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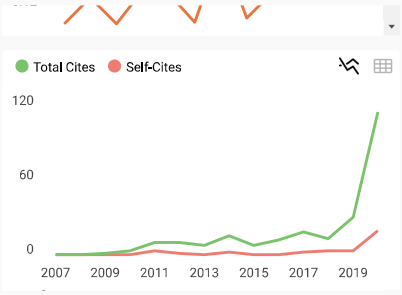

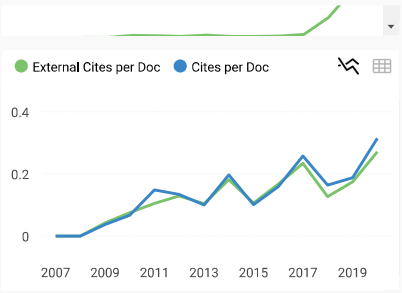

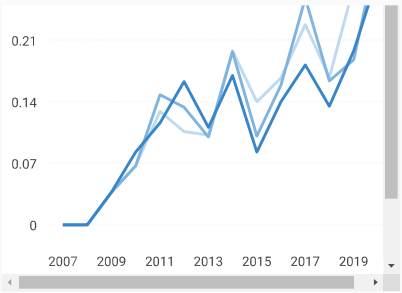

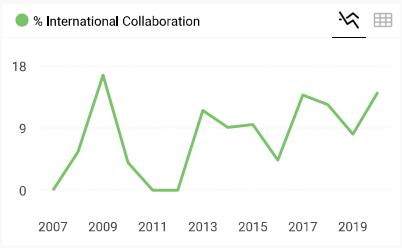

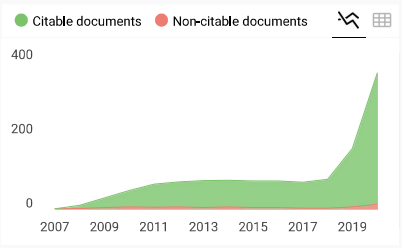

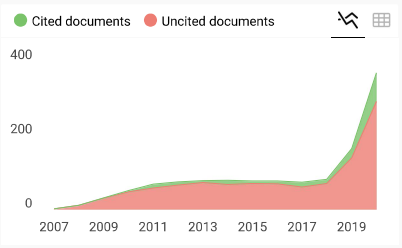

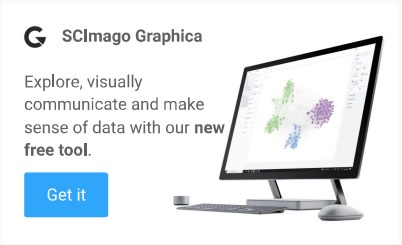

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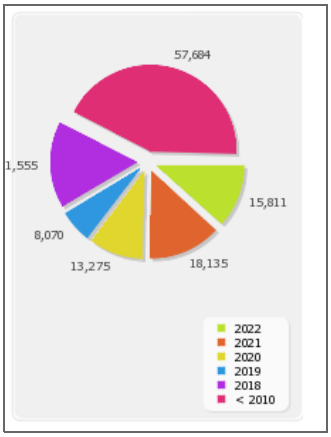

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The effectiveness of dry and wet teeth-brushing technique using 1.5% enzyme toothpaste as plaque prevention (article-view.php?id=173870)
(article-view.php?id=173870)
View abstract
View references
9
Ari Kurniawati; Nurul Inayati; Lalu Srigede.
The association between individual characteristics, personal hygiene, and environmental sanitation to pediculosis capitis in students of Mentokok Elementary School, West Praya, Central Lombok (article-view.php?id=173871)
(article-view.php?id=173871)
View abstract
View references
10
Dony Chrismanto; Miyayu Soneta Sofyan; Ira Sari Yudaniayanti.
Analysis of the cellular and humoral immune response (IgG, CD4) in rabbits immunized with the antigenic protein of leucocytozoon caulleryi (article-view.php?id=173875)
(article-view.php?id=173875)
View abstract
View references
11
Anis En Nabiilah; Yunan Jiwintarum2; Erlin Yustin Tatontos2.
Effect of temperature on viability of normal flora bacteria (ESCHERICHIA coli and STAPHYLOCOCCUS AUREUS) (article-view.php?id=173881)
(article-view.php?id=173881)
View abstract
View references
12
Heribertus Agustinus B Tena; Jenny Sunariani; Ahmad Yudianto; Budi Santosa; Tulus Ariyadi.
Alteration in organic elements of sediment in delayed examinations of alkaline pH urine sample using conventional method (article-view.php?id=173886)
(article-view.php?id=173886)
View abstract
View references
13
Ahmad Yudianto; Agung Sosiawan; Reni Sumino.
Kinship analysis on paternity test through str codis locus [CSF1PO, THOI, TPOX & vWA] from Maduranese siblings in Surabaya (article-view.php?id=173889)
(article-view.php?id=173889)
View abstract
View references
14
Ade Nahdia Nandarini; Anggraeni Puspitasari; Ahmad Yudianto.
Differences of height estimation using Karl Pearson formulation and calculation of multiplication factor using trotter and glesser formulation (article-view.php?id=173890)
(article-view.php?id=173890)
View abstract
View references
15
Ivan Panji Teguh; Diffah Hanim; Suminah Suminah.
The relationship between protein intake and vitamin d with the quality of life of the elderly (article-view.php?id=173891)
(article-view.php?id=173891)
View abstract
View references
16
Wiwit Sulistyasmi; Almurdi2, Renowati.
Comparing the degree of direct sputum Afb smear-positive with the sedimentation in patients suspected of pulmonary tuberculosis (article-view.php?id=173892)
(article-view.php?id=173892)
View abstract
View references
17
Mutia Hariani Nurjanah.
Description of erythrocyte morphology with blood smear method of giemsa staining in patients at the Thalassemia Patients Parents Association Indonesia (TPPAI) Kediri (article-view.php?id=173893)
(article-view.php?id=173893)
View abstract
View references
18
Devi Eka Juniarti; Tuti Kusumaningsih; Adioro Soetojo; Eric Priyo Prasetyo; Yulianti Kartini Sunur.
Antibacterial activity and phytochemical analysis of ethanolic purple leaf extract (Graptophyllum Pictum L.griff) on LACTOBACILLUS ACIDOPHILUS (article-view.php?id=173895)
(article-view.php?id=173895)
View abstract
View references

19
Akhmad Muzamil; Suryani Dyah Astuti; Kamelia; Suhariningsih.
Fat suppression Spectral Adiabatic Inversion Recovery (SPAIR) to optimize the quality of MRI pelvis image (article-view.php?id=173896)
(article-view.php?id=173896)
View abstract
View references
20
Selvy Wikan Garini; Suryani Dyah Astuti; Idha Kusumawati; Yunus Susilo.
Combination of curcumin photosensitizer with laser diode to reduce antibiotic resistant bacterial biofilms (article-view.php?id=173897)
(article-view.php?id=173897)
View abstract
View references
21
Elfrida Melisa Ngamal; Maria Immakulata Diah Pramudianti; Edy Prasetya.
Correlation between eosinophil to Leukocyte Ratio (ELR) and HbA1c in Type 2 diabetes mellitus patients (article-view.php?id=173898)
(article-view.php?id=173898)
View abstract
View references
22
Nunuk Dyah Retno Lastuti; Lucia Tri Suwanti; Poedji Hastutiek; Dyah Ayu Kurniawati; Heni Puspitasari.
Molecular detection of entamoeba spp in long-tailed macaque (Macaca FASCICULARIS) at Baluran National Park, Indonesia (article-view.php?id=173900)
(article-view.php?id=173900)
View abstract
View references
23
Beta Novia Rizky; Mieke Sylvia Margaretha Amiatun Ruth; Ahmad Yudianto.
DNA purity and concentration analysis from toothpick as the evidence for forensic examination (article-view.php?id=173901)
(article-view.php?id=173901)
View abstract
View references
24
Mohammad Abdul Rahman Usman; Reinaldy Octavianus Yan Dimpudus; Ledy Ana Zulfatunnadiroh; Rachmadita Yoga Pratiwi; Azizah Sastrawati Paneo; Chairil Anjasmara Robo Putra1.
The relationship between diet pattern and gastritis prevalence in nursing Semester II study program students (article-view.php?id=173904)
(article-view.php?id=173904)
View abstract
View references
25
Sawitri Dwi Indah Pertami; Theresia Indah Budhy.
The role of moringa oleifera l. leaves extract in increasing caspase 3 expressions in carcinoma of oral squamous cells (article-view.php?id=173906)
(article-view.php?id=173906)
View abstract
View references
26
Achmad Faisol; Ahmad Yudianto; Hartono Kahar; Suryani Dyah Astuti.
Relationship of therapeutic communication and healing between nurse and patient (article-view.php?id=173909)
(article-view.php?id=173909)
View abstract
View references
27
Pratiwi Soesilawati; Tantiana; Annisa Zahra.
Anti immunogenicity evaluation of bovine demineralized dentine membrane material (article-view.php?id=173910)
(article-view.php?id=173910)
View abstract
View references
28
Wd. Syafitri Salsabila; Yusuf Ahmad Husein; Zainal Rahmadsyah; Rahmaniar; La Ode Ahmad Nur Ramadhan; Alimin.
Transparent collagen soap from shellfish (Anadara GRANOSA) with additional oils from olae plant (Etlingera CALOPHRYS) (K.SCHUM.) A.D.POULSEN (article-view.php?id=173911)
(article-view.php?id=173911)
View abstract
View references
29
Marselaonety La'lang; Theresia Indah Budhy; Rini Prastyawati.
Description of hematocrit in malaria tropica (PLASMODIUM falciparum) patients at Jayapura Regional General Hospital (article-view.php?id=173916)

(article-view.php?id=173916)
View abstract
View references
30
Catur Retno Lestari; Harsono Salimo; Adi Magna Patriadi Nuhriawangsa.
The relationship between energy intake and fat intake with fine motor skill in infants aged 6-11 months (article-view.php?id=173919)
(article-view.php?id=173919)
View abstract
View references
31
Cita Rosita Sigit Prakoeswa; Anang Endaryanto; Tri Wahyu Martanto; Joni Wahyuhadi; Thinni Nurul Rochmah; Moses Glorino Rumambo Pandin.
Mapping survey of community satisfaction at an academic hospital in Surabaya (article-view.php?id=173920)
(article-view.php?id=173920)
View abstract
View references
32
Nunuk Nugrohowati; Melly Kristanti; Muhammad Fiki Fauzan; Abdul Kolib.
The physical symptoms and risk factors of COVID-19 among academic community during the large-scale social restriction period in the Faculty of Medicine UPN Veteran Jakarta (article-view.php?id=173983)
(article-view.php?id=173983)
View abstract
View references
33
Meggy Wulandari Kai; Rahayu Anggraini; Khamida; Wesiana Heris Santy.
The effect of combination of classical music therapy and breathing exercise towards the stress and cortisol level in hemodialysis patients in Dr. Mm. Dunda Regional Public Hospital in Limboto Gorontalo Regency (article-view.php?id=173985)
(article-view.php?id=173985)
View abstract
View references
34
Heru Hananto; Arief Rahman; Muhammad Fahmi.
Antibacterial activity of ethanolic extract of morel berry (PHYSALIS angulata L.) towards STAPHYLOCOCCUS AUREUS (article-view.php?id=173986)
(article-view.php?id=173986)
View abstract
View references
35
Endah Sekar Palupi; Nurul Faiza; Chairil Anjasmara Robo Putra.
Dexamethasone for Covid-19: a literature review (article-view.php?id=173987)
(article-view.php?id=173987)
View abstract
View references
36
Fery Setiawan; Ahmad Yudianto; Jenny Sunariani; Latief Mooduto.
New normal to achieve high threshold herd immunity by (Ro and Pc) post pandemic COVID-19 (article-view.php?id=173993)
(article-view.php?id=173993)
View abstract
View references
37
Mega Moeharyono Puteri; Andra Rizqiawan; Pratiwi Soesilawati.
The role of MMP-1 gene in the osseointegration of dental implant (article-view.php?id=173995)
(article-view.php?id=173995)
View abstract
View references
38
Jenny Sunariani.
The dual effects of capsaicin: benefits or disadvantages? (article-view.php?id=174000)
(article-view.php?id=174000)
View abstract
View references
39
Yan Fuana; Arief Rakhman1; Gatot Soegiarto; Theresia Indah Budhy.
Pitfalls in the diagnosis or screening of COVID-19 cases based on antibody detection: review and solution (article-view.php?id=174005)
(article-view.php?id=174005)
View abstract
View references
40
Fransiscus Arifin; K. Kuntaman.
Achieving behaviour change in COVID 19 pandemic: lessons learnt from cancer prevention and antibiotic stewardship programs based on social cognition framework (article-view.php?id=174009)

(article-view.php?id=174009)
View abstract
View references
41
Yessy Andriani Fauziah; Mohamad Rulianto; Deka Bagus Binarsa.
Challenges of dentistry in Coronavirus pandemic (article-view.php?id=174013)
(article-view.php?id=174013)
View abstract
View references
42
Nur Fadhilah; Gatot Soegiarto; Theresia Indah Budhy.
Potential of IL-10 as targeted therapy in severe COVID-19 patients (article-view.php?id=174015)
(article-view.php?id=174015)
View abstract
View references
43
Ayuningtyas Wahyu Nurani; Jenny Sunariani; Willy Sandhika; Theresia Indah Budhy.
T2R38 taste receptors can be affected by cancer (article-view.php?id=174020)
(article-view.php?id=174020)
View abstract
View references
44
Puji Rahayu; Ahmad Yudianto.
Death due to violence and sharp force injury on the neck: a case report (article-view.php?id=174022)
(article-view.php?id=174022)
View abstract
View references
45
Edwin Tambunan; Ahmad Yudianto; Galih Endradita.
Death caused by the blunt trauma on the prisoner's chest: a case report (article-view.php?id=174025)
(article-view.php?id=174025)
View abstract
View references
46
Vernando Parlindungan; Puji Rahayu; Sudjari Solichin.
Medico-legal neck stab wound on deadly masseuse: a case report (article-view.php?id=174026)
(article-view.php?id=174026)
View abstract
View references
47
Desy Martha Panjaitan; Ahmad Yudianto; Tutik Purwanti.
An autopsy review of liver injuries resulting from blunt trauma: case report (article-view.php?id=174027)
(article-view.php?id=174027)
View abstract
View references
48
Ria Kumala; Ahmad Yudianto; Tia Maya Affrita.
Natural or unnatural death in COVID-19 pandemic? a case report (article-view.php?id=174030)
(article-view.php?id=174030)
View abstract
View references
49
Ma'rifatul Ula; Abigael Samsi Pagatiku; Ahmad Yudianto.
A case report of extrafamilial child homicide (article-view.php?id=174031)
(article-view.php?id=174031)
View abstract
View references
50

Back

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REVIEW ARTICLE

Pitfalls in the Diagnosis or Screening of COVID-19 Cases Based on Antibody Detection: Review and Solution

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ABSTRACT

SARS-CoV-2 was found in Wuhan, China and has become a global pandemic until now. To achieve control of COVID-19, we need accurate and rapid diagnostic tests. There are two kinds of diagnostic: molecular tests to detect viral RNA and serological tests to detect anti-SARSCoV-2 immunoglobulins. Serological tests become an alternative or a complement to RT-PCR as it might be cheaper and easier. Combining IgM and IgG detection resulted in higher sensitivity than detecting either isotype alone. However, the tests have some limitations to measure IgM or IgG antibodies. Therefore, using merely such tests to diagnose COVID-19 will miss any infections. Consequently, the diagnosis or screening for COVID-19 using antibody test needs to be evaluated. We aim to decrease the risk of false-negative or false-positive in the tests.

Keywords: Diagnosis, Screening, COVID-19, Antibody detection, Molecular testing

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INTRODUCTION

In December 2019, a novel RNA coronavirus was found in Hubei Province, China. It causes acute respiratory distress syndrome (ARDS) and rapid multi-organ failure (1). Coronaviruses are viruses containing a single strand of positive-sense RNA (2). Spike protein is one of the main antigen proteins and structure in viral. It made of a highly glycosylated protein. The envelope, membrane, and nucleocapsid proteins are other structural proteins (3). Most of the patients experience difficulty to breathe in one week and the severely ill patients soon develop ARDS and other inflammations (4). SARS-CoV-2 is transmitted from human to human through droplets (5).

SARS-CoV-2 particles are responsible for virus entry and inducing the innate and adaptive immune response. Host of ACE-2 protein will be bound by viral spike1 (S1) protein. Then, the virus particles will carry out to endocytosis process. SsRNA viral will be detected by the immune system through TLR7 and TLR8 and transcription factor will active in NF-κB and MAPK pathways to induce the expression of pro-inflammatory cytokines in host. ssRNA and dsRNA virus generated as intermediate in virus replication, and it will be recognized by RIG-I and MDA5 which further induce the expression of type I IFN that leads to antiviral state (5). Major histo compatibility complex (MHC) class I will present viral peptides to CD8 + cytotoxic T cells. CD8+ cytotoxic T cells will become active and begin to divide and show clonal expansion and develop virus-specific effectors and memory T cells. Infected tissue cells will be lysed by CD8 + T cells. All viruses and their particles will be recognized by APC and then it will be presented to CD4 + T cells via MHC class II. B cells themselves can be directly recognize the virus and it will automatically activate. B cells can also make interaction with CD4 + T cells. B cells develop into plasma cells and increase the production of specific antibodies for viruses with IgM, IgA and IgG types (6).

In the early 1st week of symptom onset, IgM and IgA were detected, while IgG was detected at around 14 days after the initiation of symptoms (7). One of the tests to diagnose COVID-19 is serological antibody detection, and this test detects IgM, IgG, or total antibodies (typically in the blood) against SARS-CoV-2. There are many methods for serological antibody detection including ELISA, LFIA, and

chemiluminescent immunoassay (8). Nevertheless, the LFA, CLIA and ELISA were limited in sensitivity and specificity (9). In some of the tests, false-positive and false-negative results were still found (10). Failure to detect people with COVID-19 can cause a delayed treatment and risk of further spreading infections to others (11). Therefore, in this article, the writer wants to discuss the evaluation for diagnosis or screening of COVID-19 cases based on antibody detection.

ANTIBODY PROFIL IN COVID-19

The production of IgA, IgM, and IgG antibodies was positive inearly times after the onset of symptoms. The IgM/IgA antibodies as well as the IgG antibodies can be detected from 5 to 14 days PSO, respectively. The IgA antibodies were at a higher positivity rate compared with the IgM antibodies. IgG levels were consistently higher than IgM levels which cause the IgG antibodies to be in the body for quite a long time and able to contribute to long-term immune memory against SARS-CoV-2 (12). Interestingly, Zhang et al. found that increasing of IgG response has a correlation to severity of disease. It can be a marker to differentiate between severe and non-severe cases. Another study also showed that the specificity was also excellent for IgG (100%), but the specificity was significantly different between IgA (78.9%) and IgM (95.8%) in 14 days after onset of symptoms (13). Combining both IgM and IgG SARS-CoV2 detection provides the basis of COVID-19 diagnosis and screening. For early diagnosis, we can use IgM and IgG to help monitor the COVID-19 status (14).

ANTIBODY TESTING OF COVID-19

Utility of antibody testing: i) to establish the diagnosis of patients with special conditions and experiencing some symptoms; ii) to track the transmission; iii) to know the potential for immune disease or other disease; and iv) for sero-epidemiological studies, to understand the spread of COVID-19 (15). The methods used for antibody detection are ELISA, CLIA, and LFIA. All methods are made to detect Immunoglobulin G and/or Immunoglobulin M antibodies (or sometimes in some cases to detect the total of antibodies) against S (mainly RBD) and/or N viral proteins of human sera/blood samples (16).

EVALUATION OF ANTIBODY TESTING

The results of a Taiwanese study about the ability of rapid test- IgM and IgG antibodies in 14 COVID-19 patients are sensitivity (78.6%) and specificity (100%), respectively (12). While based on Kontou et al, on (15) studies from LFIA tests, it has been shown that a combination of IgG and IgM is more sensitive than detecting one of the antibodies alone (15). Combination of Immunoglobulin G-Immunoglobulin M ICT cassette is suitable for the rapid screening of SARS-CoV-2 infection among positive COVID-19 patients, suspect patients and asymptomatic SARSCoV-2 carriers (17). Several LFIA tests have shown false-positive results; there are several causes in false resulted like cross-reactivity of non-specific antibodies (e.g. have been exposed to other types of corona virus). We have to collect full information including the patients' hometown or native areas, ethnic groups, children, as well as those with immunology disease (18).

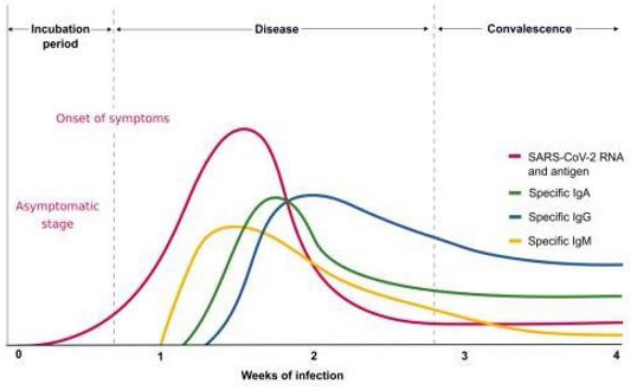

Figure 1 : The ELISA test showed that the detection of IgG, IgA, and IgA combined IgG has higher sensitivity and specificity at > 10 days after symptom onset.

Study result based on the ELISA test showed that the detection of IgG, IgA, and IgA combined, IgG has higher sensitivity and specificity at > 10 days after symptom onset. In addition, for CLIA method, the sensitivity was 65.5%, 88.8% and 100.0% when tested within 0-6 days, 713 days and 14 days after the onset of symptoms with great specificity (99.8%) (14).

The meta-analysis showed that ELISA and LFIA have high specificity. CLIA and ELISA have better sensitivity (90%–96%) and followed by LFIA and FIA (80% to 89%) (11). Based on the three methods, the specificity was high when tested on COVID-19 patients but not suspected.

Meanwhile, the specificity of LFIA and CLIA was lower when tested on positive patients with COVID-19. The Specificity of LFIA was lower when estimated in patients with other viral infections, while ELISAs or CLIAs is higher (19). False-positive and false-negative were still found in many tests. Cross-reaction with other types of coronaviruses can make antibody tests less specific and create false-positive results (16). Meanwhile, false-negative is caused by incorrect timing of diagnosis and low antibody because of inter-individual differences in the immune response (20).

PROPOSED SOLUTION

A serological diagnostic is useful to diagnose patients with acute respiratory distress syndrome and a negative PCR assay. Specimen collection is recommended in

the 2nd week of the disease. There are 2 things that must be understood for early diagnosis of COVID-19 based on the antibody detection: the window period for diagnosis must be shortened and a good specificity must be increased (21). Antibody sensitivity can be higher than the RNA test in 8-21 days after PSO. During acute and convalescent phase, understanding viral and host interactions is important to be able to know both the timing of early seroconversion after an exposure to SARS-CoV-2 and the following duration of antibodies (22). Some studies have shown that the use of S antigen is more sensitive than N antigen in ELISA tests. Because the S antigen has a higher sensitivity, earlier immune response to this antigen, more specifically, and cross-reactivity with less conserved regions of spike proteins existing in other coronaviruses is lower (16). The condition of the patients and the stage of the disease can be taken into consideration when collecting samples to support the accuracy of the diagnosis. Good quality of sampling when the initial day of illness or symptoms occur is the upper respiratory tract, while for the later stage, the use of sputum is more sensitive (23).

CONCLUSION

We found that sensitivities of LFIA method were lower compared with the ELISA and CLIA methods. CLIAs had a lower specificity among the three tests. The level of sensitivity and specificity of each test is different and it depends on commercial kits used. Cross reactivity between anti–SARS-CoV-2 with other types of coronaviruses can occur and be the cause of falsepositive results. Incorrect timing of diagnosis can lead to false-negative due to low antibody. Therefore, to avoid a missed diagnosis in people infected with SARS-CoV-2, we recommend using ELISA or CLIA instead of the widely used LFIA method.

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