Original Article

Assessment of a rapid pan-antibody dot test for detection of antibodies against SARS-CoV-2

Md. Ahsanul Haq[1], Maha Jamiruddin[1], Mohib Ullah Khondoker[1, 2], Md. Firoz Ahmed[3], Shahad Saif Khandker[1], Tamanna Ali[1], Mamun Mostafi[2], Bijon Kumar Sil[1], Nihad Adnan[1, 3], Mohd Raeed Jamiruddin[1, 4]

Abstract

Background: *With the drastic spread of COVID-19 and mass mortality of people globally, detection of the progression of this disease has stood out to be a necessity. Hence, we set out to identify the prevalence of COVID-19 antibodies in Bangladesh using the in-house rapid pan-immunoglobulin dot-blot test kit and evaluate the performance of this kit.* ***Methods:*** *In this cross-sectional study, we tested serum collected between mid-May and mid-June 2020 for COVID-19 antibodies by using the in-house rapid pan-immunoglobulin dot-blot test kit in RT-PCR confirmed patients with symptoms for 1-7 days (Group Ia; n =100) and 8-14 days (Group Ib; n = 100); symptomatic RT-PCR negative patients (Group II; n = 100) and convalescent patients (Group III; n = 109) while comparing with pre-pandemic sera samples collected prior two years to December-2019 (Group IV; n = 100).* ***Results:*** *Our kit detected that almost 70% of the convalescent patients produced antibodies against COVID-19 compared to other groups. However, the group with individuals at the end phase of COVID-19 exhibited the second-highest percentage of seroprevalence (41%). We also observed that though Group II was RT-PCR negative, 20% of them showed COVID-19 antibodies. Conclusion: With a specificity of 96% in our kit, we can say that our kit will be a potential device for the detection of SARS-CoV-2 antibodies and to understand herd immunity in Bangladesh.*

Keywords: Cross-sectional study; SARS-CoV-2; seroepidemiological study; immunoblotting; herd-immunity; antibodies.

Bangladesh Journal of Medical Science, Special Issue on COVID-19. 2021. Page : 131-139
DOI: https://doi.org/10.3329/bjms.v20i5.55407

Introduction

Coronaviruses (CoVs), the vast group of *Nidovirales* order, are enveloped, non-segmented positive-sense RNA viruses comprising 26 to 32 kilobases RNA genomes[1]. These are known to infect different animal species, including humans. In some cases, they can also cause zoonotic infection in humans, as with SARS-CoV, MERS-CoV, and SARS-CoV-2[2]. The latter is responsible for the COVID-19 pandemic, which has claimed more than 4.29 million deaths worldwide[3]. The symptoms mainly associated with SARS-CoV-2 infection are fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle or body aches, headache, the new loss of

1. Gonoshasthaya-RNA Molecular Diagnostic and Research Center, Dhaka, Bangladesh.
2. Gonoshasthaya Samaj Vittik Medical College, Savar, Dhaka, Bangladesh and Center for Multidisciplinary Research, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh.
3. Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh.
4. Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Correspondence

1. Mohd. Raeed Jamiruddin, Department of Pharmacy, BRAC University, Dhaka-1212. Email: mohd.raeed@bracu.ac.bd
2. Nihad Adnan, Department of Microbiology, Jahangirnagar University Savar, Dhaka, Bangladesh.Email: nihan@juniv.edu

taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea, and/or maybe mild to severe lower respiratory tract infections. These symptoms usually appear 2-14 days after being exposed to the virus[4].

Since the avowal of Covid-19 as a pandemic by the World Health Organization (WHO), globally detection of viral RNA in clinical specimens using real-time fluorescent RT-PCR became a benchmark assay for the diagnosis of SARS-CoV-2[5-7]. Though nucleic acid detection has the advantages of early-stage sensitivity, high specificity, and easy operation, the accuracy of nucleic acid detection results needs to be comprehensively analyzed from influencing factors such as sample type, quality, experimental factors, kit performance, patient infection cycle, especially for difficult-to-diagnose cases, the accuracy of detection should be more strictly controlled[8]. Therefore, complementary serological immunoassays such as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), and loop-mediated isothermal amplification (LAMP), rapid high-throughput flow-through membrane immunoassay (FMIA) are utilized nowadays for the pathogen detection, infection progression evaluation, and transmission dynamics analysis[9-11].

ELISA is one of the most popular assays; it requires plate reader assistance, long, laborious hours to perform the assay[12]. In contrast to other blotting methods, the dot blot technique offers a lowrisk of laboratory-acquired infections without needing equipment and rapid visibility of circular spots to the naked eye[10,13]. It also saves considerable time since complex gel blotting procedures or multiple user steps are unnecessary[10,14-16]. Consequently, dot blot, a rapid high-throughput flow-through membrane immunoassay, can be a convenient alternative amongst the point-of-care techniques.

Dot blot is an abridged molecular biology blottingtechnique for detecting, analyzing, and identifying proteins[10,17,18]. Principally, this benchtop assay involves direct application of reagents and samples of serum, plasma, or blood through the nitrocellulose (NC) membrane spotted with immobilized molecules and detection of visible colored signals using gold nanoparticle-labeled secondary antibodies[19].

This study utilized an in-house rapid pan-immunoglobulin dot-blot test kit to identify SARS-CoV-2 antibodies in COVID patients. The kit was developed to determine whether a person has been infected without discriminating whether the person is presenting a past or present infection. The purposes of this population-based cross-sectional prospective diagnostic study are to (i) detect the presence of antibodies in the serum of COVID-19 patients using in-house rapid antibody dot-blot test kits and (ii) determine the sensitivity, specificity, positive and negative predictive values of this kit.

Methods

Sample Collection

Using the quota sampling technique, 509 patient samples were collected from the Fever clinic of BSMMU and Central Police Hospital, Razarbag, Dhaka. Among the 509 patients, both blood samples and nasopharyngeal swabs were collected from 300 symptomatic suspected COVID-19 infections and 109 convalescent patients from mid-May to mid-June 2020. An additional 100 serum samples from archived pre-pandemic specimens collected between the years of 2017 to 2018 were used as control.

One-Step Quantitative Reverse transcription-polymerase Chain Reaction (RT-qPCR)

Beforethe examination of the nasopharyngeal specimens for SARS-CoV-2 RNA using Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR- Fluorescence Probing) (Sansure Biotech Inc., China), the samples were stored in -80°C. The assay includes SARS-CoV-2 2019-nCoV markers N and ORF1ab gene for screening and confirmation and human RNase P genes (hRNaseP) (Sansure Biotech EUA Kit, China) internal and extraction control. RNA was extracted from clinical samples (5µl) with the Sansure Biotech Sample Release Reagent (5µl). In accordance with the manufacturer's instructions, a 25µL reaction contained 10µL of extracted RNA, 13µL of 2019-nCoV-PCR Mix and 2µL of 2019-nCoV-PCR-Enzyme Mix. The one-step RT-qPCR was performed at 50°C for 30 min for reverse transcription, followed by cDNA predenaturation at 95 °C for 1 min and then 45 cycles of denaturation, annealing, extension, and fluorescence collection at 95°C for 15s, 60°C for the 30s and device cooling at

25°C for 10s using CFX96 Deep Well Dx Systems instrument (Bio-Rad Laboratories). A SARS-CoV-2 positive sample displays a typical S-shape amplification curve detected either or both at N and ORF1ab gene with a cycle threshold, Ct≤40.

Serological Test Kit Preparation

The antibody kit was prepared by assembling nitrocellulose membrane and absorbent pads purchased from Bhat Biotech Ltd., India. A well-characterized goat-anti-mouse IgG (Fapon Biotech) as the control dot and a fusion of 1:10-800 dilution of SARS-CoV-2 antigens, namely, receptor binding domain, nucleocapsid, and spike (S1 and S2) recombinant proteins (Fapon, China; MP Biomedicals, Singapore; The Native Antigens, UK; Creative Diagnostics, USA) as the test dot were immobilized on the nitrocellulose membrane (Ken Biotech, China; Bhat Biotech, India; Sartorius, France). The absolute cassettes were stored in an air-tight bag at 4°C until later use.

Gold Suspension

Commercially prepared Protein A-gold conjugate (Bhat Biotech Ltd., India) was used in this study. The conjugate was stored at 4°C in the dark for further use.

Serological Testing Procedure

To implement the in-house SARS-CoV-2 rapid antibody dot-blot procedure, initially, serum was separated from every 5 ml of patient's blood collected in vacutainer by centrifugation at 3500 rpm for 5 minutes at ambient temperature. All the separated serum was stored at -80°C until further use.

The unprocessed serum samples were heated at 56°C for 30 minutes before the test procedure to inactivate and mitigate the associated risks of any latent virus. Subsequently, the heat-inactivated serum samples were cooled to room temperature in the BSL2 cabinet[20]. The samples were immediately diluted at 1:2 with the commercial dilution buffer (Bhat Biotech Ltd., India). Further, the addition of diluted serum (50µl) onto the cassette was accompanied by 50µl of wash buffer. The test was completed by adding one drop of gold conjugate followed by 50µl of wash buffer. The semi-quantitative presence of the antibody was demonstrated spontaneously by the formation of a pink test blot, the strength of which is interpreted naked-eye by the preset five-point gradient scale. The validity of the test was confirmed by the visibility of a pink control spot.

Statistical Analysis

All the collected data were populated in an SPSS spreadsheet (IBM SPSS Statistics Version 23.0), and analysis was done using the STATA program (Version 13, Stata Corp, College Station, Texas, USA).A p-value of <0.05 was considered significant. Sensitivity, specificity, positive and negative values, and area under curve (AOC) with 95% confidence interval were characterized.

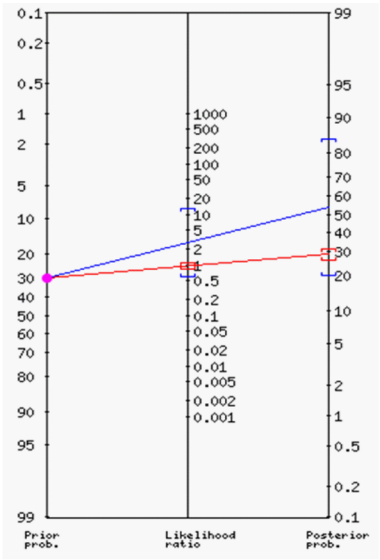
Ethical approval

Our work was undertaken with ethical approval from the Directorate General of Drug Administration (DGDA) (Memo No. DGDA/CLT/132/13/98/66, dated 29-04-2020) and the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU) (Memo No. BSMMU/2020/6016, dated 13-5-2020), including donor written informed consent for the use of their specimen for research. Each ethical guideline, such as Helsinki declaration, CIOMS guideline, GINA act, BMRC and FDA guidelines, etc. were followed during this cross-sectional study.

Results

Sample Grouping

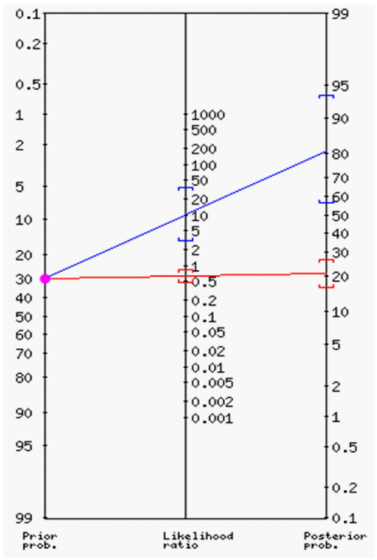
Based on the analyzed statistics from RT-PCR, the data set was classified into three groups; namely, Group I (n-200) comprising of RT-PCR positive symptomatic patients having symptoms for 1-14 days; Group II (n-100) involved RT-PCR negative symptomatic patients with symptoms lasting 1-14 days, and Group-III (n-109) included of convalescent patients who were RT-PCR positive, recovered and passed at least 14 days of symptom-free period. Group I was further divided into Group Ia (n-100), consisting of patients having symptoms for 1-7 days, and Group Ib (n-100), which comprises patients having symptoms for 8-14 days. A Control group (Group-IV, n-100) entailed of archived (pre-pandemic) patient's serum samples collected more than two years ago was included in this study (Table 1).



(A) Prior Probability (%) = 30

LR_Positive = 2.75
Post_Prob_Pos (%) = 54

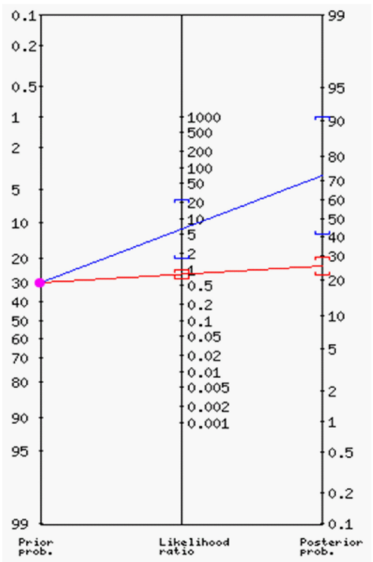
LR_Negative = 0.93
Post_Prob_Neg (%) = 28



(B) Prior Probability (%) = 30

LR_Positive = 10.25
Post_Prob_Pos (%) = 81

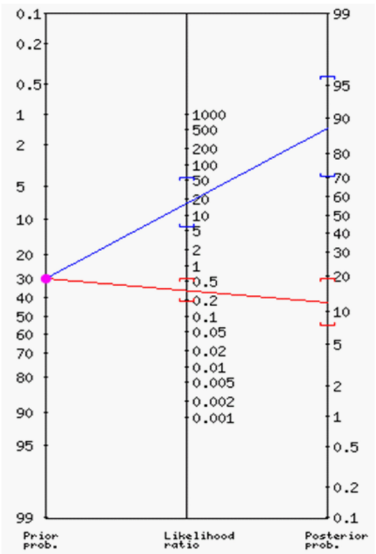
LR_Negative = 0.61
Post_Prob_Neg (%) = 21



(C) Prior Probability (%) = 30

LR_Positive = 6.15
Post_Prob_Pos (%) = 72

LR_Negative = 0.79
Post_Prob_Neg (%) = 25



(D) Prior Probability (%) = 30

LR_Positive = 17.4
Post_Prob_Pos (%) = 88

LR_Negative = 0.32
Post_Prob_Neg (%) = 12

Figure 1: Fagan nomogram shows the post-test probability of different groups in assessing the antibody production against COVID-19 disease using the in-house antibody dot blot kit. On the top left (A) Group Ia; on the top right side (B) Group Ib; on the bottom left (C) Group II and on the bottom right side (D) Group III.

Table 1: Sampling of patients based on RT-PCR.

Groups	No. of patients	Investigations
Group Ia	100	RT-PCR positive patients for SARS-CoV-2 with illness duration of 1-7 days.
Group Ib	100	RT-PCR positive patients for SARS-CoV-2 with illness duration of 8-14 days.
Group II	100	RT-PCR negative patients for SARS-CoV-2 with suspected illness duration 1-14 days
Group III	109	RT-PCR positive patients for SARS-CoV-2 but symptom-free for more than 14 days
Group IV	100	Archived pre-pandemic samples well preserved at -70^{0}C (Control group)

Pan-antibody dot-blot results in different groups:

Table 2: Test outcomes with sensitivity and specificity in different groups according to rapid antibody dot blot test.

Groups	COVID-19 antibody test outcomes		Total	Sensitivity	Specificity
	Positive	Negative			
Group Ia	11	89	100	11%	96%
Group Ib	41	59	100	41%	96%
Group II	20	80	100	20%	96%
Group III	76	33	109	69.72%	96%
Group IV	04	96	100	04%	96%

Between mid-May and mid-June,2020 we observed a steady specificity of 96% (95% CI: 90.07% to 98.90%) and a prevalence of 30% (Table 3) in every group.From Table 2, according to the rapid antibody dot blot test analysis amongst 509 subjects, there were 152 individuals whose screening test was positive, with the highest number of positive outcomes in convalescent patients (Group III; 76) and second in patients who are in the second week of illness (Group Ib; 41).

Individuals who were SARS-CoV-2 RT-PCR positive (Group I) showed a higher (52%) than those who were clinically suspected but tested SARS-CoV-2 RT-PCR negative (20%) (Table III). However, individuals in the second week of illness had an almost four-fold increase in seroprevalence (41%; 95% CI: 31.26% to 51.29%) relative to individuals in the first week of illness (11%; 95% CI: 5.62% to 18.33%). Seroprevalence was highest among those who were previously SARS-CoV-2 RT-PCR positive but had recovered for more than 14 days ago, i.e., Group III. The percentage of patients with developed antibodies in this group almost doubled to 69.7% (95% CI: 60.19% to 78.16%).

With regard to PPV and NPV, we noted that the group with the highest percentage was, again, Group III (88.2%; 95% CI: 73.94%-95.16% and 88.1%; 95% CI: 84.73%-90.80%, respectively), followed by Group Ib (81.46%; 95% CI: 62.04%-92.19% and 79.15%; 95% CI: 76.24%-81.79%) and by Group II (72.51%; 95% CI: 47.99%-88.29% and 74.82%; 95% CI: 72.00% to 77.44%).

The estimates of serologic-based antibody testing were also represented with Fagan nomogram plots for positive and negative results of various groups to determine the clinical efficacy (Figure 1). With a prior probability or prevalence of 30%, if patients in Group Ia, Ib, and II test positive for SARS-CoV-2 antibody, the post-test probability that the patients truly have a history of SARS-CoV-2 infection would be approximately 54%, 81%, and 72%, respectively. On the other hand, if the patients in Groups Ia, Ib, and II test negative, the post-test probability that the patients have a history of SARS-CoV-2 infection would be approximately 28%, 21%, and 25%, respectively.

The LR+ of Group III is 17.4, which is quite large, indicating that a positive result from the dot blot test supports the likelihood of the antibody being present. Whereas the LR- is 0.32, which is near zero, indicating that a negative result from the dot blot test does not support the likelihood of antibody being absent. Using Fagan's Nomogram and LR+ (Fig. 1D), we found that the patient's probability of having the antibody in group III increases from 30% to 88% with a positive test result. In contrast, by using LR- and Fagan's Nomogram, we deduced that the patients' probability of having the antibody production dropped from 30% to 12%.

In addition, from Table III, we also observed that the prevalence odds ratio (OR) for Group III was almost seven times higher (55.3; 95% CI: 18.76-162.83) than Group II participants (7.83; 95% CI: 2.48-24.71). The second highest prevalence odds ratio (16.67; 95% CI: 5.68-48.94) was found in Group Ib, whereas Group Ia had a minuscule odds ratio (2.96; 95% CI: 0.9-9.65), implying that the individuals who are exposed to SARS CoV-2 for one week are least likely to produce antibody against the COVID-19 in comparison to rest of the groups.

Discussion

We developed a rapid pan dot-blot kit to detect SARS-CoV-2 antibodies in the blood sample of infected individuals to address the urgent need for immunoassay tests in Bangladesh. BSMMU

Table 3: Statistical Analysis of Different Groups.

Statistics	Group Ia		Group Ib		Group II		Group III	
	Values	95% Confidence Interval	Values	95% Confidence Interval	Values	95% Confidence Interval	Values	95% Confidence Interval
Sensitivity, %	11.0	5.62 to 18.33	41.0	31.26to 51.29	20.0	14.77to 36.87	69.7	60.19 to 78.16
Specificity, %	96.0	90.07 to 98.90	96.0	90.07to 98.90	96.0	90.07to 98.90	96.0	90.07to 98.90
Prevalence, %	30.0	-	30.0	-	30.0	-	30.0	-
Positive Predictive Value (PPV), %	54.10	27.97 to 78.15	81.46%	62.04to 92.19	72.51	47.99 to 88.29	88.2%	73.94 to 95.16
Negative Predictive Value (NPV), %	71.57	69.92 to 73.16	79.15	76.24 to 81.79	74.82	72.00 to 77.44	88.1	84.73 to 90.80
***LR^+**	2.75	0.91 to 8.35	10.25	3.81 to 27.55	6.15	2.15 to 17.59	17.4	6.62 to 45.90
***LR^-**	0.93	0.86 to 1.00	0.61	0.52 to 0.73	0.79	0.68 to 0.91	0.32	0.24 to 0.42
Odds Ratio (OR)	2.96	0.9 to 9.65	16.67	5.68 to 48.94	7.83	2.48 to 24.71	55.3	18.76 to 162.83

*In likelihood ratio analysis, the further away the value is from 1 (in any direction), the more valuable the test.

subsequently verified the kit. During the early stage of the pandemic, COVID-19 detection utilizing RT-PCR based method was minimal and was affected by adequately trained human resources. Therefore, the kit might help to address contact tracing and the spread of the virus in the population.

In our evaluation, we found that in convalescent patients, the kit could detect 70% of the population, while only 11% of the patients were detected during the first week of infection. An earlier study with SARS reported that during the 14 days post-onset of SARS, RT-PCR based technique is the most sensitive in detecting the viral RNA; however, during the convalescent phase of SARS, detection of serum antibodies is more critical than detecting the RNA of SARS virus[21]. This is in line with the early development of antibodies. It has been previously mentioned that antibodies against SARS-CoV-2 generally develop 5-7 days after the onset of symptoms.

However, it is to be noted that there are some limitations to our study. Due to the lack of enough information and human resources, we could not collect samples from the same person over three to four weeks. Furthermore, reports have been that a small percentage of the population fails to develop antibodies in their first infection[22]. This may be due to the activation of the cell-mediated immunity[23]. Additionally, the test kit needed to be evaluated against other antibody kits; however, during the early stage of the pandemic, when we conducted our study getting such items was difficult to impossible because of ongoing worldwide lock-down.

It is to be noted that the 96% specificity of the kit is indicative of the efficiency of the kit in detecting SARS-CoV-2 specific antibodies. The reduction in sensitivity in subsequent validation may result from the implementation of Protein A in the detection of antibodies. It is well established that Protein A can detect IgG, IgA, and IgM with varying degrees of efficiency[24,25]. Nonetheless, our kit identifies the first step in the development of SARS-CoV-2 antibody detection in the population. It may indicate whether a person has been infected with SARS-CoV-2 in the past and can help understand the circulating immunity against SARS-CoV-2 in the population. Additionally, it may help us understand the antibody profile of the people vaccinated against SARS-CoV-2.

Conclusion

No successful medication has been developed yet to tackle COVID-19, and the ones in use have either no confirmatory effect rather have adverse side effects[26-29].In this hopeless situation, physicians have used repurposed prophylaxis without knowing their exact outcomes[30]. Moreover, intensive-care-unit (ICU) requirements for ventilation have increased the chances of nosocomial infection and use of antimicrobials, resulting in antimicrobial resistance and death[31-33]. COVID-19 vaccines are the only hope. SARS-CoV-2 infection or COVID-19 vaccines induces both humoral and cell-mediated immunity (CMI)[34-36].It is difficult and expensive to assess the CMI post-vaccination or natural infection; as a result, serological assays are the easiest and cheapest alternative to indicatethe efficacies of vaccines. A pan-antibody assay kit can detect a broadrange of immunoglobulins against specific antigens and thus be implemented in serosurveillance studies. The assay kit developed in this work can detect a wide range of immunoglobulins. Moreover, due to the incorporation of a unique antigen cocktail combination comprising a wide range of SARS-CoV-2 surface antigens used in this assay kit, it can detect immunoglobins specific to multiple targets in a single run.

Recommendations

I. This rapid antibody dot blot may be recommended for observing the development of antibodies during the first week of illness. However, the test shall not be used as a substitute for RT-qPCR in places where such tests are available.

II. This rapid antibody dot blot may be recommended for diagnosis of COVID-19 from the first week of illness where RT-PCR facilities are not available and in clinically suspected but RT-PCR negative patients.

III. This assay may be recommended for screening prospective plasma donors.

IV. This immunoassay may be recommended for observing the antibody development after vaccine administration.

V. This assay may be recommended for serological surveillance and planning of lock-down exit strategies.

Acknowledgment

The authors express their thanks to all the participants who were willing to participate in the study. They also thank their team members for their support work during the current pandemic situation. The authors are also grateful to Professor Dr. Mainul Haque for his guidance, suggestions,and careful reading of this manuscript.

Data Availability Statement

All data underlying the findings in our study are freely available in the manuscript. For additional information, please refer to: http://www.grblbd.com

Funding source

No funding sources

Conflict of interest

The authors declare that they have no competing interest

References

1. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol*. 2015;**1282**:1-23. doi: 10.1007/978-1-4939-2438-7_1.
2. Abdi M. Coronavirus disease 2019 (COVID-19) outbreak in Iran: Actions and problems. *Infect Control Hosp Epidemiol*. 2020; **41(6)**:754-755. doi:10.1017/ice.2020.86
3. World Health Organization 2021, *WHO Coronavirus (COVID-19) Dashboard*, Accessed 15 March 2021, https://covid19.who.int/.
4. Centers for Disease Control and Prevention 2021, *Symptoms of Coronavirus*. Accessed 15 March 2021, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html.
5. Cucinotta D., Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed*. 2020; **91(1):**157–160. doi: 10.23750/abm.v91i1.9397.
6. OisheeMJ, Ali T, Jahan N, Khandker SS, Haq MA, Khondoker MU, et al. COVID-19 Pandemic: Review of Contemporary and Forthcoming Detection Tools. *Infect Drug Resist*. 2021;**17**;14:1049-1082. doi: 10.2147/IDR.S289629.
7. Zakariah SZ, Tengku Jamaluddin TZM, MahayidinH, Dahari Z, and Ibrahim R. Laboratory Diagnostics in COVID-19: What We Know So Far. *Bangladesh Journal of Medical Science*. 2020; **19**: S93–S100. https://doi.org/10.3329/bjms.v19i0.48199
8. Emery SL, Erdman DD, Bowen MD, Newton BR, Winchell JM, Meyer RF, et al. Real-time reverse transcription-polymerase chain reaction assay for SARS-associated coronavirus. *Emerg Infect Dis*. 2004;**10(2)**:311-316. doi:10.3201/eid1002.030759
9. Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020; **26**:1033–1036.
10. SilBK, Jamiruddin MR, Haq MA, Khondoker MU, Jahan N, Khandker SS, et al. AuNP Coupled Rapid Flow-Through Dot-Blot Immuno-Assay for Enhanced Detection of SARS-CoV-2 Specific Nucleocapsid and Receptor Binding Domain IgG. *Int J Nanomedicine.* 2021;**16**:4739-4753. doi: 10.2147/IJN.S313140. PMID: 34267520; PMCID: PMC8277418.
11. Sil BK, Jahan N, Haq MA, Oishee MJ, Ali T, Khandker SS, et al.Development and performance evaluation of a rapid in-house ELISA for retrospective serosurveillance of SARS-CoV-2. *PLoS ONE*.2021; **16(2)**: e0246346.
12. Wen T, Huang C, Shi F-J, Zeng X-Y, Lu T, Ding S-N, Jiao Y-J. Development of a lateral flow immunoassay strip for rapid detection of IgG antibody against SARS-CoV-2 virus. *Analyst*. 2020;**145**:5345–5352. doi: 10.1039/d0an00629g
13. Kubina R and Dziedzic A. Molecular and Serological Tests for COVID-19. A Comparative Review of SARS-CoV-2 Coronavirus Laboratory and Point-of-Care Diagnostics. *Diagnostics*. 2020; **10**:434. doi:10.3390/diagnostics10060434
14. Renart J, Behrens MM, Fernández-Renart M, and Martinez JL. Immunoblotting Techniques. *Immunoassay* 1996, 537– 554, DOI: 10.1016/B978-012214730-2/50024-8
15. Kawashima Y, Shikawa II. Simple and rapid detection of serum antibody to periodontopathic bacteria by dot blotting. *J Periodontal Res.* 2002;**37(3)**:223-9. doi: 10.1034/j.1600-0765.2002.01608.x.
16. Gerbig Jr DG, Fenk CJ, and Goodhart AS. The Dot Blot ELISA: A Rapid & Simple Experiment to Demonstrate Antibody-Antigen Interactions. *The American Biology Teacher*. 2000; **62(8)**: 583-587. Doi: 10.2307/4450982
17. Justiz Vaillant AA, McFarlane-Anderson N, Akpaka PE, Smikle MP, Ramirez N, and Cadiz A. Use of Dot Blots Analysis in the Separation of Anti-HIV Antibodies in Animals. *J Chromat Separation Techniq*. 2013; **4(5)**:181. doi: 10.4172/2157-7064.1000181
18. Hawkes R, Niday E, and Gordon J. A Dot-lmmunobinding Assay for Monoclonal and Other Antibodies. *Anal Biochem.* 1982;**119(1):**142-7. doi: 10.1016/0003-2697(82)90677-7.
19. Ramachandran S, Singhal M, McKenzie KG, Osborn JL, Arjyal A, Dongol S, et al. A Rapid, Multiplexed, High-Throughput Flow-Through Membrane Immunoassay: A Convenient Alternative to ELISA. *Diagnostics*. 2013; **3**:244-260. doi:10.3390/diagnostics3020244
20. Hu X, Zhang R, An T, Li Q, Situ B, Ou Z, et al. Impact of heat-inactivation on the detection of SARS-CoV-2 IgM and IgG antibody by ELISA. *Clinica Chimica Acta*. 2020; **509**:288–292. https://doi.org/10.1016/j.cca.2020.06.032
21. Wu HS, Chiu SC, Tseng TC, Lin SF, Lin JH, Hsu YH, et al. Serologic and molecular biologic methods for SARS-associated coronavirus infection, Taiwan. *Emerg Infect Dis*. 2004; **10**(2): 304-10. doi: 10.3201/eid1002.030731.
22. Wang J, Chen C, Li Q, Cai P, Wang Z, Wang L. COVID-19 confirmed patients with negative antibodies results. *BMC Infect Dis.* 2020;**20(1):**698. doi: 10.1186/

s12879-020-05419-3.

23. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell*. 2020; **183**(1): 158-168.e14. https://doi.org/10.1016/j.cell.2020.08.017

24. Falugi F, Kim HK, Missiakas DM, and Schneewind O. Role of Protein A in the Evasion of Host Adaptive Immune Responses by *Staphylococcus aureus*. *mBio*. 2013; **4(5)**: e00575-13. doi:10.1128/mBio.00575-13.

25. Inganäs M, Johansson SGO, and Bennich HH. Interaction of Human Polyclonal IgE and IgG from Different Species with Protein A from *Staphylococcus aureus*: Demonstration of Protein-A-reactive Sites Located in the Fab: Fragment of Human IgG. *Scand J Immunol.* 1980;**12(1):**23-31. doi: 10.1111/j.1365-3083.1980.tb00037.x.

26. Dutta S, Kaur R, Bhardwaj P, Deora S, Singh K, Ambwani S, et al. Hydroxychloroquine as therapeutic option in COVID-19: analysis of suspected cardiovascular adverse drug events reported in the vigibase. *Bangladesh J Med Sci*. 2021;**20(4):**897–910. doi:10.3329/bjms.v20i4.54150

27. Charan J, Dutta S, Kaur R, Bhardwaj P, Sharma P, Ambwani S, et al. Tocilizumab in COVID-19: a study of adverse drug events reported in the WHO database. *Expert Opin Drug Saf*. 2021;1–12. doi:10.1080/14740338.2021.1946513

28. Charan J, Kaur RJ, Bhardwaj P, Haque M, Sharma P, Misra S, et al. Rapid review of suspected adverse drug events due to remdesivir in the WHO database; findings and implications. *Expert Rev Clin Pharmacol*. 2021;**14(1):**95–103.

29. Kaur RJ, Charan J, Dutta S, Sharma P, Bhardwaj P, Sharma P, et al. Favipiravir Use in COVID-19: analysis of suspected adverse drug events reported in the WHO database. *Infect Drug Resist*. 2020;**13**:4427–4438. doi:10.2147/IDR.S287934

30. Rahman SZ and Khan S. Patients' case scenario as well as approaches and strategies adopted to manage COVID-19 pandemic at Aligarh Muslim University, Aligarh, India. *Bangladesh JMed Sci*. 2020; **19**: S28–S35. https://doi.org/10.3329/bjms.v19i0.47832

31. Dutta S and Haque M. COVID-19: Questions of Antimicrobial Resistance. *Bangladesh JMed Sci*. 2021; **20(2)**: 221–227. https://doi.org/10.3329/bjms.v20i2.51527

32. Haque M and Godman B. Potential Strategies to Improve Antimicrobial Utilisation in Hospitals in Bangladesh Building on Experiences Across Developing Countries. *Bangladesh J Med Sci*.2021; **20(3)**: 469–477. https://doi.org/10.3329/bjms.v20i3.52787

33. Haque M and Godman B. Potential strategies to reduce inappropriate prescribing and dispensing of antimicrobials in Bangladesh building on the experiences in other developing countries. *Bangladesh JMed Sci*. 2021;**20(4)**: 700–706. https://doi.org/10.3329/bjms.v20i4.54123

34. JamiruddinMR, Haq MA, TomizawaK, Kobatake E, Mie M, Ahmed S, et al. Longitudinal Antibody Dynamics Against Structural Proteins of SARS-CoV-2 in Three COVID-19 Patients Shows Concurrent Development of IgA, IgM, and IgG. *J Inflamm Res.* 2021; **14**, 2497–2506. https://doi.org/10.2147/JIR.S313188

35. BonifaciusA, Tischer-Zimmermann S, Dragon AC, GussarowD, VogelA, KrettekU, et al. COVID-19 immune signatures reveal stable antiviral T cell function despite declining humoral responses. *Immunity*.2021; **54(2)**, 340–354.e6. https://doi.org/10.1016/j.immuni.2021.01.008

36. Hasan A, Al-Ozairi E, Al-Baqsumi Z, Ahmad R, Al-Mulla F. Cellular and Humoral Immune Responses in Covid-19 and Immunotherapeutic Approaches. *Immunotargets Ther*. 2021;**10**:63-85. https://doi.org/10.2147/ITT.S280706