Diagnostic Accuracy of SARS-CoV-2 Rapid Antigen Test (Oral Fluid) with Unsupervised Self-sampling in the Omicron Period

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ABSTRACT

Background: To assess the performance of rapid antigen tests between unsupervised oral fluid self-sampling and nasal self-sampling during the omicron period.

Objective: To diagnostically validate SARS-CoV-2 Rapid Antigen Test (Oral Fluid) by comparing results with those of nasal self-sampling in the omicron period.

Method: Run rapid in vitro diagnostic tests for detection of antigen to SARS-CoV-2 in nasal and oral fluid sampling, compared to a leading commercial test using clinical specimens for validation of performance.

Result: When nasal self-sampling compared with oral fluidself-sampling, sensitivities were found to be slightly higher in confirmatory testers.

Sensitivity (94.3%): In total 297 PCR in the Clinitest group were confirmatory testers (previously tested positive by a self-test at their own initiative), 280 PCR confirmed positive samples were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid). 17 false negative cases were reported.

Specificity (99.4%): In total 350 PCR confirmed negative samples were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid). Only 2 false positive cases were reported.

Accuracy (97.1%): In total 649 PCR confirmed samples: 630 PCR confirmed samples were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid). Among which, overall sensitivities with nasal self-sampling were 79.0% (95% confidence interval 74.7% to 82.8%) for SARS-CoV-2 by Rapid Antigen Tests on Saliva. Sensitivities were substantially higher in confirmatory testers with Citest SARS-CoV-2 Rapid Antigen Test (Oral Fluid) than in those who tested for other reasons.

Conclusion: Sensitivities of three rapid antigen tests with nasal self-sampling decreased during the emergence of omicron but was only statistically significant for Clinitest. Sensitivities appeared to be substantially influenced by the proportion of confirmatory testers. Sensitivities of Citest SARS-CoV-2 Rapid Antigen Test improved after the addition of Oral Fluid self-sampling. A positive self-test result justifies prompt self-isolation without the need for confirmatory testing. Individuals with a negative self-test result should adhere to general preventive measures because a false negative result cannot be ruled out.

Keywords: Rapid Antigen Test; Oral Fluid; Self-sampling; Omicron Period

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INTRODUCTION

As 2020 rages on, the novel COVID-19 has spread worldwide, resulting in growing numbers of infected individuals and mortality throughout the world. Now that researchers have discovered a wide asymptomatic spread of the virus, testing recommendations have vastly changed as well. Diagnosis of COVID-19 involves molecular or antigen tests.

Antigen COVID-19 tests, or rapid tests, typically provide results faster than a molecular test, but also have a higher chance of missing an active infection. Antigen tests can provide results within minutes, however, compared to a molecular test, more of the virus needs to be present in order to test positive. Sometimes, if an antigen test comes back with a negative result, healthcare provider may suggest patients to complete a molecular test to confirm the result.

FACTS ABOUT OMICRON

Characteristic: Omicron mutant was first found in Africa. The Pango lineage evolutionary classification system classified the mutant into B.1.1.529 mutant, and the World Health Organization (WHO) identified the mutant as a "concerned virus strain" and named it Omicron mutant. Compared with the previous "concerned virus strains", the Omicron mutant has many mutation sites, spreads quickly, and has numerous changes in infectivity and immunological characteristics. There is a risk of immune escape and breaking through the protective effect of existing vaccines. In more than a month since it was found, the mutant has spread rapidly in 77 countries and regions around the world, and has attracted extensive attention worldwide. This article summarized the discovery process, epidemic status, variation characteristics, immune escape and prevention and control strategies of Omicron mutant.

Compared with Delta strain, Omicron strain spreads faster and is more likely to cause individual infection and population transmission. Wei Sheng, director of the Department of Epidemiology and Health Statistics of the School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, and doctoral supervisor, introduced that, through the epidemiological analysis of existing cases, the intergenerational gap between Omicron infection cases was on average 3 days, which was further shortened than Delta strain, and the transmission capacity was about twice that of Delta strain.

Chen Baozhong, director of Xi'an Center for Disease Control and Prevention (CDC), introduced that the Omicron mutant has the following characteristics: 32 different mutations were carried on the spike protein of neocoronavirus, while 16 mutations were found in the spike protein of delta virus strain; Incubation period short, infectivity strong, and the transmission speed fast, foreign data show that the intergenerational days of transmission can be as short as 2-3 days, and the transmission power is 5 times stronger than that of Delta mutant. Symptoms are obscure, and more likely to be sporadic or concentrated outbreak.

PREVENTION FOR COVID-19

The impact of the coronavirus disease-2019 (COVID-19) pandemic has been profound and global. Mitigating future waves and overcoming the pandemic is a global public health priority. Thus, key public health measures include physical distancing, restricting the number of contacts and hygiene measures (including simple respiratory hygiene measures and hand hygiene). Each measure is not recommended in isolation, but as part of a wider package of measures and there is currently limited evidence to confidently quantify the absolute risk reduction attributed to each mitigation method.

DIAGNOSIS FOR COVID-19

PCR testing: Initially, quantitative real-time reverse transcriptase PCR (RT-PCR) testing remains the most widely used test and the current gold standard. This is usually done on a swab taken from the nose and/or throat and is used to detect viral genetic material, if present, in those who are currently infected and 'shedding' the virus (both symptomatic and asymptomatic persons). Of note, other sample types have also been evaluated and a recent meta-analysis of the accuracy of diagnostic tests for COVID-19 showed that PCR testing on sputum and saliva is also sensitive to detecting the virus [1].

PCR tests have a theoretical assay sensitivity and specificity that approaches 100%. However, in operational use, the sensitivity is lower, 73.3% using nasopharyngeal swabs in a recent meta-analysis. This may be due to many reasons, including: a false-negative test, timing of sampling, poor sampling technique, and technical/operational issues such as labelling errors and degradation of the samples/swabs or transport medium if not processed in a timely manner. The timing of the swab test in relation to symptoms is likely to be the biggest factor impacting the test result and potentially leading to a false-negative result [2]. Thus, recommendations (government and NHS) are to take a swab test as early as possible after symptom onset, ideally within 24 to 48 hours to obtain an accurate result. This fits with documented patterns of viral shedding, peaking just before or at the onset of symptoms, although further work is needed to fully understand the exact time course and length of viral shedding and its relationship to infectiousness [3].

As with any diagnostic test, COVID-19 laboratory tests, both positive and negative, should be interpreted in the context of the clinical picture. A single positive PCR test effectively confirms the diagnosis, although there is a very small false-positive rate. PCR testing is sometimes said to be 'overly sensitive' as it can detect viral shedding and dead virus particles after the infectious period (usually approximately 9 days) with people testing positive for a mean of 17 days [3,4]. False-negative tests are more common, although when the background

prevalence is low, they have little impact on the reliability of a negative result. Where the prior probability of having the virus is high, a negative test may be necessary to help exclude the presence of the virus. The false-negative results may paradoxically increase transmission risk with a potential increase in risky behaviors following a negative test [5].

Of note, point-of-care rapid PCR tests for use as near patient-testing devices (e.g. on arrival in Emergency departments to ensure appropriate isolation/cohorting) or in mobile laboratories have also been developed. This test can be almost as sensitive as quantitative PCR [4].

SEROLOGY

Serology tests detect those who have had an antibody response from previous infection with SARS CoV-2; therefore, they need to be taken after a time lag of at least 2 to 3 weeks to allow for the development of a sufficient detectable immune response. A range of commercially available SARS-CoV-2 antibody immuno-assays exist using enzyme-linked immunosorbent assays or chemiluminescence immunoassays on venous blood. The main tests currently used in United Kingdom laboratories are the Abbott SARSCoV-2 assay that detects IgG and the Roche Elecsys assay that detects both IgM and IgG, although there are several other tests, which have been approved have a sensitivity of 83.9% to 92.7% with a specificity of 100% (dependent on the test) in laboratory conditions [6]. A recent Cochrane review of SARS-CoV-2 antibody tests (across 25 assays, 54 studies and 15,976 samples of which 8,526 were confirmed infections) showed a maximum sensitivity for combined IgG or IgM tests at 96% at 22 to 35 days after symptom onset. For IgG tests alone, the sensitivity was 88.2% at 15 to 21 days after symptom onset [7]. The overall specificity was 98% (reported in 35 of the 54 studies) [6]. However, the accuracy of a serology test is determined by comparing the result with a gold standard (in this case PCR testing), which itself may be limited by its own sensitivity [7]. Therefore, the results should be interpreted with caution.

Recent evidence suggests that past infection confers a robust cellular immunity, which persists for at least 6 months post-infection, even in mild or asymptomatic disease [9]. However, the immune response against SARS-CoV-2 at 6 months was 50% greater in those who had symptomatic disease [8]. It is still unclear how long an immune response may persist post-infection and previous infection does not mean that people cannot be re-infected [9]. This is an active area of research and further work is needed to understand this field, particularly the interaction between an antibody response and T-cell immunity on further transmission potential of an individual.

LATERAL FLOW

Lateral flow testing assays detect viral antigens and commercially available assays have been validated and incorporated into the NHS Test and Trace program alongside lab-based PCR tests. Several governments are now purchasing them in large quantities [5]. These tests are easy to use, much like a sophisticated pregnancy test and relatively cheap.

Lateral flow devices (LFDs) are thought to be useful in the detection of infectious cases, not infections per se [10]. They are less sensitive than PCR testing, thus generating more false-negative results [11]. This is particularly true if used during the incubation period (5 to 7 days following the infectious exposure) before the viral antigen can be detected through shedding in the nose and throat, which is possible approximately 1 to 2 days before symptom onset [43,44]. The difference in sensitivity between lateral flow assays and PCR testing is, in part, due to the threshold of detection of virus between the two methods. However, in theory, due to the rapid increase in viral shedding after the incubation period, the difference between the two thresholds (PCR versus LFD) translates to only a short period of time where the two tests may practically differ [12]. The false positive rate for LFDs is low, and this can be overcome by using confirmatory PCR testing (in a low prevalence setting). They are particularly sensitive to the sampling quality and the ideal window of use is narrow [11].

RAPID TESTING TECHNOLOGIES

Initially, the testing capacity was limited as quantitative PCR diagnostics require a molecular laboratory with specialized technical equipment and fully trained staff. Additionally, there is a time lag between test and result, which means that infections can spread before the result is known. Testing technologies that shorten this interval and allow decentralized local testing could play a key role in minimizing onward transmission. Thus, the identification of rapid testing technologies became a major area or research and development, particularly those that could be undertaken as near-patient testing requiring little or no technical expertise to successfully undertake the test. The main rapid technologies developed include LFDs (outlined above), Loop-mediated isothermal amplification (LAMP), and Next generation sequencing (LamPORE – a diagnostic platform combining LAMP with nanopore sequencing) testing. The first two are already in use as part of the wider testing strategy. Table 5 outlines LAMP and LamPORE. Point-of-care PCR testing has also been used as detailed above.

Numerous tests are currently in different stages of development, validation, MHRA approval and roll out. The performance characteristics of each test is different, and each test may be useful in different settings, for example, rapid point-of-care tests for universal screening for Obstetrics and Gynecology patients according to local policies. Understanding the utility of each type of test (and each specific brand) is essential to ensure they are optimal and effective, particularly in a time of urgent need and limited resources.

EVALUATION OF CITEST SARS-COV-2 (COVID-19) ANTIGEN RAPID TEST (ORAL FLUID)

MATERIALS AND METHOD

649 samples were enrolled in the study including 297 with positive results and 352 with negative results. Samples were qualified for the study at laboratory approval. Specimens were collected among those routinely analyzed by RT-PCR for SARS-CoV2 diagnosis, and then swirled in 3ml of viral transport medium (VTM), to allow the same sample of control.

Specimens were as follows:

- 352 PCR negative for SARS-CoV2 without any request for other diagnosis.
- 297 PCR positive for SARS-CoV2 with condition of Ct<37, without any request for further.

SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid) is a single-use test kit intended to detect the novel coronavirus SARS-CoV-2 antigens in human oral fluids, the virus causes COVID-19. This test is designed for home use with self-collected oral fluid samples from symptomatic/asymptomatic individuals who are suspected of being infected with SARS-CoV-2. This test is designed for use by a layperson.

RESULTS ANALYSIS

Clinical performance

A clinical evaluation was conducted comparing the results obtained using the SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid) with RT-PCR (Nasopharyngeal swab) test result.

The clinical trial included 649 oral fluid specimens. The results demonstrated 99.4% specificity and 94.3% sensitivity with an overall accuracy of 97.1%.

94.3% Sensitivity: In total 297 PCR confirmed positive samples: 280 PCR confirmed

Positive samples were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid). There were 17 false negative cases.

99.4% Specificity: In total 352 PCR confirmed negative samples: 350 PCR confirmed

Negative samples were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid). There were only 2 false positive cases.

97.1% Accuracy: In total 649 PCR confirmed samples: 630 PCR confirmed samples

Were correctly detected by SARS-CoV-2 (COVID-19) Rapid Test (Oral Fluid).

The observed accuracy may vary depending on the prevalence of the virus in the population.

Cross-reactivity

Test results will not be affected by other respiratory viruses and commonly encountered microbial flora and low pathogenic coronaviruses listed in table below at certain concentrations.

	PCR confirmed sample numbers	Correct identified	Rate
Positive samples	297	280	94.3% (Sensitivity) (95% CI*: 91.0% ~96.6%)
Negative samples	352	350	99.4% (Specificity) (95% CI*: 98.0% ~99.9%)
Total	649	630	97.1% (Total Accuracy) (95% CI*: 95.5% ~98.2%)

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SUMMARY

The 649 samples tested positive for SARS-CoV-2 by RT-PCR also tested positive by the Citest SARS-CoV-2 Rapid Antigen Test (Oral Fluid), 99.4% in concordance with SARS-CoV-2 negative results. Citest SARS-CoV-2 (COVID-19) Antigen Rapid Test (Oral Fluid) has potential benefit to screening with short turnaround times, simplified operation procedure and decentralized testing environment. Moreover, test results will not be affected by other respiratory viruses and commonly encountered microbial flora and low pathogenic coronaviruses listed in table below at certain concentrations. This finding suggests that rapid antigen testing could be an effective tool for COVID-19 control and prevention. In addition, these tests can also be performed by laypersons at home to identify COVID-19 infections and help limit the spread of disease.

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