

# Application and Interpretation of Antibody-based Rapid Test Kits in the Context of Laboratory Diagnosis of COVID-19

Selvaraj Stephen<sup>1</sup>, Jothimani Pradeep<sup>2</sup>

## ABSTRACT

Rapid kits, which are known as point-of-care (POC) tests, are extremely helpful in the diagnosis of infectious diseases, especially in remote rural areas as well as in physicians' clinics. Since the results are available within 30 minutes, appropriate treatment can be initiated without delay and thus avoiding any complications/mortality. However, in the present scenario of COVID-19 pandemic, the need for adequate validation of the antibody-based rapid kits as an emergency is a challenge. The test results of these kits are to be interpreted with sufficient caution and proper clinical correlation. Track records of the kit manufacturers need proper scrutiny before taking a decision to use a particular test kit.

**Keywords:** Coronavirus, COVID-19, Immunochromatographic test, SARS-CoV 2.

SBV Journal of Basic, Clinical and Applied Health Science (2020): 10.5005/jp-journals-10082-02252

## INTRODUCTION

By definition, the rapid kits are those which yield the test results within 30 minutes.

These point-of care (POC) tests have made a tremendous impact in the laboratory diagnosis of several infectious diseases caused by various bacteria/parasites<sup>1-8</sup> (Table 1). Rapid diagnostic kits are also manufactured and applied in few countries—South Korea, the United states, Hong Kong, Singapore, Australia, the United Kingdom, etc. Immunochromatography is also available for the diagnosis of many infectious agents, viz., *Streptococcus pneumoniae*, gonococcus, syphilis, onchocerciasis, influenza, rotavirus, herpes viruses, rubella viruses, adenovirus, and dengue virus. The rapid test can be used for screening or epidemiological purpose and not for diagnosis unless supported by clinical findings. The rapid kits are suitable to be run by physicians in their own clinics and also in remote rural areas and resource-poor setting laboratories.<sup>1,2</sup> They are known for their ease of performance, affordability, and clear interpretation of the results without any ambiguity. Nevertheless, regular monitoring of the performance of these kits must be an integral part of the healthcare system.<sup>2</sup> The general feature of the rapid kits is also discussed here to enable the clarity regarding a very high percentage of false positivity of the antibody-based rapid COVID-19 kits.<sup>9,10</sup>

## CATEGORIES OF RAPID KITS

### Principle of Rapid Immunochromatography

Immunochromatographic kits targeting IgM and IgG combined antibody contain a colloidal gold-labeled recombinant antigen (on nitrocellulose membrane). There are three lines: one for IgM, another for IgG (G and M lines), and one for control (C) fixed on a nitrocellulose membrane. IgM is fixed with monoclonal antihuman IgM and IgG with antihuman IgG for detecting the antibody. When the specimen is added to the sample well of the cassette, it will move forward along the test card by capillary action. If IgM/IgG is present, it will bind to the colloidal gold-labeled antigen. The antibody/antigen complex will be captured by the antihuman IgM/antihuman IgG antibody, which are

<sup>1,2</sup>Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India

**Corresponding Author:** Selvaraj Stephen, Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India, Phone: +91 9894383368, e-mail: selvarajstephen@mgmcri.ac.in

**How to cite this article:** Stephen S, Pradeep J. Application and Interpretation of Antibody-based Rapid Test Kits in the Context of Laboratory Diagnosis of COVID-19. J Basic Clin Appl Health Sci 2020;3(3):104–106.

**Source of support:** Nil

**Conflict of interest:** None

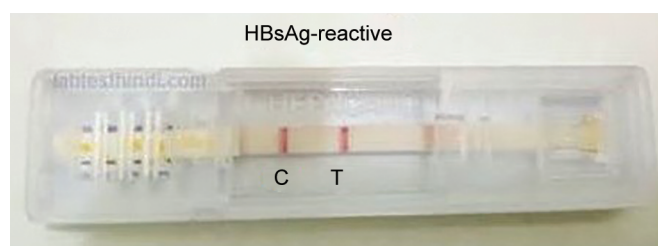
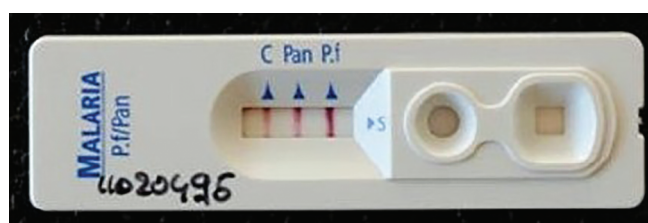
immobilized on the membrane, forming a line against M or G and indicating a positive result for the IgM/IgG antibody. The card also contains a quality control line (C). Regardless of what antibodies are present, the C line should appear to indicate that the sample has been transported properly through the membrane. If the C line does not appear, it indicates that the test result is invalid and a new, unopened test cassette is required to retest the sample.<sup>9</sup> For doubtful results, the testing can be repeated with another batch of the test kit or by some other manufacturers' test kit.

Three different kits are available for the rapid diagnosis of infectious diseases, based on immunochromatography (dot ELISA/cassette ELISA/comb ELISA):

- Kits detecting microbes/their proteins/nucleic acid in clinical specimens—kits for hepatitis B surface antigen (HBsAg) (Fig. 1), kits for detecting malarial parasites (Fig. 2), and NS1 dengue antigen kits are some examples.
- Kits detecting antibodies—IgM and/or IgG: Several kits are available targeting antibodies to viruses, rickettsiae, and parasites.
- Kits detecting both the microbes as well as their antibodies in the same cassette.

**Table 1:** Details of some commonly used rapid kits

S. no.	Name of the kit and manufacturers	Antigen/nucleic acids detection	Antibody detection		Total antibodies
			IgM	IgG	
1	HIV Tri-Dot, J. Mitra & Co Pvt. Ltd.	–	+	+	+
2	Hepacard, J. Mitra & Co Pvt. Ltd.	+	–	–	–
3	SD Dengue Duo kit, Standard Diagnostics, South Korea	+	+	+	–
4	SD Orientia tsutsugamushi IgM/IgG/IgA	–	+	+	+
5	SD BIOLINE Malaria Ag Pf/Pan test	+	–	–	–
6	Leptocheck WB, Tulip Diagnostics	–	+	–	–
7	COVID-19 IgM/IgG Duo, Standard Q	–	+	+	–
8	HCV Tri-Dot, J. Mitra & Co Pvt. Ltd.	–	+	+	+
9	Rubella virus IgG/IgM, Rapid test, Biogate, Laboratories Ltd., Canada	–	+	+	–
10	OnSite HSV-2 IgG/IgM Rapid test	–	+	+	–
11	One-step immunochromatographic test, (Zhuhai Livzon Diagnostics Inc.)	–	–	+	+

**Fig. 1:** HEPACARD positive for HBsAg**Fig. 2:** Malarial rapid card test positive for PAN and *P. falciparum***Fig. 3:** HIV TRI-DOT positive for HIV1 and HIV2 antibodies

HIV TRIDOT (Fig. 3) and Dengue DUO kits (Fig. 4) belong to this category.

There are several commercial kits that have stood the test of time and proved their reliability and reproducibility.

### Sensitivity and Specificity of Rapid Kits

Sensitivity refers to the ability of the kit to pick up the maximum number of positive cases (or minimum number of antibody molecules), while specificity is the kit's result to be negative in noninfectious persons or any other infections. In general, the specificity and sensitivity of these kits is in the range of 85–90%,

**Fig. 4:** Dengue Duo kit positive for NS1Ag and presence of IgM and IgG antibodies

notwithstanding manufacturers' claims to 95–99% range. False positivity could be due to several factors, but the most common ones are the following:

- Presence of impurities, which is a manufacturing defect *per se*.
- Antigenic cross-reactivity due to closely related microbes and sometimes unrelated organisms.
- Presence of the rheumatoid factor in the serum of autoimmune disease conditions.
- False negativity could be due to absence of an important antigenic determinant.
- Technically, the failure to maintain the "cold chain" from the manufacturing plant—during transportation and storage conditions in the testing laboratory.<sup>2</sup>

The most important material that is not provided in almost all the rapid kits is positive controls with grading of +, ++, and +++, so as to differentiate the strong positives from the weak positives.

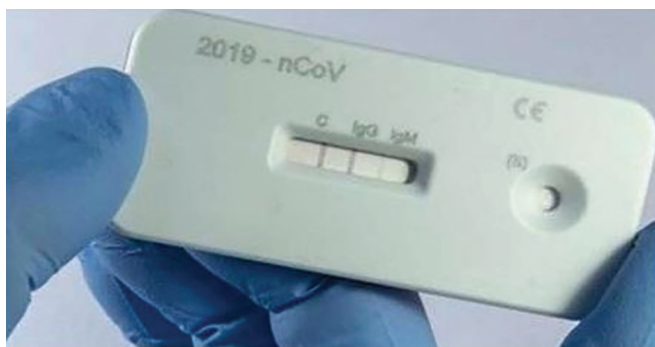


Fig. 5: COVID-19 rapid test kit positive for IgM and IgG

This is needed to pick up even those samples with low levels of antibodies.

Some kits detect the total immunoglobulins (IgM/IgG/IgA) for a particular infectious disease while others target IgM and/or IgG only. The rapid kits recommended for serological diagnosis of COVID-19 belongs to the second category<sup>2</sup> (Fig. 5). In any infectious disease diagnosis, the “gold standard” test is the isolation of the agent in culture/demonstration of nucleic acid (DNA/RNA) in the clinical samples.<sup>2</sup> However, serological gold standard tests are already in place. The immunofluorescence assay (IFA) is generally considered as the gold standard test for viral and rickettsial diseases. In view of its technical complexity and subjectivity, the enzyme-linked immunosorbent assay (ELISA) and its modification of immunochromatographic-based rapid kits are considered to be equivalent to IFA, which is affordable and objective.<sup>3–5</sup> The time lines for the appearance of antibodies in blood for COVID-19 from the time of exposure to this virus are as follows:<sup>4</sup>

- 7–21 days for IgM
- 14 days onward for IgG
- 1–28 days for SARS CoV2 RNA

Generally, IgM antibodies disappear after 28 days, whereas IgG antibodies might persist longer for several weeks to months.<sup>4</sup> This can vary depending upon the immune status of the individual, age, and other comorbid conditions as well as the infecting viral load. IgM positivity is therefore generally regarded as indicating a recent/acute infection.<sup>4</sup> Due to false positivity and false negativity in the antibody tests, the confirmation of the infection is through RT-PCR, which detects viral RNA/antigens. Interpretation of the rapid kit results is made in correlation with the clinical findings.<sup>7</sup> With our limited knowledge of COVID-19, the sufficient care and caution has to be exercised in the interpretation of IgM antibody-based rapid kits, since the knowledge about false positivity/false negativity related to this disease is poorly understood. Even kits with excellent performance of 95% or more were found to show a

very poor 30–40% sensitivity within 4–5 years.<sup>2,6</sup> The low sensitivity of the kit may be due to not maintaining the “cold chain” during the transportation and storage process or it may be due to defect in the process of incorporating the antigen molecule in the kit by the manufacturers.

## CONCLUSION

The track record of the manufacturing companies needs to be scrutinized in advance and prior to importing the test kits. Due to the enormity and seriousness of the current scenario of COVID-19 pandemic, the rapid kits based only on antibodies must be subjected to a very thorough and frequent scrutiny with intra- and interlaboratory validation by both governmental and nongovernmental agencies.

## REFERENCES

1. Stephen S, Sangeetha B, Ambrose S, Sarangapani K, Gunasekaran D, Hanifah M, et al. Outbreak of scrub typhus in Puducherry & Tamil Nadu during cooler months. *Indian J Med Res* 2015;142(5):591–597. DOI: 10.4103/0971-5916.171289.
2. Stephen S, Anitharaj V, Pradeep J. Poor Sensitivity of a rapid kit for diagnosing Scrub typhus: Need for continuous monitoring and regular quality check. *J Pure App Microbiol* 2018;4(3):1–4.
3. Dall C. Antibody tests may hold clues to COVID-19 exposure, immunity—but it’s complicated. <https://www.cidrap.umn.edu/news-perspective/2020/04/antibody-tests-may-hold-clues-covid-19-exposure-immunity-its-complicated>.
4. COVID-19: No more use of rapid test kits! ICMR issues latest advisory to States and UTs; details here. <https://www.financialexpress.com/lifestyle/health/covid-19-no-more-use-of-rapid-test-kits-icmr-issues-latest-advisory-to-states-and-uts-details-here/1941876/> Accessed on 15.05.2020.
5. Kim YJ, Park S, Premaratna R, Selvaraj S, Park SJ, Kim S, et al. Clinical evaluation of rapid diagnostic test kit for scrub typhus with improved performance. *J Korean Med Sci* 2016;31(8):1190–1196. DOI: 10.3346/jkms.2016.31.8.1190.
6. Pote K, Narang R, Deshmukh P. Diagnostic performance of serological tests to detect antibodies against acute scrub typhus infection in central India. *Indian J Med Microbiol* 2018;36(1):108–112. DOI: 10.4103/ijmm.IJMM\_17\_405.
7. Sethuraman N, Jeremiah SS, Ryo A. Interpreting the diagnostic tests for SARS-CoV-2. *JAMA*. 2020(22):E1–E3. DOI: 10.1001/jama.2020.8259.
8. Rao M, Amran F, Aqilla N. Evaluation of a rapid kit for detection of IgM against leptospira in human. *Canadian J Infect Dis Med Microbiol*. 2019;2019:5763595. DOI: 10.1155/2019/5763595.
9. COVID-19 IgM-IgG Rapid Test Instructions for use. file:///C:/Users/Lenovo/Downloads/51-PI-002.EN-04%20(1).pdf Accessed on 15.05.2020.
10. Pan Y, Li X, Yang G, Fan J, Tang Y, Zhao J, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. *J Infect* 2020;81(1):E28–E32. DOI: 10.1016/j.jinf.2020.03.051.