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Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jmv.25727.

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Abstract

The outbreak of the novel coronavirus disease (COVID-19) quickly spread all over China and to more than 20 other countries. Although the virus (SARS-CoV-2) nucleic acid RT-PCR test has become the standard method for diagnosis of SARS-CoV-2 infection, these real-time PCR test kits have many limitations. In addition, high false negative rates were reported. There is an urgent need for an accurate and rapid test method to quickly identify large number of infected patients and asymptomatic carriers to prevent virus transmission and assure timely treatment of patients.

We have developed a rapid and simple point-of-care lateral flow immunoassay which can detect IgM and IgG antibodies simultaneously against SARS-CoV-2 virus in human blood within 15 minutes which can detect patients at different infection stages. With this test kit, we carried out clinical studies to validate its clinical efficacy uses. The clinical detection sensitivity and specificity of this test were measured using blood samples collected from 397 PCR confirmed COVID-19 patients and 128 negative patients at 8 different clinical sites. The overall testing sensitivity was 88.66% and specificity was 90.63%. In addition, we evaluated clinical diagnosis results obtained from different types of venous and fingerstick blood samples. The results indicated great detection consistency among samples from fingerstick blood, serum and plasma of venous blood. The IgM-IgG combined assay has better utility and sensitivity compared with a single IgM or IgG test. It can be used for the rapid screening of SARS-CoV-2 carriers, symptomatic or asymptomatic, in hospitals, clinics, and test laboratories.


Introduction

Since December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia. Subsequently, pathogenic gene sequencing confirmed that the infected pathogen was a novel coronavirus, named 2019 novel coronavirus (SARS-CoV-2). Similar to previous outbreaks of coronavirus infection in humans, 2003 SARS-CoV and 2012 MERS-CoV, SARS-CoV-2 infection caused the novel coronavirus disease (COVID-19), its outbreak.
developed into an epidemic that quickly spread all over China and to more than 20 other countries.\textsuperscript{6} It has been listed as a public health emergency of international concern.\textsuperscript{7} The outbreak of this disease has caused the Chinese government to take drastic measures to contain the outbreak, including the quarantine of millions of residents in Wuhan and other affected cities. Countrywide interventions include delaying resumption of workplaces, and encouraging citizens to stay and work from home, and so on.

However, these efforts are limited by one hard problem: how to differentiate the COVID-19 cases from the healthy. For confirmed COVID-19 cases, reported the common clinical symptoms include fever, cough, myalgia or fatigue.\textsuperscript{8} Yet these symptoms are not unique features of COVID-19 because these symptoms are similar to that of other virus-infected disease such as influenza.\textsuperscript{9} Currently, virus nucleic acid Real Time-PCR (RT-PCR), CT imaging and some hematology parameters are the primary tools for clinical diagnosis of the infection.\textsuperscript{10} Many laboratory test kits have been developed and used in testing patient specimens for COVID-19 by Chinese CDC, US CDC and other private companies. The virus nucleic acid RT-PCR test has become as the current standard diagnostic method for diagnosis of COVID-19. Yet these real-time PCR test kits suffer from many limitations: 1) These tests have long turnaround times and are complicated in operation; they generally take on average over 2 to 3 hours to generate results. 2) The PCR tests require certified laboratories, expensive equipment and trained technicians to operate. 3) There are some numbers of false negatives for RT-PCR of COVID-19.\textsuperscript{11} These limitations make RT-PCR unsuitable for use in the field for rapid and simple diagnosis and screening of patients. It limits the outbreak containment effort. Therefore, there is an urgent need for a rapid, simple to use, sensitive, and accurate test to quickly identify infected patients of SARS-CoV-2 to prevent virus transmission and to assure timely treatment of patients.

Testing of specific antibodies of SARS-CoV-2 in patient blood is a good choice for rapid, simple, highly sensitive diagnosis of COVID-19. It is widely accepted that IgM provides the first line of defense during viral infections, prior to the generation of adaptive, high affinity IgG responses that are important for long term immunity and immunological memory.\textsuperscript{12} It was reported that after SARS infection, IgM antibody could be detected in
patient blood after 3 - 6 days and IgG could be detected after 8 days.\textsuperscript{13,14} Since COVID-19 belongs to the same large family of viruses as those that cause the MERS and SARS outbreak, we assume its antibody generation process is similar, and detection of the IgG and IgM antibody against SARS-CoV-2 will be an indication of infection. Furthermore, detection of IgM antibodies tends to indicate a recent exposure to SARS-CoV-2, whereas detection of COVID-19 IgG antibodies indicates virus exposure some time ago. Thus, we believe that detection of both IgM and IgG could provide information on virus infection time course. The rapid detection of both IgM and IgG antibodies will add value to the diagnosis and treatment of COVID-19 disease.

Based on these, we developed a point-of-care lateral flow immunoassay (LFIA) test product, which can detect IgM and IgG simultaneously in human blood within 15 minutes. We tested the product in 8 hospitals and Chinese CDC agencies to validate its clinical efficacy. The results demonstrated this rapid antibody test has high sensitivity and specificity. It can be used in hospitals, clinics, and testing laboratories. The test can also be effectively deployed in businesses, schools, airports, seaports and train stations, etc., giving it the potential to become a compelling force in the fight against this global threat.

**Methods and Materials**

*The materials for the manufacture of IgG-IgM combined antibody test of COVID-19*

Anti-human IgG and IgM (LF201001, LF201002) were purchased from Nanjing Lefushidai Inc., COVID-19 recombinant antigen (MK201027) was developed and purified at Medomics. The recombinant antigen (MK201027) is receptor binding domain of SARS-CoV-2 Spike Protein, which is transient transfected in cell culture and purified by protein A affinity chromatography and size-exclusion chromatography (SEC). The design of the antigen was based on the published SARS-CoV-2 sequence. Several different designs of antigen were tested and optimized. Eventually, MK201027 was picked into testing product. Bovine serum albumin (BSA), and goat anti-human IgG and IgM antibodies, rabbit IgG and goat anti-rabbit IgG antibodies were obtained from Sigma. 40nm gold nanoparticle (AuNP) colloids, NC membrane and plastic pad were obtained from Shanghai KinBio Inc., the glass fiber conjugate (GFC) pad was obtained from Whatman. The phosphate buffer

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saline was purchased from Sigma. Inactivated COVID-19 positive serum and negative serum samples of patients with were supplied by Hunan CDC, China.

**Preparation of AuNP conjugates**

To prepare the AuNP conjugate, SARS-CoV-2 recombinant protein dissolved in PBS (1 mg/mL) was added to the mixture of 1mL AuNP colloid (40nm in diameter, OD=1) and 0.1mL of borate buffer (0.1 M, pH 8.5). After incubation for 30 min at room temperature, 0.1mL of 10mg/mL BSA in PBS was added to the solution to block the AuNP surface. After incubation for 15 min at room temperature, the mixture was centrifuged at 10,000rpm and 4°C for 20 min. The supernatant was discarded, and 1mL of 1mg/mL BSA in PBS was added to the AuNP conjugate to be re-suspended. The centrifugation and suspension process were repeated twice, and the final suspension solution was PBS. The AuNP-rabbit IgG conjugates was prepared and purified by the same procedure.

**Preparation of COVID-19 rapid test of IgG-IgM**

The main body of the test strip consist of five parts, including plastic backing, sample pad, conjugate pad, absorbent pad and NC membrane. Every component of the strip should be given a pretreatment described as follows: the NC membrane was attached to a plastic backing layer for cutting and handling. The anti-human-IgM, anti-human-IgG and anti-rabbit-IgG were immobilized at test M, G and control line (C line), respectively. Conjugate pad was sprayed with mixture of AuNP-COVID-19 recombinant antigen conjugate and AuNP-rabbit-IgG. Sample pad was pretreated with BSA (3%, w/v) and Tween-20 (0.5%, w/v) before use.

**Testing of COVID-19 samples using the LFIA system**

1) Patient and sample collection

The patients were recruited who conform to the diagnostic criteria of suspected case of COVID-19 according to guideline of diagnosis and treatment of COVID-19\textsuperscript{15} including typical epidemiological history and clinical characteristics. These samples were collected from various hospitals and CDC testing laboratories (total 8) at 6 different provinces in
China. The tests were conducted at the sites by clinical staffs who followed test procedure described in the product inserts. The respiratory tract specimen, including pharyngeal swab and sputum, was used to confirm COVID-19 cases, and the blood, including serum and plasma, was used to test the IgM and IgG antibody.

2) Sample Testing

Prior to testing, the pouched device was opened immediately before use. When refrigerated blood samples were used for the test, they were warmed to room temperature (15 -30°C). During testing, 20 ul whole blood sample (or 10 ul of serum/plasma samples) was pipetted into the sample port followed by adding 2-3 drops (70-100 ul) of dilution buffer (10mM PBS buffer) to drive capillary action along the strip. The entire test took about 15 mins to finish.

3) Display of results

A total of three detection lines are on the stip. The control (C) line appears when sample has flowed through the cartridge. The presence of anti- SARS-CoV-2 IgM and anti- SARS-CoV-2 IgG will be indicated by a red/pink test line in the M and G region. If only the control line (C) showed red, the sample is negative. Either M or G line or both lines turning into red indicates presence of anti- SARS-CoV-2 -IgM or anti- SARS-CoV-2 -IgG or both antibodies in the specimen. If the control line does not appear red, the test is invalid, and the test should be repeated with another cartridge.

Data analysis

The rapid SARS-CoV-2 IgG-IgM combined antibody test kits were tested at 8 hospitals and Chinese CDC laboratories in different provinces, with a total of 397 clinical positive and 128 clinical negative patient blood samples. The test data was collected and analyzed. The specificity and sensitivity of the rapid test kits were calculated according to the following formulas:

Specificity (%) = 100 x [True negative / (True Negative + False Positive)].
Sensitivity (%) = 100 x [True Positive/(True Positive + False Negative)]

Results

Design and finished product of SARS-CoV-2 rapid test of IgG-IgM combined antibody kit

The SARS-CoV-2 rapid IgG-IgM combined antibody test kit is designed and manufactured by Jiangsu Medomics Medical Technologies, located in Nanjing, China. It is a lateral flow qualitative immunoassay for the rapid determination of the presence or absence of both anti-SARS-CoV-2-IgM and anti-SARS-CoV-2-IgG in human specimens (whole blood, serum, and plasma). The test kit comes with a test cartridge, sample dilution buffer, and a package insert. The testing cartridge has three detection bands, including a distal control band that appears when the sample has flowed to the end of the testing strip. The presence of SARS-CoV-2 IgG and IgM antibodies are indicated by a red/purple line in the specific region indicated on the device. The SARS-CoV-2 rapid IgG-IgM combined antibody test strip, as shown in the Figure 1, has two mouse anti-human monoclonal antibodies (anti-IgG and anti-IgM) stripped on two separated test lines. A surface antigen from SARS-CoV-2 which can specifically bind to SARS-CoV-2 antibodies (including both IgM and IgG) is conjugated to colloidal gold nanoparticles and sprayed on conjugation pads. The AuNP-rabbit IgG conjugates was also sprayed on conjugation pads for binding to anti-rabbit IgG antibody which is immobilized on control line (Figure 1A).

When testing, 10-15ul of specimen is added into the sample port followed by the addition of sample dilution buffer. The mechanism of the assay is based on the hydration and transport of reagents as they interact with the specimen across the strip via chromatographic lateral flow (Figure 1 A). As the specimen flows through the device, anti-SARS-CoV-2 IgG and IgM antibodies, if present in the specimen, are bound by the SARS-CoV-2 antigen labeled gold colorimetric reagent fixed on the conjugate pad. As the conjugated sample continues to travel up the strip, the anti-SARS-CoV-2 IgM antibodies are bound on the M(IgM) line, and the anti-COVID-19 IgG antibodies are bound to the G (IgG) line. If the specimen does not contain SARS-CoV-2 antibodies, no labeled complexes bind at the Test
Zone and no lines could be observed. The remaining colloidal gold travels up the nitrocellulose to the Control line Zone, which captures the excess conjugate demonstrating that the fluid has migrated adequately through the device. A reddish-purple line will appear at the Control line Zone during the performance of all valid tests whether the sample is positive or negative for SARS-CoV-2 infection. During the test, excess reagent including AuNP-rabbit IgG conjugates migrate passes the control line zone, where the AnNP-rabbit IgG conjugates binds to anti-rabbit IgG to form red line on the control line. Figure 1 B is the illustration of different testing results reading for negative, IgM positive, IgG positive and IgM/IgG both positive situations.

As one of example to show real testing results, Figure 2 shows a testing result photo for 6 different test cartridges from 6 patients which represents several different types of results. In cartridge #13, the photo reading represents detection of both IgM and IgG; in cartridge #14 IgM only in low concentration; in cartridge #15, no IgM and IgG; in cartridge #16 IgG only in low concentration; in cartridge #17 IgG only in high concentration and in cartridge #18, IgM only in high concentration in patient bloods, respectively.

**The detection sensitivity and specificity of SARS-CoV-2 IgG-IgM combined antibody kit**

In order to test the detection sensitivity and specificity of SARS-CoV-2 IgG-IgM combined antibody test, blood samples were collected from COVID-19 patients from multiple hospitals and Chinese CDC laboratories. The tests were done separately at each site. A total of 525 cases were tested: 397 (positive) clinically confirmed (including PCR test) SARS-CoV-2-infected patients and 128 non- SARS-CoV-2-infected patients (128 negative). The testing results of vein blood without viral inactivation were summarized in the Table 1. Of the 397 blood sample from SARS-CoV-2-infected patients, 352 tested positive, resulting in a sensitivity of 88.66%. 12 of the blood samples from the 128 non-SARS-CoV-2 infection patients tested positive, generating a specificity of 90.63%. It was also found that 64.48% (256 out of 397) of positive patients had both IgM and IgG antibodies (Table 1).
For the test, it is very important to know the data of infection time point from clinical samples which will be helpful to compare the data of single or double positive in Table 1. Due to limited time, we do not have complete detailed information for how long each patient was infected or for how long each patient had symptoms when blood sample was collected at all the clinical sites. We only had such data from one of clinical site- Wuhan Red Cross Hospital. As a reference, by analyzing one subset data of 58 patients in Wuhan, it was found that 94.83% of the positive patients had both IgM and IgG positive test lines, and 1.72%, 3.45% had only IgM or only IgG positive lines respectively (table 2). The test time was at day 8 to day 33 after infection symptoms appeared. Further studies and information collection are needed for this.

SARS-CoV-2 IgG-IgM combined antibody test in different types of blood samples

The above results have verified the sensitivity and specificity of kit detection in un-inactivated vein blood. However, it is more convenient to collect fingerstick blood outside hospitals and clinics. In order to achieve a simpler operating process, we tested the performance of SARS-CoV-2 IgG-IgM combined antibody kit with peripheral blood. Patient fingerstick blood and vein blood and plasma from the same patient were tested. As showed in the Table 3, seven COVID-19 patients and three healthy volunteers were recruited. We took the blood samples from fingerstick, serum of venous blood, and plasma of venous blood and tested them with the kits. Within the 7 patients, 3 patients have IgM only positive and 4 patients have both IgM and IgG positive. All healthy volunteers tested negative. The results showed that all of the positive and negative test results matched with 100% consistency among the corresponding blood samples. This result demonstrates that the SARS-CoV-2 IgG-IgM combined antibody test kit can be used as a point-of-care test (POCT). It can be performed near the bedside with fingerstick blood.

Discussion

Here, we successfully developed a rapid IgG-IgM combined antibody test kit for COVID-19 diagnosis. The sensitivity and specificity of the kit were verified via the lab and

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clinical practice. This test kit provides a product to meet the urgent need for immunoassay tests in Chinese hospitals for the diagnosis of COVID-19.

In order to make the kit suitable for different stages of the disease, we developed an IgG-IgM combined antibody test for COVID-19 infection (Figure 1). It was also been confirmed that the detection sensibility was higher in IgG-IgM combined antibody test than in individual IgG or IgM antibody test (Table 1). Therefore, we more recommend the development of IgG-IgM combined antibody test kits than the separate IgG or IgM antibody test kits, if there is a reliable technical system available. It is a better test for screening COVID-19 patients.

This new develop test kit, the IgG-IgM combined antibody test kit, has sensitivity of 88.66% and specificity of 90.63%. However, there were still false positive and false negative results (Table 1). The reasons for the false negative may be due, first, to low antibody concentrations. When IgM and IgG levels are below the detection limit (not determined yet) of this rapid test, the test results will be negative. Second, the difference in individual immune response antibody production could be one reason for the false negative results in COVID-19 patients. The last but not least, IgM antibody will decrease and disappear after 2 weeks. In some cases, it is hard to know exactly when the patient was infected or how long the patient was infected. Thus, when the patient was tested, the IgM level might well be below its peak and not detectable by this test. Therefore, we encourage more research and development of the COVID-19 IgG-IgM combined antibody test kit to improve the diagnostic sensitivity and specificity for patients. Since COVID-19 recombinant antigen was used in the test, our test is specific for COVID-19 infection. The clinical testing data in this paper at different clinical sites from east, south, west and middle of China confirmed the specificity of the test kit. However, because of the emergency of outbreak of COVID-19, we could not carry out normal research activities and perform enough tests to verify if there is interference from other IgM and IgG induced by different virus infections such as typical flu viruses. This work need be done later.

This new rapid SARS-CoV-2 IgG-IgM combined antibody test kit has several advantages. Compared to RT-PCR, it saves time and it does not require equipment, it is
simple to perform and only requires minimal training. It can be performed at the bedside, in any clinic or laboratory, at airports or at railway stations\textsuperscript{16}. It will be more convenient to use fingerstick blood or heel blood instead of vein blood for out-of-clinic screening. Our initial test results using fingertip blood were as good as that of vein blood (Table 3), which suggests that the SARS-CoV-2 IgG-IgM combined antibody test kits can be developed as agents for rapid field detection. Another potential application of this test is screening asymptomatic SARS-CoV-2 carriers, it was reported that asymptomatic carriers could spread SARS-CoV-2 virus\textsuperscript{17,18}. This finding made the current COVID-19 outbreak control more difficult, because there is no method available to screen asymptomatic carriers. This rapid IgM-IgG combined antibody test kit makes large scale screening of asymptomatic carriers possible. At least some if not all of the carriers are likely to have anti-SARS-CoV-2 antibodies, as demonstrated by asymptomatic Zika virus carriers\textsuperscript{19}. Because this test can detect IgM and IgG simultaneously, it could be used for both early diagnosis (IgM) and for monitoring during treatment. SARS-CoV-2 infection starts at the lungs, not in the upper respiratory tract\textsuperscript{20}, therefore, sampling during the early infection stage using throat swab or sputum may not detect the virus. This is one possible explanation for high false negatives in nucleic acid PCR test. However, this sampling effect should not have any effect on IgM and IgG detection with this rapid test.

Based on our knowledge and information, there are several other Chinese IVD companies are developing or have developed similar products ranging from IgM only and IgM-IgG combined tests. We do not know the details about their technical performance since there is no publication on them. We believe the good test products will be used in clinical sites and the information will emerge. We will carry out comparison studies later.

Certainly, this test cannot confirm virus presence, only provide evidence of recent infection, but it provides an important immunological evidence for physicians to make the correct diagnosis along with other tests and to start treatment of patients. In addition, possible cross-reactivity with other coronaviruses and flu viruses were not studied, and the change level of antibody was not compared in the different stages of SARS-CoV-2 infection. We believe the combination of nucleic acid RT-PCR and the IgM-IgG antibody test can provide more accurate SARS-CoV-2 infection diagnosis.

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Conclusion

We developed a rapid SARS-CoV-2 IgG-IgM combined antibody test using lateral flow immune assay techniques. It takes less than 15 minutes to generate results and determine whether there is recent SARS-CoV-2 infection. It is easy to use, and no additional equipment is required. Results from this study demonstrated that this test is sensitive and specific. This rapid test has great potential benefit for the fast screening of SARS-CoV-2 infections, and it has already generated tremendous interest and increased clinical uses after a short time testing in Chinese hospitals.

Acknowledgments

The authors would like to thank the technical staff of the Department of State Key Laboratory of Respiratory Disease for excellent assistance. Furthermore, we also like to thank Mrs. Cuiwei Qiu for manuscript content assistance and Dr. Alan Schreier for editing this manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


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**Figures**

*Figure 1: Schematic illustration of rapid SARS-CoV-2 IgM-IgG combined antibody test.* A: Schematic diagram of the detection device; B: An illustration of different testing results, C means control line, G means IgG line, M means IgM line.
Figure 2: Representative photo for different patient blood testing results. #13) Both IgM and IgG positive, #14) IgM weak positive, #15) Both IgM and IgG negative, #16) IgG weak positive, #17) IgG positive, #18) IgM positive.

Table 1. The detection sensitivity and specificity of SARS-CoV-2 IgG-IgM combined antibody reagent.

<table>
<thead>
<tr>
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<th>Clinical positive samples</th>
<th>Clinical negative samples</th>
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<tr>
<td>Sample Quantity</td>
<td>397</td>
<td>128</td>
</tr>
<tr>
<td>IgG&amp;IgM Positive</td>
<td>256</td>
<td>1</td>
</tr>
<tr>
<td>IgG Positive</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>IgM Positive</td>
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<td>10</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>88.66%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>90.63%</td>
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Table 2 IgM and IgG in positive patient blood samples from Wuhan Red Cross Hospital

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<tr>
<th></th>
<th>Number in positive samples</th>
<th>Percentage %</th>
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<tr>
<td>IgM only</td>
<td>1</td>
<td>1.72</td>
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<tr>
<td>IgG only</td>
<td>2</td>
<td>3.45</td>
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<tr>
<td>Both IgM and IgG</td>
<td>55</td>
<td>94.83</td>
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Table 3. The evaluation of detection consistency in different types of blood samples

<table>
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<tr>
<th></th>
<th>COVID-19 patients</th>
<th>Healthy person</th>
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<tbody>
<tr>
<td></td>
<td>Total samples</td>
<td>IgM Positive</td>
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<tr>
<td>Fingerstick blood</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Serum of venous blood</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Plasma of venous blood</td>
<td>7</td>
<td>3</td>
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<tr>
<td>Detection consistency</td>
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