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Update of Guidelines for Laboratory Diagnosis of COVID-19 in Korea

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Korean Society for Laboratory Medicine and the Korea Disease Prevention and Control Agency have announced guidelines for diagnosing coronavirus disease (COVID-19) in clinical laboratories in Korea. With the ongoing pandemic, we propose an update of the previous guidelines based on new scientific data. This update includes recommendations for tests that were not included in the previous guidelines, including the rapid molecular test, antigen test, antibody test, and self-collected specimens, and a revision of the previous recommendations. This update will aid clinical laboratories in performing laboratory tests for diagnosing COVID-19.

Key Words: COVID-19, SARS-CoV-2, Laboratory diagnosis, Guidelines, Antigen, Antibody, Pooled test, Rapid molecular test, Saliva, Nasal swab

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INTRODUCTION

We propose an update of the Guidelines for Laboratory Diagnosis of Coronavirus Disease (COVID-19) in Korea issued by Korean Society for Laboratory Medicine (KSLM) and the Korea Disease Control and Prevention Agency (KDCA) that elaborate on the tests recommended for diagnosing COVID-19 [1]. This update includes recommendations for the rapid molecular test, antigen test, antibody test, and self-collected respiratory specimens, which were not covered in the previous guidelines, and a revision of the previous recommendations based on new scientific data (Table 1).

MOLECULAR TESTS

KSLM and KDCA recommend real-time reverse transcription (rRT)-PCR as a molecular test for diagnosing COVID-19. In addition to rRT-PCR, there are various isothermal amplification methods, including loop-mediated isothermal amplification and clustered regularly interspaced short palindromic repeats-based tests [2-7]. However, meta-analyses of these methods revealed insufficient performance or insufficient data; therefore, they should be used with caution in Korea at present [3-7].

Specimen types

Nasopharyngeal and oropharyngeal swabs collected simultaneously and placed in the same transport medium are no longer recommended for routine use because of the high risk of droplet generation in the process of placing two swabs in the same transport medium and because the viral loads are similar between the two specimen types; nasopharyngeal swabs are sufficient [8]. The use of an inactivating agent-containing transport medium (e.g., a chaotropic agent) for molecular tests provides safer specimen handling [9, 10].

Self-collected respiratory specimens

KSLM and KDCA generally do not recommend using self-collected respiratory specimens for diagnosing asymptomatic patients. Studies have investigated diagnosing COVID-19 using self-collected respiratory specimens, such as saliva, anterior nasal swabs (ANS), and mouthwash, as alternatives to nasopharyngeal and oropharyngeal swabs [11-14]. The advantage of these specimens is that they are easily obtained. However, recent prospective studies have revealed that the test sensitivities for saliva and ANS in asymptomatic patients were significantly lower than those for nasopharyngeal swabs [15-19]. In addition, pooled tests using saliva or ANS have lower sensitivities than individual tests using saliva or ANS [20-23]. Therefore, collecting such specimens can be considered for patients who need repeated specimen collection or in whom nasopharyngeal swabs are difficult to collect. However, the possibility of false negatives should be carefully considered in advance [24, 25]. The possibility of viral transmission during the process of self-collection should also be considered.

Test interpretation

KSLM and KDCA recommend using a molecular test that targets two or more sites of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome and to consider a COVID-19 diagnostic test positive only when all targets are positive. If the initial test result is inconclusive in a patient with no history of confirmed COVID-19, collecting a new specimen for retesting is recommended.

The following criteria apply only to the newly confirmed COVID-19 cases and in the course of treatment of a patient with confirmed COVID-19. The rRT-PCR test result can be judged

Table 1. Types of COVID-19 laboratory tests in Korea

Type of test	Intended use	Caution
rRT-PCR	Confirmatory diagnosis in acute symptomatic patients or screening of asymptomatic individuals	Not suitable for quantitative interpretation. Infectivity cannot be determined on the basis of PCR results alone.
Pooled test using rRT-PCR	Screening of asymptomatic individuals	Sensitivity may vary with the transport medium, nucleic acid extraction method, and PCR reagent used, and the pool size.
Rapid molecular test	Rapid screening under emergency situations	Positive results are recommended to be confirmed with a validated rRT-PCR.
Antigen test	Diagnosis for symptomatic patients within seven days from symptom onset	The false-negative rate is high in pre-symptomatic or asymptomatic patients. The false-positive rate is high when prevalence is low.
Antibody test	Confirmation of past infection or multisystem inflammation syndrome or serosurveillance	Assays with high specificity should be used. Not recommended for evaluating the risk of infection.

Abbreviation: rRT-PCR, real-time reverse transcription PCR.

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positive even if the fluorescent signal reaches the threshold after the cut-off cycle because of a decrease in the viral load.

- All genes positive: COVID-19-positive (SARS-CoV-2 detected)
- All genes negative: COVID-19-negative (SARS-CoV-2 not detected)
- Some genes positive as compared to the reference values: inconclusive

Considerations for test interpretation

Molecular tests that target a single site are not recommended for diagnosing COVID-19 as various mutations affecting test sensitivity have been reported [26-35]. Multiple targets can also discriminate non-specific reactions, background fluorescence signals, and cross-reactivity, which can cause false-positive results [36-38]. The targets do not necessarily have to be located in different genes. Some reagents target multiple sites but employ the same fluorescent dyes; in such cases, the manufacturer has to establish a method to differentiate between non-specific reaction and SARS-CoV-2 genome amplification for the user.

Solutions for inconclusive results

If the results are inconsistent after retesting, they can be judged false positive, and when the results are consistent, the results can be judged positive. If there is a possibility of cross-contamination, re-amplification of the extracted nucleic acids is not sufficient; the specimen must be re-extracted or a new specimen must be collected.

The possibility of COVID-19 can be judged by reviewing the patient's medical history. In some cases, an antibody test may help in interpreting inconsistent or inconclusive results. To determine the risk of cross-contamination, it is recommended to follow-up the laboratory's positive-test rate or to consider the location of other SARS-CoV-2-positive specimens on the PCR plate [39].

Result reporting

KSLM and KDCA do not recommend routine reporting of threshold cycle (Ct) values [40-43]. All currently approved COVID-19 molecular tests are qualitative tests. Respiratory specimens are not homogeneous in quality and the amount collected, and there are differences between reagents used, leading to large fluctuations in Ct values. In external quality programs of COVID-19 molecular tests conducted in Korea and other countries, a very wide range of Ct values (10-27) has been observed [44-47]. Making clinical judgments, e.g., on quarantine release, based on Ct values has a high probability of error [42, 43, 48, 49]. Ct values should be interpreted cautiously in consultation with the laboratory director.

Pooled test using rRT-PCR

KSLM and KDCA recommend that test performance must be verified before using in the pooled specimen test method for the screening of asymptomatic patients [40, 50]. The pooled rRT-PCR test for COVID-19 uses pooled upper respiratory tract specimens for the screening of asymptomatic patients with COVID-19 [50-52]. If the pooled test result is positive, all specimens included in the pooled test are subjected to rRT-PCR individually for confirmation. This method cannot be used for confirming suspected COVID-19 and should be limited to the screening of asymptomatic patients. For suspected patients, rRT-PCR of individual specimens is recommended.

As the pooled specimen test method is a screening test, sensitivity should be maintained as high as possible; the possibility of a decrease in sensitivity should be considered [52, 53]. Sensitivity may vary with the transport medium, nucleic acid extraction method, and PCR reagent used and the pool size [54, 55]. Therefore, it is necessary to verify whether the pooled specimen test method can be applied to the conditions used in each laboratory before testing.

- For general matters and considerations, refer to the previous guidelines and the protocol of KSLM for the pooled specimen test method [50].
- Regarding the determination of the pool size, various factors should be considered, including the virus amount of the newly diagnosed patient, the nucleic acid extraction method used, and the performance of the PCR reagent. Generally, a pool of no more than five to six specimens is recommended.
- Before starting a new test or changing the test method, a combination of the nucleic acid extraction method and PCR reagent should be tested with sufficient positive specimens to confirm that 100% sensitivity is obtained for the pooled test. In the verification process, sufficient specimens with high Ct values (e.g., at least 30% specimens with a Ct value $\geq$30) should be used. These recommendations are based on the distribution of the Ct values of newly diagnosed cases in Korea [50, 56].

RAPID MOLECULAR TEST

KSLM and KDCA recommend using reagents and equipment with verified performance when using a rapid molecular test [57]. The rapid molecular test for COVID-19 is used for the confirmation of a COVID-19 diagnosis within a short time [58]. The test principle is the same as that of other molecular tests, but the use of optimal reagents and equipment saves time; results can

generally be obtained within one hour. Rapid molecular tests can provide accurate results under emergency situations. To this end, the entire process from specimen input to obtaining test results must be automated.

Considering the performance of the currently available rapid molecular tests and the prevalence of COVID-19 in Korea, a rapid molecular test should be used only for screening, and positive specimens should be confirmed using a validated rRT-PCR test. A rapid molecular test is not recommended for the pooled specimen method, which requires maximum sensitivity. Rapid molecular tests that employ a general thermocycler and rRT-PCR reagent with a short reaction time are not suitable for emergency situations as not all processes are automated and the equipment is not suitable for detecting short-time reactions.

Test procedure

As most rapid molecular tests do not use positive and negative controls, an internal control must be included in the same reaction well as the specimen.

Before using the rapid molecular test, it is recommended to verify its performance with sufficient positive specimens to confirm that 100% sensitivity is obtained for multiple pooled specimens. In the verification process, it is recommended to include sufficient specimens with high Ct values (e.g., at least 30% of specimens with a Ct value ≥30). These recommendations are based on the distribution of Ct values of newly reported cases in Korea [50, 56].

Interpretation

Even if only one of the target genes is present, it is considered presumptive positive, and a validated rRT-PCR test is recommended for confirmation. As rapid molecular tests are for screening, inconclusive results should be considered presumptive positive. The positive predictive value may be low when the prevalence is low as the specificity of the rapid molecular test is not sufficiently high. In such a case, both positive and inconclusive rapid molecular test results should be considered only as screening test results and the results should be confirmed using a conventional rRT-PCR test.

ANTIGEN TEST

KSLM and KDCA do not recommend using a SARS-CoV-2 antigen test for asymptomatic individuals. Considering the low sensitivity and specificity of the antigen tests and the relatively low prevalence of COVID-19 in Korea, SARS-CoV-2 antigen tests have a very limited role in the screening of asymptomatic patients in Korea [56, 59-63]. The European Centers for Disease Control and Prevention recommend that antigen tests should not be used for the screening of asymptomatic patients if the prevalence is <10% [60]. The sensitivity of antigen tests using self-collected specimens, such as saliva or ANS, is even lower [63-67].

The use of a SARS-CoV-2 antigen test may be considered for symptomatic patients within seven days from symptom onset (1) when the prevalence and positive predictive value are high, (2) when the molecular test results are delayed for more than 48 hrs, and (3) when effective prophylactic treatment can be administered on the basis of clinical suspicion even after suspected false-negative results with the antigen test. A molecular test is recommended simultaneously with the antigen test to reduce the possibility of false positives or false negatives [68]. The manufacturer's instructions for immunochromatography must be followed strictly as result interpretation may be subjective [69].

ANTIBODY TEST

KSLM and KDCA do not recommend using an antibody test for diagnosing acute COVID-19 or for evaluating the risk of infection for individuals who have recovered from COVID-19 or who have been vaccinated for COVID-19 [40, 70-73]. SARS-CoV-2 antibody tests are indicated (1) for serosurveillance studies, (2) in the case of strong suspicion of past SARS-CoV-2 infection based on epidemiological and clinical results and repeated negative or indeterminate molecular tests, (3) in the case of suspected COVID-19-related multisystem inflammatory syndrome, (4) for the selection of convalescent plasma donors from patients who have recovered from COVID-19 for therapeutic purposes, (5) for studies aimed at investigating the efficacy or effectiveness of a vaccine, and (6) for entry into a country where a SARS-CoV-2 antibody test result is a prerequisite [74]. An antibody test with very high specificity (e.g., ≥99.5%) should be used [75-77]. To diagnose a breakthrough infection in a vaccinated patient, an antibody test detecting antigens not targeted by the vaccine received should be used.

LABORATORY GUIDELINES FOR BIOSAFETY AND INFECTION CONTROL

KSLM and KDCA recommend that only fully COVID-19-vaccinated healthcare personnel perform the tests [78-82].

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AUTHOR CONTRIBUTIONS

Hong KH and Kim GJ reviewed the literature on the currently used general recommendations and wrote the manuscript. Roh KH and Sung H contributed to general concepts and recommendations. Lee J and Kim SY contributed to recommendations for antigen tests, pooling tests, and rapid molecular tests. Kim TS and Kim J-S contributed to recommendations for biosafety. Park Y and Kim HS contributed to recommendations for antibody testing. Park J-S, Huh HJ, Seong M-W, and Ryoo N collated protocols and interpreted results and contributed to recommendations. Lee H, Song SH, Kwon GC, and Yoo CK organized the task force and contributed to the concept and design of the guidelines.

CONFLICTS OF INTEREST

None.

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