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Longitudinal Monitoring of SARS-CoV-2 IgM and IgG Seropositivity to Detect COVID-19

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Summary:

The clinical performance of the Diazyme SARS-CoV-2 assay was evaluated and deemed appropriate for patient testing using a cohort of 54 PCR positive patients and an additional 235 negative samples. The kinetics of IgM and IgG seroconversion in 14 PCR confirmed SARS-CoV-2 patients were characterized by SARS-CoV-2 IgM/IgG serology. Serology testing should be considered a complimentary test to support PCR testing to aid in the detection of asymptomatic cases and is useful for documenting previous exposures to SARS-CoV-2.

Abstract

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a novel beta-coronavirus that has recently emerged as the cause of the 2019 coronavirus pandemic (COVID-19). Polymerase chain reaction (PCR) based tests are optimal and recommended for the diagnosis of an acute SARS-CoV-2 infection. Serology tests for viral antibodies provide an important tool to diagnose previous exposure to the virus. Here we evaluate the analytical performance parameters of the Diazyme SARS-CoV-2 IgM/IgG serology assays and describe the kinetics of IgM and IgG seroconversion observed in patients with PCR confirmed COVID-19 who were admitted to our hospital.

Methods. We validated the performance of the Diazyme assay in 235 subjects to determine specificity. Subsequently, we evaluated the SARS-CoV-2 IgM and IgG seroconversion of 54 PCR confirmed COVID-19 patients and determined sensitivity of the assay at three different timeframes.

Result. Sensitivity and specificity for detecting seropositivity at ≥ 15 days following a positive SARS-CoV-2 PCR result, was 100.0% and 98.7% when assaying for the panel of IgM and IgG. The median time to seropositivity observed for a reactive IgM and IgG result from the date of a positive PCR was 5 days (IQR: 2.75-9 days) and 4 days (IQR: 2.75-6.75 days), respectively.

Conclusions. Our data demonstrates that the Diazyme IgM/IgG assays are suited for the purpose of detecting SARS-CoV-2 IgG and IgM in patients with suspected SARS-CoV-2 infections. For the first time, we report longitudinal data showing the evolution of seroconversion for both IgG and IgM in a cohort of acutely ill patients in the United States. We also demonstrate a low false positive rate in patients who were presumed to be disease free.

Key words: SARS-CoV-2; antibody; serology; diagnosis; COVID-19

Introduction

Most known coronaviruses are common respiratory viruses causing cold-like symptoms (1). However, two other beta-coronaviruses, SARS and MERS, caused severe respiratory infections with increased fatality rates of 10% and 35%, respectively (2,3). In December 2019, an outbreak of a third novel beta-coronavirus, SARS-CoV-2, has led to a global pandemic of the acute respiratory disease, COVID-19 (4,5). Common symptoms of this infection include fever, cough, shortness of breath, and viral pneumonia (6,7). Severe cases develop acute respiratory distress syndrome (ARDS) and septic shock. An estimated 14% have severe symptoms, 5% develope critical conditions, with fatality rates estimated to be 3.4% as of March 3, 2020 (8,9). The mortality risk is increased with age (>65 years old) and in individuals with comorbidities (*e.g.* hypertension, diabetes) (10).

As of May 8th, 2020 over 4 million people were infected with 275,000 deaths worldwide (11). While several countries have taken an approach to massively screen the general population combined with contact tracing to effectively control COVID-19 (12–15), implementation of testing in the United States (US) has been slower and therefore prioritized for specific groups in the population(16). The current standard for the diagnosis of acute COVID-19 is real-time reverse transcriptase polymerase chain reaction (RT-PCR) for oral or nasal specimens (17,18).

Clinically validated serologic tests can be used to document previous exposure to the virus. Serologic assays are essential for epidemiological studies, development of vaccines, evaluation of passive antibody therapy, and risk assessment. Accurate serological tests will improve our understanding of viral spread in the community, particularly in the asymptomatic population, and help guide control measures. Recent reports have described the analytical characteristics of newly developed serology assays and the seroconversion of SARS-CoV-2 IgM and IgG (6,19–24). Most assays described are ELISAs measuring IgG and IgM targeting the surface spike protein (S-protein), the receptor binding

domain of the spike protein (RDB), and the internal viral nucleoprotein (N-protein). The time to seroconversion is dependent on the antibodies being measured and the sensitivity of the assay. The reported time to IgM positivity ranges from 5 to 10 days following disease onset, whereas IgG positivity occurs between 13-21 days (19–22,24). Importantly, combining serology testing with PCR results can enhance COVID-19 detection sensitivity and accuracy. A study from Guo and colleagues demonstrated that the positive detection rate of a single PCR test went from 51.9% to 98.6% when combined with an IgM ELISA assay (20). Further characterization of SARS-CoV-2 antibody response is necessary to support COVID-19 diagnosis, epidemiological studies, and vaccine evaluation.

Here we investigate a patient cohort of 54 SARS-CoV-2 PCR confirmed patients along with 235SARS-CoV-2 presumed negative subjects. SARS-CoV-2 IgM and IgG reactivity was quantified in samples from these subjects using the Diazyme SARS-CoV-2 IgM/IgG platform. This includes the longitudinal sampling of 14 hospitalized patients who were seronegative for SARS-CoV-2 IgM and IgG on admission. Clinical validation parameters of the assay were also evaluated with the goal of achieving a better understanding of the utility and analytical performance of this assay.

Materials and Methods

Study Design and Patient Cohort

Excess serum and plasma samples from a total of 289 subjects were collected in BD Vacutainer collection tubes (K-EDTA, lithium-Heparin plasma separator tubes, and/or serum separator tubes) under UCSD IRB protocol 181656. This included serum or plasma samples from 54 patients which tested PCR positive for SARS-CoV-2, 21 patients which tested PCR positive on a respiratory panel nucleic acid (RPNA) test for other infections, 24 patients which tested positive for antinuclear antibodies (ANA) or anti-double stranded DNA (dsDNA), 10 HIV positive patients, 78 apparently healthy subjects (no respiratory symptoms per self-report), and 102 patient samples that had been stored frozen (-20^{o}C)

since 2018. The SARS-CoV-2 positive patient cohort consists of 54 patients at University of California San Diego Health (UCSD). Three additional groups within the cohort were generated to calculate sensitivity and specificity for SARS-CoV-2 IgM and IgG at three time-frames relative to a confirmed positive SARS-CoV-2 PCR result: a ≤ 7 day group (n = 271), a between 8 – 14 day group (n=258), and a ≥ 15 day group (n=253). These three groups contained all the presumed negative subjects and all the PCR positive patients who had a blood sample draws in the specified timeframe.

Confirmation of SARS-CoV-2 Positive Patients

All 54 SARS-CoV-2 patients were confirmed positive for COVID-19 by nucleic acid amplification test that had been clinically validated in our laboratory and had an emergency use authorization (EUA) listing. For the purposes of sensitivity and specificity calculations, all 54 SARS-CoV-2 confirmed patients were treated as a true positive for IgM and IgG serology. All remaining samples were considered true negatives.

Serology

Serology was performed on the Diazyme DZ-Lite 3000 plus clinical analyzer platform, with the Diazyme DZ-LITE 2019-nCoV IgG (CLIA) Assay Kit (Cat # 130219015M) and the Diazyme DZ-LITE 2019-nCoV-2 IgM (CLIA) Assay Kit (Cat # 130219016M), in accordance with the manufacturer's product insert. Plasma (Li-Heparin or K-EDTA) and serum samples were analyzed in a manner consistent with the package insert. Briefly, the Diazyme DZ-LITE SARS-CoV-2 IgG/IgM CLIA kit uses magnetic microbeads and SARS-CoV-2 recombinant nucleocapsid (N) and spike (S) proteins to capture IgG and IgM (Personal Communication with Diazyme). Detection of formed immune-complexes are achieved with N-(4-Amino-Butyl)-N-Ethyl-Isoluminol (ABEI). The light signal is measured by a photomultiplier and is reported as calculated luminescence units per mL (AU/mL); values ≥ 1.00 AU/mL are considered reactive, while values < 1.00 AU/mL are considered non-reactive.

GenMark ePlex Respiratory Pathogen Nucleic Acid Test

To identify patient specimens containing other PCR confirmed microbes than SARS-Cov-2, the respiratory pathogen nucleic acid (RPNA) test was performed on the GenMark ePlex. This panel detects Adenovirus (A-F), Coronavirus (229E, HKU1, NL63, OC42), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, B and C, Influenza 2009 H1N1, Parainfluenza (1-4), Respiratory Syncytial Virus (A and B), Chlamydia pneumoniae and Mycoplasma pneumoniae.

Validation of Li-Heparin Plasma Separator Tubes (PST)

Paired potassium EDTA and lithium heparin PST tubes were compared to cross-validate PST as an alternative to manufacturer validated collection tubes. This was done for 12 SARS-CoV-2 PCR positive patients as well as 20 apparently healthy subjects. Samples were subjected to a single freeze thaw (-80 °C) and analyzed in parallel on the Diazyme SARS-CoV-2 IgM/IgG serology assay.

Precision & Stability

Precision was calculated across 4 days by running 5 batches of 5 replicates of the quality controls (QCs) supplied with the DZ-LITE SARS-CoV-2 IgG/IgM CLIA kit and with patient pools (Hi, Low and Negative) (n=25). Reactive patient pools were made by pooling SARS-CoV-2 PCR positive patient samples to create two sample pools with two different concentrations of IgG and IgM against SARS-CoV-2 (Hi and Low). Non-reactive patient pools were created by combining patient serum samples that were collected in 2018.

Dilutional linearity

Dilutional linearity for IgG across the lower end of the analytically measurable range (AMR) was performed by mixing one high (26.9 AU/mL) and one low (0.117 AU/mL) sample in 20% increments prior to analysis. Linearity for IgG across the upper end of the AMR was evaluated by mixing pooled patient

samples from a high pool (114.1 AU/mL) and low pool (0.011 AU/mL) in 10% increments prior to analysis. Dilutional linearity for IgM across the lower end of the AMR was evaluated by mixing one high (4.517 AU/mL) and one low (0.507 AU/mL) sample in 20% increments prior to analysis. Dilutional linearity for IgM across the upper end of the AMR was performed by mixing pooled patient samples from a high pool (11.85 AU/mL) and low pool (0.327 AU/mL) in 10% increments prior to analysis.

Statistical Analyses

Data was analyzed using R in Rstudio and linear regression analysis for all figures was performed in Excel. Precision (%CV) was calculated by analysis of variance (ANOVA) and total precision (%CV) was calculated by the sum of squares.

Results

Validation of plasma collected in Li-heparin plasma separator tubes

The Diazyme SARS-CoV-2 IgM/IgG serology assays have been validated by the manufacturer to analyze plasma collected in EDTA anti-coagulant tubes or serum. We investigated the use of Li-heparin plasma separator tubes (PST) as an alternative collection tube to improve testing flexibility. Linear regression analysis comparing the observed and measured AU/mL values for IgM and IgG in K-EDTA and PST collected plasma demonstrated strong agreement for IgM (R^2 = 0.9719) and IgG (R^2 = 0.9862) (**Supplemental Figure 1A and 1B**). Bland-Altman plots describing the observed bias between the AU/mL values for IgM and IgG indicate agreement over a wide range of values (**Supplementary Figure 1C and 1D**). AU/mL values for samples collected in K-EDTA tubes ranged from 0.6 – 8.4 AU/mL for IgM and 4.9 – 108.5 AU/mL for IgG, with the average biases being 1.0% for IgM and 0.4% for IgG.

Precision profile

The precision profiles (%CV) of the SARS-CoV-2 IgM and IgG serology assays can be found in **Table 1** for the positive and negative quality controls (QCs) as well as three patient pools that were non-reactive, moderately reactive, and highly reactive for both IgM and IgG. Within run precision ranged from 1.4% - 5.0% for IgM and from 0.1% -14.6% for IgG. Between-run precision ranged from 4.1% - 7.3% for IgM and from 0.2% - 34.1% for IgG. Total precision ranged from 4.3% - 8.0% for IgM and from 0.2% - 37.1% for IgG. The high variation observed for IgG was only seen in the negative patient pool where all measured AU/mL values were well below the cutoff (near zero). Outside of the negative patient pool, the highest within-run, between-run, and total precision observed for IgG was 7.6%, 11.56%, and 13.25%.

Sample stability

QC and patient sample pools were found to be stable on the DZ-Lite 3000 Plus analyzer for 12 hours at 25°C (**Supplementary Table 1**). The maximum bias observed, ignoring the non-reactive patient pool, was 8.33% for IgM and -8.02% for IgG.

Dilutional linearity

Dilutional linearity was determined and linear regression analysis was used to describe the relationship between observed and expected AU/mL values following sample dilution (**Supplementary Figure 2**). The IgM assay was linear from 0-12 Au/L (R^2= 0.946, y=0.9296x + 0.5441) and IgG assay was linear from 0-27 AU/mL (R^2= 0.996, y=1.0082x - 0.4738) (**Supplementary Figure 2A and 2B**). Significant saturation was observed at the upper end of the measured range for IgG.

Cross-reactivity

The cross-reactivity of the SARS-CoV-2 IgM and IgG assays were evaluated against 55 patients, with other conditions or respiratory pathogens (**Table 2**). We observed no cross-reactivity for either IgM or IgG in these patient samples, including 8 samples positive for non-COVID-19 coronavirus.

Sensitivity and Specificity

To calculate sensitivity and specificity for IgM and IgG, patient groups that fell into three different time-frames, were generated (**Table 3**, **Supplementary Table 2 and Supplementary Table 3**). The ≤ 7 day group included 36 PCR positive patients, the between 8 – 14 day group included 23 PCR positive patients, and the ≥ 15 day group included 18 PCR positive patients. As expected, sensitivity for the ≤ 7 day group was poor for IgM (58.3%), IgG (69.4%) and the IgM/IgG panel (69.4%) (**Table 4**). In contrast, sensitivity observed in the between 8 – 14 day group was greatly improved for IgM, IgG, and the IgM/IgG panel; 94.4%, 94.4%, and 94.4%, respectively. The ≥ 15 day group saw reduced sensitivity of for IgM (89.5%), however improvements were observed for IgG and the IgM/IgG panel of 94.7% and 100.0%, respectively. Specificity of the assay was excellent for both IgM, IgG, and IgM/IgG panel; 99.6%, 99.1%, and 98.7%, respectively.

Positive and negative predictive values and the impact of prevalence

Positive and negative predictive values (PPV and NPV) were calculated for the ≥ 15 day patient group as well at different levels of COVID-19 disease prevalence (**Table 5**). The prevalence for COVID-19 in the ≥ 15 day patient group was 7.1%, and the observed PPV for IgM, IgG, and IgG/IgM panel was 94.4%, 89.4%, and 85.6%, respectively. The observed NPV for IgM, IgG, and IgM/IgG panel was 99.6%, 99.6%, and 100.0%. The impact of disease prevalence on the PPV and NPV of the SARS-CoV-2 IgM and IgG assays has been calculated for different levels of disease prevalence as well (**Table 5**). The prevalence of 4.3% (139/3272) represents the percentage of positive SARS-CoV-2 PCR tests performed on patients presenting with acute symptoms of COVID at the UCSD as of April 15, 2020.

Longitudinal patient sampling

We evaluated the time to seroconversion of SARS-CoV-2 IgM and IgG in 14 PCR confirmed patients that were initially admitted to the hospital and presented as seronegative (**Figure 1A and 1B**). Observed median seroconversion to IgM and IgG was 5 days and 4 days following a positive PCR result, respectively. Interquartile range (IQR) for IgM and IgG was calculated as 2.75-9 days for IgM and 2.75 – 6.75 days for IgG. All 14 patients seroconverted to IgM and IgG over the course of their hospital admission.

Discussion

We report a cohort of 289 subjects which includes 54 confirmed as PCR positive for SARS-CoV-2, 21 which tested positive on a respiratory panel nucleic acid (RPNA) test for other infections, 24 which tested positive for ANA or dsDNA, 10 HIV positive patients, 78 apparently healthy subjects, and 102 serum samples from 2018 that were considered SARS-CoV-2 naïve. Our study evaluated several analytical parameters of the Diazyme SARS-CoV-2 IgM and IgG serology assays (precision, dilutional linearity, and sample stability) and found them to be appropriate for clinical testing for both SARS-CoV-2 IgM and IgG reactivity. The sensitivity of the combined IgM/IgG assays observed in the ≤ 7 day group was poor (69.4%), confirming that acutely sick patients presenting shortly after symptom onset should receive PCR testing. However, when testing samples that fell between 8 – 14 days after the date of a confirmed SARS-CoV-2 PCR result, the clinical sensitivity was 94.4% when using IgM/IgG panel. Sensitivity was 100.0% when testing samples that were ≥ 15 days after a confirmed SARS-CoV-2 PCR result using the combined IgM/IgG panel. Specificity was highest when using IgM alone to detect seropositivity (99.6%) but the specificity was also high when using the panel (98.7%). Interestingly, the sensitivity for IgM seropositivity was lower (89.5%) in the ≥ 15 day group compared to the between 8 – 14 day group (94.4%), which probably reflects antibody class switching. The clinical utility of this test is

still being determined, but it appears to be useful to complement PCR-based methods where getting an adequate sample is difficult and to detect past or current SARS-CoV-2 infections.

The importance of disease prevalence on predictive values is often overlooked. **Table 5**, shows that the PPV is high when the disease prevalence is high, but that the PPV drops as low as 17.3% at a disease prevalence of 0.3%. As of April 15th 2020, the estimated prevalence of SARS-CoV-2 in acutely ill patients that had been prescreened for COVID symptoms at UCSD Health was about 4.3%, which leads to a PPV of 77.7% and NPV of 99.8% when using the panel of IgM/IgG (**Table 5**). Thus, the PPV will be poor if this test is used in a broader swath of the general population without symptoms and a low prevalence for SARS-CoV-2. This is not a negative reflection on the assay, but a reality of any screening technique.

A look at the precision profile indicates acceptable between-run, within-run, and total precision for all samples tested. The negative patient pool had a total CV of 37.1%, but this is acceptable for a blank sample where the small variance is magnified and not clinically significant (**Table 1**). Sample stability was robust at 25 °C as maximum biases observed for reactive samples was less than 10% (**Supplementary Table 1**). Dilution studies revealed good linearity up to an AU/mL of 12 AU/mL for IgM and 27 AU/mL for IgG. Due to the wider dynamic range of the IgG assay, saturation was observed for samples with AU/mL values that ranged from 48.1 – 107.6, with clear saturation at values around 100 AU/mL (**Supplementary Figure 2**). Cross-reactivity of the assay towards other conditions and respiratory pathogens was not observed for 55 individual patients. Importantly, 8 patients with non-COVID coronavirus did not cross react with either the IgM or IgG (**Table 2**). Cross-reactivity for IgM and IgG of 24 patients which had positive ANA titers > 1:40 or anti-dsDNA (>100), was not observed (**Table 2)**. We also tested 10 HIV positive patient samples and found no cross reactivity for IgM or IgG (**Table 2**).

The median time to seroconversion of the 14 longitudinally sampled patients was observed to be 5 days for IgM and 4 days for IgG, with IQRs of 2.75 – 9 days and 2.75 – 6.75 days, respectively (**Figure 1).** We also investigated how these findings would translate relative to the number of days post-symptom onset (PSO). Relative to symptom onset, the median time to seropositivity for IgM and IgG was 10.5 days PSO and 9.5 days PSO, respectively. Inter-quartile ranges of the median PSO seroconversion were 8.0 – 12.3 days for IgM and 7.0 – 12.0 days for IgG.

The fact that we observe IgG becoming seropositive in these patients before IgM indicates that our cutoff for IgM seropositivity may be too stringent or that the assay lacks sensitivity, as IgM is known to become seropositive before IgG (20). This could also be due to the presence of high levels of viral antigen, that could compete with the labeled antigen used to detect the presence of IgM against SARS-CoV-2. We evaluated a 0.7 AU/mL cutoff for IgM and recalculated the median time to seropositivity observed for IgM in the 14 longitudinally sampled patients. As expected, the observed median time to IgM seropositivity was shortened to 3.5 days relative to the positive PCR result (**Supplementary Table 4**). However, utilizing the lower cutoff would have generated two additional false positives across the entire cohort and thus we followed the manufactures recommendation and used a 1.0 AU/mL as the cutoff for a reactive IgM result.

One of the limitations of our study is that 50 out of 54 of our SARS-CoV-2 PCR confirmed patients were admitted to the hospital and are likely to be more acutely sick compared to the average person that contracts COVID-19. However, 56% (30/54) of the patients were not intubated at the time of writing, suggesting that most patients were not in critical condition. Another limitation of our study is that one of our non-admitted patients (#37) had a past-medical history of common variable immunodeficiency for IgG (**Table 3**). This medical condition could explain why this patient was classified as being falsely negative for IgG 22 days following a positive SARS-CoV-2 PCR result. If this patient was dropped from the cohort, the sensitivity if the IgG assay at >15 days would have been 100%. More

serologic data is needed on SARS-CoV-2 PCR positive patients with mild symptoms who recovered at home as compared with our acutely ill patients who were generally hospitalized.

Some reports have suggested that SARS-CoV-2 IgM could be used as an acute phase marker for recent infection (20,25). There are several reports on various viruses, including SARS coronavirus, that suggest IgM antibodies against viral proteins can persists months and even years after an acute infection (26–34). Therefore, care must be used when interpreting seropositivity for IgM; if there is a question about acute exposure and infectivity, PCR testing should be performed.

In conclusion, our data demonstrates that the Diazyme IgM/IgG assay is suited for the purpose of detecting SARS-CoV-2 IgM and IgG in patients with suspected SARS-CoV-2 infections. This is the first report of longitudinal data showing the evolution of seroconversion for both IgM and IgG in a cohort of patients in the United States. We also demonstrate a low false positive rate in patients who were presumed to be disease free. This validation study serves as a potential template for other studies evaluating the analytical and clinical performance of SARS-CoV-2 assays.

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References

1. Myint SH. Human Coronavirus Infections. In: Siddell SG, editor. The Coronaviridae [Internet]. Boston, MA: Springer US; 1995. page 389–401. Available from: https://doi.org/10.1007/978-1-4899-1531-3_18

2. Poon L, Guan Y, Nicholls J, Yuen K, Peiris J. The aetiology, origins, and diagnosis of severe acute respiratory syndrome. Lancet Infect Dis. 2004;4:663–71.

3. Donnelly CA, Malik MR, Elkholy A, Cauchemez S, Van Kerkhove MD. Worldwide Reduction in MERS Cases and Deaths since 2016. Emerg Infect Dis. 2019/09/17. Centers for Disease Control and Prevention; 2019;25:1758–60.

4. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–3.

5. Ren L-L, Wang Y-M, Wu Z-Q, Xiang Z-C, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl) [Internet]. 2020 [cited 2020 Apr 14];Publish Ahead of Print. Available from: https://journals.lww.com/cmj/Abstract/publishahead/Identification_of_a_novel_coronavirus_causing.99423.aspx

6. Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. Taylor & Francis; 2020;9:386–9.

7. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med [Internet]. Massachusetts Medical Society; 2020 [cited 2020 Apr 14]; Available from: https://www.nejm.org/doi/10.1056/NEJMoa2001017

8. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239–42.

9. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 3 March 2020 [Internet]. 2020 [cited 2020 Apr 16]. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---3-march-2020

10. Ioannidis JPA, Axfors C, Contopoulos-Ioannidis DG. Population-level COVID-19 mortality risk for non-elderly individuals overall and for non-elderly individuals without underlying diseases in pandemic epicenters. medRxiv. 2020;2020.04.05.20054361.

11. COVID-10 Coronavirus Pandemic [Internet]. Worldmeter. 2020 [cited 2020 Apr 15]. Available from: https://www.worldometers.info/coronavirus/

12. Hellewell J, Abbott S, Gimma A, Bosse NI, Jarvis CI, Russell TW, et al. Feasibility of controlling COVID-19 outbreaks by isolation of cases and contacts. Lancet Glob Health. 2020;8:e488–96.

13. Kwon KT, Ko J-H, Shin H, Sung M, Kim JY. Drive-Through Screening Center for COVID-19: a Safe and Efficient Screening System against Massive Community Outbreak. J Korean Med Sci [Internet]. The Korean Academy of Medical Sciences; 2020;35. Available from: http://synapse.koreamed.org/DOIx.php?id=10.3346%2Fjkms.2020.35.e123

14. Salath M, Althaus CL, Neher R, Stringhini S, Hodcroft E, Fellay J, et al. COVID-19 epidemic in Switzerland: on the importance of testing, contact tracing and isolation. Swiss Med Wkly [Internet]. 2020 [cited 2020 Apr 16]; Available from: https://doi.emh.ch/smw.2020.20225

15. Wang CJ, Ng CY, Brook RH. Response to COVID-19 in Taiwan: Big Data Analytics, New Technology, and Proactive Testing. JAMA. 2020;323:1341–2.

16. Cohen J, Kupferschmidt K. Countries test tactics in 'war' against COVID-19. Science. 2020;367:1287.

17. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. European Centre for Disease Prevention and Control (ECDC); 2020;25:2000045.

18. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clin Chem. 2020;66:549–55.

19. Lou B, Li T, Zheng S, Su Y, Li Z, Liu W, et al. Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. medRxiv. 2020;2020.03.23.20041707.

20. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis [Internet]. 2020 [cited 2020 Apr 13]; Available from: https://doi.org/10.1093/cid/ciaa310

21. OKBA NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients. medRxiv. 2020;2020.03.18.20038059.

22. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv. 2020;2020.03.30.20047365.

23. Amanat F, Stadlbauer D, Strohmeier S, Nguyen T, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. medRxiv. 2020;2020.03.17.20037713.

24. Long Q, Deng H, Chen J, Hu J, Liu B, Liao P, et al. Antibody responses to SARS-CoV-2 in COVID-19 patients: the perspective application of serological tests in clinical practice [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Mar. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.03.18.20038018

25. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol [Internet]. [cited 2020 Apr 17];n/a. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25727

26. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, et al. Virus and Antibody Dynamics in Acute West Nile Virus Infection. J Infect Dis. 2008;198:984–93.

27. Murray KO, Garcia MN, Yan C, Gorchakov R. Persistence of Detectable Immunoglobulin M Antibodies Up to 8 Years After Infection with West Nile Virus. Am J Trop Med Hyg. 2013;89:996–1000.

28. Chien Y-W, Liu Z-H, Tseng F-C, Ho T-C, Guo H-R, Ko N-Y, et al. Prolonged persistence of IgM against dengue virus detected by commonly used commercial assays. BMC Infect Dis [Internet]. 2018 [cited 2020 Apr 17];18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880084/

29. Griffin I, Martin SW, Fischer M, Chambers TV, Kosoy O, Falise A, et al. Zika Virus IgM Detection and Neutralizing Antibody Profiles 12–19 Months after Illness Onset. Emerg Infect Dis. 2019;25:299–303.

30. Meurman OH. Persistence of immunoglobulin G and immunoglobulin M antibodies after postnatal rubella infection determined by solid-phase radioimmunoassay. J Clin Microbiol. 1978;7:34–8.

31. Woo PCY, Lau SKP, Wong BHL, Chan K, Chu C, Tsoi H, et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. Clin Diagn Lab Immunol. 2004;11:665–8.

32. Liaw YF, Yang CY, Chu CM, Huang MJ. Appearance and persistence of hepatitis A IgM antibody in acute clinical hepatitis A observed in an outbreak. Infection. 1986;14:156–8.

33. Cumberland P, Everard CO, Wheeler JG, Levett PN. Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979-1989. Eur J Epidemiol. 2001;17:601–8.

34. Varghese GM, Rajagopal VM, Trowbridge P, Purushothaman D, Martin SJ. Kinetics of IgM and IgG antibodies after scrub typhus infection and the clinical implications. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. 2018;71:53–5.

Table 1. Precision profile of the Diazyme SARS-CoV-2 IgM/IgG serology assay. Between-run, within-run and total precision was calculated from a total of 25 replicates run across 4 days. Mean AU/mL and standard deviations (N=25) are shown in parentheses.

Precision of Concentration Values (AU/mL)			
Sample (Mean AU/mL, SD)	IgM Between-run %CV	IgM Within-run %CV	IgM Total %CV
Positive QC (3.88, 0.17)	7.3	3.3	8.0
Negative QC (0.48, 0.08)	4.1	1.4	4.3
Patient Pool Hi (5.87, 0.25)	5.2	4.1	6.6
Patient Pool Low (1.71, 0.08)	3.5	4.7	5.9
Patient Pool Negative (0.55, 0.03)	3.9	5.0	6.3
Sample	IgG Between-run %CV	IgG Within-run %CV	IgG Total %CV
Positive QC (3.97, 0.21)	9.5	3.8	10.2
Negative QC (0.00, 0.004)	0.2	0.1	0.2
Patient Pool Hi (71.5, 5.4)	7.5	7.6	10.7
Patient Pool Low (33.4, 2.5)	11.6	6.5	13.3
Patient Pool Negative (0.07, 0.01)	34.1	14.6	37.1

Table 2. Cross-reactivity of the Diazyme SARS-CoV-2 IgM/IgG serology assay towards serum or plasma from 55 patients with other conditions.

Conditions	Number of Samples Tested	Number of Reactive (IgG or IgM)
Human Metapneumovirus	3	0
Influenza A H1-20009 PCR	1	0
Mycoplasma Pneumoniae	1	0
Non COVID-19 Coronavirus	8	0
Parainfluenza 4 PCR	1	0
Respiratory Syncytial Virus A	2	0
Respiratory Syncytial Virus B	1	0
Rhinovirus/Enterovirus	4	0
Anti-dsDNA (>100)	4	0
Antinuclear Antibodies	20	0
HIV+	10	0

Table 3. Clinical Information for COVID-19 Positive Patients at UC San Diego Health from March – April 2020.

Patient	Sex	Age	BMI	Symptoms	Day* of First Symptoms	Day* Hospitalized	Day* of Intubation	Past Medical History
1	M	36	38.1	fever; cough; SOB	-5	-1	2	hepatomegaly
2	M	42	31.1	fever; cough; SOB; chills; body aches; sore throat; nausea; diarrhea; weakness	-9	1	NA	None
3	M	57	29.2	fever; cough; SOB; loss of taste; nausea; vomiting; fatigue; body aches	-3	0	6	None
4	M	33	24.4	fever; cough; SOB; chills; body aches	-11	-4	-2	HIV+; hyperlipidemia
5	M	47	23.7	cough; fever; body aches; weakness; SOB	-6	0	NA	hyperlipidemia; hypertension
6**	M	82	23.3	cough; loss of appetite; lethargic	-2	0	4	dementia
7	M	71	30.8	cough; fever; SOB	-3	0	NA	alcoholic liver cirrhosis; CKD; diabetes
8**	M	73	26.6	cough; fever; SOB	-7	0	2	hypertension
9	F	25	21.9	cough; SOB; weakness	-2	-13	NA	CKD; diabetes; hypothyroidism
10	M	56	26.1	fever; cough; body aches; diarrhea; weakness; chills	-5	0	NA	None
11	F	33	32.2	fever; cough; SOB	-5	0	0	pregnant
12	M	43	28.9	fever; cough; SOB; weakness	-3	0	3	None
13	M	53	27.8	fever; cough; SOB; body aches; chills; diarrhea	-3	3	5	diabetes; HIV+; hyperlipidemia; hyertension
14	M	50	23.2	fever; cough; chills; body aches	-4	0	NA	asthma; HIV+; hyperlipidemia; meth use
15**	F	77	20.5	fever; SOB; weakness; fatigue; lethargy	-2	0	5	dementia
16	F	64	31.4	fever; cough; SOB; body aches; diarrhea	-4	0	NA	diabetes
17	M	83	25.9	weakness	0	2	NA	asthma; cancer; diabetes
18	F	65	30.6	fever; weakness; diarrhea; body aches	-4	-1	2	diabetes; hypertension
19	M	59	25.3	cough; fever; SOB; loss of appetite; lethargic; diarrhea	-8	-1	9	congestive heart failure; hypertension; hypothyroidism
20	M	61	36.7	cough; fever; SOB	-7	0	1	None
21	M	41	22.1	cough; fever; SOB; nausea; diarrhea	-9	0	NA	None
22	M	34	26	cough; fever; SOB	-1	9	NA	asthma
23	M	39	24.4	cough; fever; chills	-7	NA	NA	None
24**	M	76	28.6	cough; SOB	-9	-1	0	None
25	F	25	34.2	cough; fever; chills	-1	0	NA	cancer; diabetes
26	M	52	28.1	SOB; fever; body aches	-6	NA	NA	cancer

Table 3. Clinical Information for COVID-19 Positive Patients at UC San Diego Health from March – April 2020.

Patient	Sex	Age	BMI	Symptoms	Day* of First Symptoms	Day* Hospitalized	Day* of Intubation	Past Medical History
27	M	81	20.9	fever; fainting	-7	-3	NA	cancer
28	M	60	28.3	cough; SOB; body aches; diarrhea	-7	4	NA	hypertension
29	M	57	36.4	SOB; cough; diarrhea	-10	0	NA	diabetes; HIV+
30	F	37	23.2	fever; cough	-5	0	NA	None
31	M	83	25.4	weakness	-3	0	NA	dementia
32	F	40	35.9	cough; fever; SOB; body aches	-8	-2	-1	None
33	F	20	30.8	weakness; chills; chest pain	-2	-2	NA	diabetes
34	F	38	32.5	fever; diarrhea	-5	0	NA	ectopic pregnancy
35	M	45	26.7	cough; fever; SOB; body aches	-10	NA	NA	asthma; hyperlipidemia
36	F	71	32.1	cough; fever; SOB; diarrhea	-5	-1	NA	diabetes; hypertension
37	M	31	23.1	fever; cough; loss of taste	-9	NA	NA	IgG deficient
38**	F	88	30.3	cough; fever; SOB; fatigue	-1	0	2	hypertension
39	F	53	40.0	cough; fever; SOB; fatigue; body aches	-9	0	NA	None
40**	M	67	25.0	cough; fever; SOB; dizziness; loss of taste and smell	-7	0	0	hypotension; heart failure
41	F	46	45.8	cough; fever; SOB; body aches; chills	-10	2	NA	diabetes; hypertension; asthma
42	M	37	28.5	cough; fever; SOB; body aches; chills	-2	14	NA	HIV+; meth use
43	M	67	27.4	cough; SOB	-5	0	5	None
44	M	50	24.6	cough; fever; SOB; chills	-22	-15	-8	hypertension
45	M	64	34.9	cough; SOB	-9	0	1	COPD; hypertension
46	F	69	33.8	cough; fever; SOB	-3	8	NA	hypertension
47	M	41	35.5	cough; fever SOB; fatigue	-18	0	5	asthma
48	M	65	28.2	cough; fever; SOB	-2	0	8	multiple myeloma
49**	F	80	30.0	cough; fever; SOB; nausea; vomiting; chills	-1	0	17	diabetes; hypertension; anemia
50	F	90	26.2	cough; fever; nausea	-2	3	NA	hypertension
51	M	90	19.6	cough; diarrhea	-2	2	NA	hypertension; CKD; cancer
52	M	48	30.0	cough; fever; SOB; fatigue; headache	-5	0	2	None
53	F	91	12.7	cough; fever	-7	0	NA	hypertension; stroke
54**	F	46	28.8	cough; fever; SOB	0	-1	0	hypertension; diabetes; rheumatoid arthritis

Abbreviations: NA, not applicable; SOB, shortness of breath; HIV, human immunodeficiency virus; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease
*All days are calculated relative to the date of the first PCR positive result.
**Deceased at time of writing

Table 4. Sensitivity and specificity of the Diazyme SARS-CoV-2 IgM/IgG serology assay at different time-points following a positive SARS-CoV-2 PCR result

Statistical Measure	≤ 7 Post Symptom Onset (n=271)			8 - 14 Post Symptom Onset (n=258)			≥ 15 Post Symptom Onset (n=253)		
	IgM	IgG	IgM/IgG	IgM	IgG	IgM/IgG	IgM	IgG	IgM/IgG
Sensitivity	58.3%	69.4%	69.4%	94.4%	94.4%	94.4%	89.5%	94.7%	100.0%
Specificity	99.6%	99.1%	98.7%	99.6%	99.1%	98.7%	99.6%	99.1%	98.7%

Table 5: Positive and negative predictive values at various levels of disease prevalence.

Prevalence (%)	IgM				IgG				IgM + IgG			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
20.0	89.5	99.6	98.2	98.6	94.7	99.1	96.5	98.6	100.0	98.7	95.1	100.0
7.1			94.4	99.6			89.4	99.6			85.6	100.0
4.3*			90.8	99.7			83.2	99.7			77.7	100.0
2.2			82.9	99.9			70.7	99.9			63.1	100.0
1.1			70.5	99.9			54.5	99.9			45.8	100.0
0.5			54.3	100.0			37.3	100.0			29.6	100.0
0.3			37.2	100.0			22.9	100.0			17.3	100.0

Sensitivity and specificity is shown for seropositivity for samples ≥15 days after a confirmatory PCR test.

* Estimated prevalence of SARS-CoV-2 in the high risk testing population at UC San Diego Health on April 16th, 2020

Figure 1: Longitudinal Sampling Illustrates Seroconversion in SARS-CoV-2 Infected Patients.

A, B) Seroconversion to IgM and IgG was monitored in longitudinal samples for 14 patients. AU/mL values for IgG and IgM are plotted on the Y-axis on a semi-log scale and the number of days relative to a positive PCR test is shown on the X-axis. Values ≥ 1.00 AU/mL indicate reactivity, while values < 1.00 AU/mL indicate a non-reactive result. The median and interquartile ranges for the time to seropositivity, relative to a positive SARS-CoV-2 PCR result, is shown for IgG and IgM.

Figure 1