cross-reactivity with hMPV on a serum collected 31 days after the RPP, and to PIV-4 on serum from 120 days post RPP, while the BioRad IgG assay showed cross-reactivity with rhinovirus/enterovirus (collected 48 days post RPP), and CoV-229E (collected 14 days post RPP). DiaSorin showed cross-reactivity with PIV-4 collected 54 days post RPP. The Euroimmun IgA assay showed cross-reactivity to two CoV-NL63 (collected 37 and 46 days post RPP from different patients), two CoV-OC43 (collected 15 and 49 days post RPP from different patients), CoV-229E (collected 13 days post RPP), and PIV-4 (collected 54 days post RPP), while the IgG assay showed cross-reactivity against PIV-4 (collected 54 days post RPP). The Affinity IgM assay showed cross-reactivity to PIV-4 (collected 120 days post RPP).

Abbott, Roche and the Affinity IgG assay did not show any cross reactivity against other...
respiratory viruses (Table 5). Overall, PIV-4 and CoV-229E were most cross reactive across assays, with additional cross-reactivity noted for CoV-NL63 and CoV-OC43 in one assay (Table 5).

Time course of antibody development
To evaluate the progression of antibody development, eleven patients in our study had serial serum samples collected (Table 6). Samples ranged from 5 to 29 days post symptom onset, and patients had between 2 and 6 samples collected overtime. Overall, Abbott detected 63.6% (7 of 11 patients) of the earliest sample drawn from a patient, Affinity detected 100%, BioRad 72.7%, Euroimmun 45.5%, DiaSorin 36.4%, and Roche 63.6% (Table 6). Interestingly, despite four different samples collected from patient 6 (ranging from 18-29 days post symptom onset), antibodies were never detected by the Roche assay. Likewise, patient 7 antibodies were not detected by the DiaSorin, however collection of the two samples was at days 6 and 8, which is relatively early during the course of infection (Table 6). Once a patient was positive by an assay, all sera from subsequent collection days were also positive; and in no instances did a patient go from positive to negative for this timeframe.

Discussion
We conducted a head-to-head comparison of fourteen different serology assays for detection of SARS-CoV-2 antibodies, in 161 different samples from 143 patients. We found Abbott, Affinity, and BioRad to have the highest clinical sensitivity and specificity.
compared to the other EIAs. Despite the relatively small panel size, and the wide statistical confidence intervals, we believe these data are very useful and informative for evaluation, comparison and validation purposes. Most EIAs and POCTs performed well after 21 days post symptom onset, however, and most importantly, no single assay was sensitive enough to detect antibodies <7 days post symptom onset. Even assays with IgM or IgA components were unable to detect antibodies reliably before 14 days from time of symptom onset (Tables 3 and 4). Furthermore, many of the assays did not perform at a level that is considered acceptable for laboratory diagnostics (>95% sensitivity and specificity). The use of these assays for diagnostic testing is therefore not recommended.

It is worth noting that these performance characteristics were determined using a cohort of COVID-19 positive patients which represented mostly hospitalized patients with high mortality rates. Preliminary studies have suggested that more severe infection may have higher seroconversion rates and antibodies may develop earlier when compared to those with mild infection. Therefore, our study data may artificially improve performance characteristics of the assays. Further studies should be done for mostly non-hospitalized COVID-19-positive patients to ensure findings remain consistent across all cohorts.

As the time course for antibody development has not yet been fully determined for SARS-CoV-2, it is important to compare equivalent start points when analyzing and comparing these data. The sensitivity of serology assays decreases significantly when
time of symptom onset rather than time from first PCR positive result is used as the start
of infection. In our study, time of PCR positivity was 5.3 days after date of symptom
onset on average (range 0-19 days). This time difference has the potential to
significantly skew the data. If time of first PCR positive result was used as the start-
point for this study, all EIAs and POCTs would perform better earlier in the disease
course compared to when the time from symptom onset is used. However, using a
positive PCR result to define the start of the infection would be an inaccurate reflection
of the development of antibody in an individual. Therefore, whenever possible, studies
should strive to include performance data in relation to date of onset, to allow true
comparison of SARS-CoV-2 testing data.

Cross-reactivity with other respiratory viruses was observed with PIV-4, hMPV,
rhinovirus/enterovirus, and most importantly CoV-229E, CoV-NL63 and CoV-OC43
infection in some of the commercial EIA assays. To date, only small panels assessing
cross-reactivity have been performed, and very little cross-reactivity with coronaviruses
other than SARS-CoV-1 has been shown. Our larger convalescent sera panel
suggests that cross-reactivity with other respiratory viruses may influence SARS-CoV-2
serology results, particularly following a recent respiratory infection (within 13-49 days
post RPP). As the majority of individuals have been exposed to endemic coronaviruses
229E, NL63, OC43, and HKU1 during their lifetime, the cross reactivity of these
viruses in particular should be evaluated. In our hands, two different assays showed
cross-reactivity against CoV-229E (Euroimmun IgA and BioRad IgG), and one assay
(Euroimmun IgA) showed cross-reactivity to two different patient samples positive for
CoV-NL63, and an additional two different patient samples positive for CoV-OC43 (Table 5). An additional study found a single patient sample to be cross-reactive against coronavirus-OC43, and although the sample number tested was small (n=5), these results are consistent with what we observed in our panel. The potential cross-reactivity of the endemic coronaviruses with SARS-CoV-2 is a significant drawback. Cross-reactivity to other coronaviruses should therefore be considered when interpreting serological results, particularly when determining SARS-CoV-2 prevalence of infection.

We assessed the ability of EIAs and POCTs to detect antibodies against SARS-CoV-2 in human sera, however no correlation with neutralizing antibodies was performed. While a few neutralizing antibody studies have been completed, and show some antibodies are protective (namely against the receptor-binding domain (RBD) of the spike domain),\textsuperscript{11} the assays that we have evaluated in this study detect total, and not specifically neutralizing, antibodies. Additionally, not all assays target the same antigen-specific antibodies, and therefore the kinetics of detection may not be equivalent between assays (Table 1). As antigen presentation and trafficking will vary between different epitopes, variability between assays is expected. We therefore caution the use of these serology results as indicators of immunity, and recommend further studies be done to measure appropriate immunity markers. This will be particularly important for the POCT assays as the antigens used are often not described (Table 1). Additionally, because SARS-CoV-2 is a recently emerged virus, there is little data on the longevity of immunity following infection. Here we found that both IgM, IgA
and IgG were detected in samples >45 days post symptom onset, suggesting that antibodies are detectable for extended periods, however, more extensive long-term evaluations on the level and duration of immunity following infection are needed.

While the use of serology assays in clinical testing is currently under debate, some recommendations as to the appropriate use of these assays is beginning to emerge. The American Society for Microbiology (ASM) and the WHO recently published similar recommendations against using serology testing for diagnosis of acute infection\textsuperscript{12,13}. A natural delay is seen from the time a patient is exposed to the virus to the time the patient starts creating antibodies against the virus. Therefore, regardless of the sensitivity of the assay, there will always be a delay between infection and the development of antibodies. Recent studies suggest that most patients seroconvert between 7 and 14 days, with IgM and IgA detected as early as 3 and 4 days post symptom onset respectively.\textsuperscript{9} However, we found that detection of antibodies earlier than 14 days was unreliable but performance for all serology assays improved overtime. To this end, both the Centers for Disease Control\textsuperscript{14} and the Public Health Agency of Canada\textsuperscript{15} have also recommended against using serology assays as an aid in the diagnosis of acute infection. However, serology assays will be helpful in understanding the prevalence of SARS-CoV-2 infection in the population, understanding the timeline of antibody development in different patient populations, and the longevity of the antibody response.
Here, we present a comprehensive serology panel consisting of sera from known COVID-19 positive patients and known negatives. We have evaluated six different commercial EIA platforms and eight POCT with the same serum panel to give an accurate comparison across all platforms. Based on our results, serology assays should not be used for the diagnosis of acute infections, but rather in carefully designed serosurveys to facilitate understanding of seroprevalence in a population and to identify previous exposure to the virus.

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Table 1: Description of serology assays used in this study

| Company   | Detection of Antibody Class | Type of Assay | Volume and Estimated TAT | Assay Target                                                                 | Approvals                                      | Notes                                                                 |
|-----------|-----------------------------|---------------|--------------------------|-------------------------------------------------------------------------------|------------------------------------------------|----------------------------------------------------------------------|
| Abbott    | IgG                         | CMIA          | High throughput          | Recombinant antigen nucleocapsid protein                                     | FDA EUA granted April 26, 2020                 | A clean of the instrument before and after running SARS-CoV-2 samples is required (~40 min total) |
|           |                             |               | 45 min per sample        |                                                                              | HC Approved May 14, 2020                      |                                                                      |
| Affinity  | IgM and IgG                 | ELISA         | Mid volume               | Recombinant antigens of the RBD and spike protein                           | CE Marked                                      | Package insert recommends testing each sample in duplicate          |
|           |                             |               | 4 hours per 96-well plate |                                                                              |                                                |                                                                      |
| BioRad    | IgM and IgG                 | ELISA         | Mid volume               | Antibodies recognizing recombinant nucleocapsid proteins and peptides       | Submitted to HC                                | Package insert recommends testing each sample in duplicate          |
|           |                             |               | 4 hours per 96-well plate |                                                                              |                                                |                                                                      |
| Diasorin  | IgG                         | CLIA          | High throughput          | IgG antibodies directed against the S1 and S2 domains of the spike protein   | FDA EUA granted April 24, 2020                 |                                                                      |
|           |                             |               | 40 min per sample        |                                                                              | HC Approved May 12, 2020                      |                                                                      |
| Manufacturer   | Type of Test | Assay Format | Testing Time | Analyte Details | FDA EUA Approval Date | CE Marked | Package Insert Notes |
|----------------|--------------|--------------|--------------|-----------------|-----------------------|-----------|----------------------|
| Euroimmun      | IgA and IgG  | ELISA        | Mid volume   | 4 hours per 96-well plate | Recombinant S1 domain of the structural protein | FDA EUA granted May 4, 2020 | CE Marked | Package insert recommends testing each sample in duplicate |
| Roche          | IgG          | ECLIA        | High throughput | 45 min per sample | Recombinant protein representing the nucleocapsid antigen | FDA EUA granted May 2, 2020 |         |                      |
| BTNX           | IgM and IgG  | Lateral flow | POCT         | 15 min per sample | Target unspecified |           |                      |
| Biolidics      | IgM and IgG  | Lateral flow | POCT         | 15 min per sample | Recombinant protein, target unspecified |           |                      |
| Deep Blue      | IgM and IgG  | Lateral flow | POCT         | 15 min per sample | Target unspecified | Removed from FDA EUA |           |                      |
| Genruim        | IgM and IgG  | Lateral flow | POCT         | 15 min per sample | Target unspecified |           |                      |
| Getein BioTech | IgM and IgG  | Lateral flow | POCT         | 15 min per sample | Recombinant nucleocapsid and spike proteins |           |                      |
| Innovita | IgM and IgG | Lateral flow | POCT | Target unspecified |
|----------|-------------|--------------|------|--------------------|
|          |             |              | 15 min per sample |        |
Table 2: Demographic and clinical variables of patients with confirmed SARS-CoV-2 infection (COVID-19) (n=28).

| Variable                        | Value          |
|---------------------------------|----------------|
| Age (yrs)                       | Mean 70.1      |
|                                 | Median 73      |
|                                 | Range 34-102   |
| Female (n (%))                  | 12 (43)        |
| Type of specimen used in diagnosis | Nasopharyngeal (n (%)) 27 (96) |
|                                 | Endotracheal suction (n (%)) 1 (4) |
| Hospitalized (n (%))            | 26 (93)        |
| Duration of hospitalization (days) | Range 4-51    |
|                                 | Mean 17        |
|                                 | Median 11      |
### Time from symptom onset to hospitalization (days)

|                          | Range  |
|--------------------------|--------|
| **Range**                | -29 to 19* |
| **Mean**                 | 5      |
| **Median**               | 5      |

Of those hospitalized (n=26):

|                          |        |
|--------------------------|--------|
| **ICU admission required in those hospitalized (n (%))** | 9 (35) |
| **Need for mechanical ventilation in those hospitalized (n (%))** | 7 (27) |
| **Pulmonary embolism (n (%))** | 1 (4)  |
| **Development of COVID-19 pneumonia (n (%))** | 26 (92) |
| **No**                   | 1 (4)  |
| **Unknown**              | 1 (4)  |
| **Development of acute** | Yes    |
|                          | 13 (46) |
| respiratory distress syndrome (n (%)) |        |
|-------------------------------------|--------|
| No                                  | 14 (50) |
| Unknown                             | 1 (4)  |
| Died (n (%))                        | 9 (32)  |
| Receipt of investigational treatments (n (%)) | 8 (29) |
| HCQ alone                           | 3 (37)  |
| HCQ + AZT                           | 2 (25)  |
| LPV/r                               | 1 (13)  |
| HCQ + LPV/r                         | 2 (25)  |
| Viral co-pathogen (n (%))           |        |
| Coronavirus-NL63                    | 1 (4)   |
| Symptoms at presentation (n (%))    |        |
| Fever                               | 17 (61) |
| Cough                               | 24 (86) |
| Dyspnea                             | 26 (93) |
| Symptom                | Count (%) |
|------------------------|-----------|
| Myalgias               | 10 (36)   |
| Abdominal pain         | 6 (21)    |
| Diarrhea               | 4 (14)    |
| Sore throat            | 2 (7)     |
| Chest pain             | 10 (36)   |
| Malaise                | 18 (64)   |
| Anorexia               | 9 (32)    |
| **Comorbidities (n (%))** |           |
| Hypertension           | 18 (64)   |
| Diabetes mellitus      | 7 (25)    |
| COPD                   | 4 (14)    |
| Coronary artery disease| 4 (14)    |

Median HBA1c – 7.2%
Range HBA1c – 4.2-10.9%
| Condition                          | Percentage |
|-----------------------------------|------------|
| Valvular disease                  | 2 (7)      |
| Obesity (BMI >30 kg/m²)           | 4 (14)     |
| Chronic renal disease (%)         | 7 (25)     |
| Hypothyroid (%)                   | 9 (36)     |
| Asthma                            | 6 (24)     |
| Congestive heart failure          | 7 (25)     |
| Atrial fibrillation               | 4 (14)     |
| Dyslipidemia                      | 16 (57)    |
| Cancer                            | 4 (14)     |
| HIV                               | 0          |

**Exposures:**

| Yes (%) | 4 (14) |
| No (%)   | 23 (82) |
| Unknown (%) | 1 (4) |

**Location of travel (n=7)**

| United States (%) | 2 (50) |
| Category                                           | Value   | Percentage |
|----------------------------------------------------|---------|------------|
| United Arab Emirates (%)                           | 1       | 25         |
| Within Canada (%)                                  | 1       | 25         |
| Contact with traveller Yes (%)                     | 6       | 21         |
| Contact with traveller No (%)                      | 21      | 75         |
| Contact with traveller Unknown (%)                 | 1       | 4          |
| Related to outbreak in long-term care/continuing care facility (%) | 9       | 32         |

*Negative due to one health-care acquired case of COVID-19 which occurred 29 days post hospital admission. If this case is removed, the range is 0-19 days.

**Abbreviations:**
- HCQ – hydroxychloroquine
- AZT – azithromycin
- LPV/r – lopinavir/ritonavir
- COPD – chronic obstructive pulmonary disease
- BMI – body mass index (kg/m²)
HIV – human immunodeficiency virus

HBA1c – glycated hemoglobin (reported for diabetic patients only)
| Assay          | Sensitivity (equivocal considered positive) | Specificity if equivocal is considered positive | 9-14 days | 15-21 days | >21 days | All time points |
|---------------|--------------------------------------------|-------------------------------------------------|-----------|------------|----------|-----------------|
| Affinity      |                                             | 1                                               | 5         | 0          | 0        | 0               |
| Euroimmun     |                                             | 2                                               | 2         | 0          | 0        | 0               |
| Overall       |                                             | 1                                               | 3         | 0          | 0        | 0               |
| Bluestar      |                                             | 1                                               | 4         | 0          | 0        | 0               |
| Euroimmun     |                                             | 2                                               | 2         | 0          | 0        | 0               |
| Overall       |                                             | 1                                               | 5         | 0          | 0        | 0               |
| Baselog       |                                             | 1                                               | 5         | 0          | 0        | 0               |
| Euroimmun     |                                             | 2                                               | 2         | 0          | 0        | 0               |
| Overall       |                                             | 1                                               | 5         | 0          | 0        | 0               |

1 Sensitivity if equivocal is considered positive
2 Confidence intervals (CI) are calculated for sensitivity and specificity where equivocals are considered negative
3 Two invalid samples were observed for Affinity and for Euroimmun (total n = 40)
4 Specificity if equivocal is considered positive
Table 4: Performance of six SARS-CoV-2 lateral flow assays (POCT) by date of serum collection relative to date of symptom onset

| Assay | 0-14 days | 15-21 days | >21 days | All time points |
|-------|-----------|------------|----------|----------------|
| Positive Samples |             |             |          |                |
| Negative Samples |             |             |          |                |

1Sensitivity if equivocal is considered positive
2Confidence intervals (CI) are calculated for sensitivity and specificity where equivocals are considered negative
3Control failure on one Getein BioTech sample was reported (n=19 for 0-14 day time frame)
4Specificity if equivocal is considered positive

Notes:
- Specificity if equivocal is considered positive
- Confidence intervals (CI) are calculated for sensitivity and specificity where equivocals are considered negative
- Control failure on one Getein BioTech sample was reported (n=19 for 0-14 day time frame)
- Specificity if equivocal is considered positive
Table 5: Cross-reactivity of high- to mid-volume serological EIA assays with sera from patients infected for other respiratory viruses by antibody class

| Virus               | Abbott G | Affinity G | Affinity M | BioRad M | BioRad G | DiaSorin G | Euroimmun A | Euroimmun G | Roche G |
|---------------------|----------|------------|------------|----------|----------|------------|-------------|-------------|--------|
| Influenza A         | 5        | 0          | 5          | 0        | 4        | 0          | 5           | 0           | 5      | 0      | 5      | 0      | 5      | 0      |
| Influenza B         | 5        | 0          | 5          | 0        | 5        | 0          | 5           | 0           | 5      | 0      | 5      | 0      | 5      | 0      |
| RSV A               | 6        | 0          | 6          | 0        | 6        | 0          | 6           | 0           | 6      | 0      | 6      | 0      | 6      | 0      |
| RSV B               | 1        | 0          | 1          | 0        | 1        | 0          | 1           | 0           | 1      | 0      | 1      | 0      | 1      | 0      |
| Rhino/Enterovirus   | 6        | 0          | 6          | 0        | 6        | 0          | 5           | 1           | 6      | 0      | 6      | 0      | 6      | 0      |
| hMPV                | 5        | 0          | 5          | 0        | 4        | 1          | 4           | 1           | 4      | 0      | 5      | 0      | 5      | 0      |
| PIV                 | 4        | 0          | 3          | 1        | 4        | 0          | 3           | 1           | 3      | 1      | 3      | 1      | 3      | 1      |
| Coronavirus 229E    | 6        | 0          | 6          | 0        | 5        | 0          | 5           | 1           | 6      | 0      | 5      | 1      | 6      | 0      |
| Coronavirus NL63    | 11       | 0          | 11         | 0        | 11       | 0          | 11          | 0           | 11     | 0      | 11     | 0      | 11     | 0      |
| Coronavirus OC43    | 7        | 0          | 7          | 0        | 7        | 0          | 7           | 0           | 7      | 0      | 7      | 0      | 7      | 0      |
| Coronavirus HKU1    | 7        | 0          | 7          | 0        | 7        | 0          | 7           | 0           | 7      | 0      | 7      | 0      | 7      | 0      |
Table 6: Blood collection over time for SARS-CoV-2 positive PCR patients from time of symptom onset to serum collection date tested by EIA platform.

| Days from symptom onset to serum specimen collection | 1 2 3 4 5 6 | 7-14 days | 15-21 days | >21 days |
|-----------------------------------------------------|--------------|------------|------------|----------|
| 1 2 3 4 5 6 | <7 days | 7-14 days | 15-21 days | >21 days |
| Patient 1   | AB/AF/B/D/E/R | AF/B/D/E/R | D/E        |           |
| Patient 2   | AB/AF/B/R     | AB/AF/B/R  | E/D        |           |
| Patient 3   | AB/AF/B/R     | AB/AF/B/R  | E/D        |           |
| Patient 4   | AB/AF/B/R     | AB/AF/B/R  | E/D        |           |
| Patient 5   | AB/AF/B/R     | AB/AF/B/R  | D/E        |           |
| Patient 6   | AB/AF/B/R     | AB/AF/B/R  | E/D        |           |
| Patient 7   | AB/AF/B/R     | AB/AF/B/R  | E/D        |           |
| Patient 8   | AB/AF/B/D/E/R | AB/AF/B/D/E/R | E/D        |           |
| Patient 9   | AB/AF/B/D/E/R | AB/AF/B/D/E/R | D/E        |           |
| Patient 10  | AB/AF/B/D/E/R | AB/AF/B/D/E/R | D/E        |           |
| Patient 11  | AB/AF/B/D/E/R | AB/AF/B/D/E/R | D/E        |           |

1 Day of sample collection is indicated with a grey box with black outline.

2 The earliest detection of antibodies by an assay is indicated by: AB, Abbott; AF, Affinity; B, BioRad; D, DiaSorin; E, Euroimmun; R, Roche.
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