

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
স্বাস্থ্য ও পরিবার কল্যাণ মন্ত্রণালয়
স্বাস্থ্য সেবা বিভাগ
সরকারি স্বাস্থ্য ব্যবস্থাপনা-১ শাখা
বাংলাদেশ সচিবালয়, ঢাকা
www.hsd.gov.bd



স্মারক নং-৪৫.০০.০০০০.১৬০.২২.০০১.২০-৭৯৪

তারিখঃ-১৭-০৯-২০২০খ্রিঃ

বিষয়ঃ কোভিড-১৯ সনাক্তকরণের জন্য এন্টিজেনভিত্তিক টেস্ট চালুকরণ।

সূত্রঃ মহাপরিচালক, স্বাস্থ্য অধিদপ্তরের ১৫/০৯/২০২০ তারিখে স্বাক্ষরিত প্রস্তাবনা এবং বিশ্ব স্বাস্থ্য সংস্থার ১১ সেপ্টেম্বর ২০২০ তারিখের গাইডলাইন।

উপর্যুক্ত বিষয় ও সূত্রোক্ত স্মারকদ্বয়ের আলোকে সারাদেশে এন্টিজেন টেস্টের চাহিদার প্রেক্ষিতে অতি স্বল্প সময়ে কোভিড-১৯ সনাক্তকরণের জন্য মহাপরিচালক স্বাস্থ্য অধিদপ্তরের প্রস্তাবনা এবং বিশ্ব স্বাস্থ্য সংস্থার ১১ সেপ্টেম্বর ২০২০ তারিখের Interim Guidance অনুসরণপূর্বক দেশের সকল সরকারি মেডিকেল কলেজ হাসপাতাল, জেলা হাসপাতাল, সরকারি PCR Lab এবং সকল স্বাস্থ্য ইনস্টিটিউটে এন্টিজেনভিত্তিক টেস্ট চালুর অনুমতি নির্দেশক্রমে প্রদান করা হলো।

০২। তবে শর্ত থাকে যে, যাচাই-বাহাইয়ের নিমিত্তে স্বাস্থ্য অধিদপ্তরে প্রক্রিয়াধীন কোভিড-১৯ ল্যাব সম্প্রসারণ নীতিমালাটি চূড়ান্ত হলে তা যথাযথভাবে অনুসরণ করতে হবে।

০৩। এ আদেশ অবলম্বনে কার্যকর হবে।

সংযুক্তিঃ বর্ণনা মোতাবেক।

B. Begum
29/09/2020

ড. বিলকিস বেগম
উপসচিব

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বিতরণঃ

- ১। পরিচালক, মেডিকেল কলেজ হাসপাতাল (সকল)।
- ২। পরিচালক (সকল) সরকারী স্বাস্থ্য ইনস্টিটিউট।
- ৩। তত্ত্বাবধায়ক/সিভিল সার্জন, জেলা সদর/জেনারেল হাসপাতাল (সকল)।

অনুলিপি (জ্যেষ্ঠতার ক্রমানুসারে নয়):

- ১। মহাপরিচালক, স্বাস্থ্য অধিদপ্তর, মহাখালী, ঢাকা।
- ২। মন্ত্রী মহোদয়ের একান্ত সচিব, স্বাস্থ্য ও পরিবার কল্যাণ মন্ত্রণালয়, ঢাকা।
- ৩। সচিব মহোদয়ের একান্ত সচিব, স্বাস্থ্য সেবা বিভাগ, স্বাস্থ্য ও পরিবার কল্যাণ মন্ত্রণালয়, ঢাকা।
- ৪। সিস্টেম এনালিস্ট, স্বাস্থ্য সেবা বিভাগ, স্বাস্থ্য ও পরিবার কল্যাণ মন্ত্রণালয়, ঢাকা (তাকে পত্রটি মন্ত্রণালয়ের ওয়েবসাইটে প্রকাশের অনুরোধসহ)।
- ৫। অতিরিক্ত সচিব (হাসপাতাল) মহোদয়ের ব্যক্তিগত কর্মকর্তা, স্বাস্থ্য সেবা বিভাগ, ঢাকা।
- ৬। যুগ্ম সচিব (সঃ ও বেঃ স্বঃ ব্যঃ) মহোদয়ের ব্যক্তিগত কর্মকর্তা, স্বাস্থ্য সেবা বিভাগ, ঢাকা।

র‍্যাপিড অ্যান্টিজেন টেস্ট বিষয়ে প্রস্তাবনা

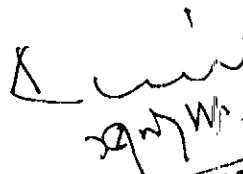
অ্যান্টিজেন টেস্ট কোভিড-১৯ ভাইরাসের উপাদান সনাক্ত করে। লক্ষণ/উপসর্গ দেখা দেয়ার পর এ টেস্ট ভাইরাসের উপস্থিতি সনাক্ত করতে পারে, তবে ভাইরাসের পরিমাণ বেশী হলে সনাক্ত করার সম্ভাবনা বৃদ্ধি পায়। র‍্যাপিড অ্যান্টিজেন টেস্টসমূহের প্রধান সুবিধা হল ৩০-৪০ মিনিটের মধ্যে টেস্টের ফলাফল পাওয়া যায় এবং ল্যাবরেটরিতে বিশেষ কোন ব্যবস্থার প্রয়োজন হয় না। ফলে এ টেস্টগুলো চিকিৎসাকেন্দ্রে করা যাবে।

মার্কিন যুক্তরাষ্ট্রের এফডিএ গত ৯ মে, ২০২০ তারিখে প্রথম র‍্যাপিড অ্যান্টিজেন টেস্ট অনুমোদন করেছে যদিও বিশ্ব স্বাস্থ্য সংস্থা এখন পর্যন্ত কোভিড-১৯ রোগ নির্ণয়ের জন্য কোন র‍্যাপিড টেস্টকে অনুমোদন দেয়নি। মার্কিন যুক্তরাষ্ট্র, ইউরোপীয় ইউনিয়নের কোন কোন সদস্য দেশ, দক্ষিণ কোরিয়া, ভারত র‍্যাপিড অ্যান্টিজেন টেস্ট ব্যবহার করছে।

বিশ্বে বেশ কিছু অ্যান্টিজেন টেস্ট থাকলেও প্রাপ্ত তথ্য অনুযায়ী বাংলাদেশে ২/৩টির বেশী টেস্ট এখনও পাওয়ার সম্ভাবনা নেই যেগুলোর গ্রহণযোগ্য মাত্রার (কমপক্ষে ৮০%) সংবেদনশীলতা (Sensitivity) ও সুনির্দিষ্টতা (Specificity) রয়েছে। এর মধ্যে মার্কিন যুক্তরাষ্ট্রের কুইডেল কর্পোরেশন এর সোফিয়া এসএআরএস অ্যান্টিজেন উচ্চ মাত্রায় সংবেদনশীল (৯৭%) ও সুনির্দিষ্ট (১০০%)। কিন্তু এই টেস্ট এর জন্য নির্দিষ্ট একটি মেশিন প্রয়োজন (আনুমানিক দাম আড়াই লক্ষ টাকা) এবং কিটের দাম বেশী (আনুমানিক প্রতিটি টেস্ট ২৭০০ টাকা)। এছাড়া রয়েছে এসডি বায়োসেন্সর এর স্ট্যান্ডার্ড কিউ কোভিড-১৯ অ্যান্টিজেন। এই টেস্ট এর জন্য কোন মেশিন প্রয়োজন নেই এবং কিটের দাম তুলনামূলক কম। স্থানীয় সরবরাহকারীর তথ্য অনুযায়ী আনুমানিক প্রতিটি টেস্ট এর দাম ১০০০ টাকা। অধিক সংখ্যায় কেনা হলে দাম কমে আনুমানিক ৭০০-৮০০ টাকার মধ্যে হতে পারে। যদি সরাসরি প্রস্তুতকারী প্রতিষ্ঠানের কাছ থেকে কেনা হয় এর দাম আরও কম হবে। তবে এর সুনির্দিষ্টতা শতকরা শতভাগ হলেও সংবেদনশীলতা কম (৮৪%), ভারতে ব্যবহার করে ৫০% পাওয়া গেছে। এর ফলে কোভিড আক্রান্ত ব্যক্তির নমুনা পরীক্ষা করে ভুলভাবে নেগেটিভ ফলাফল পাওয়ার আশংকা রয়েছে। তাই অ্যান্টিজেন টেস্টের ফলাফল নেগেটিভ হলে নিশ্চিত হওয়ার জন্য পিসিআর/জিনএক্সপার্ট পদ্ধতিতে দ্বিতীয়বার পরীক্ষা করতে হবে।

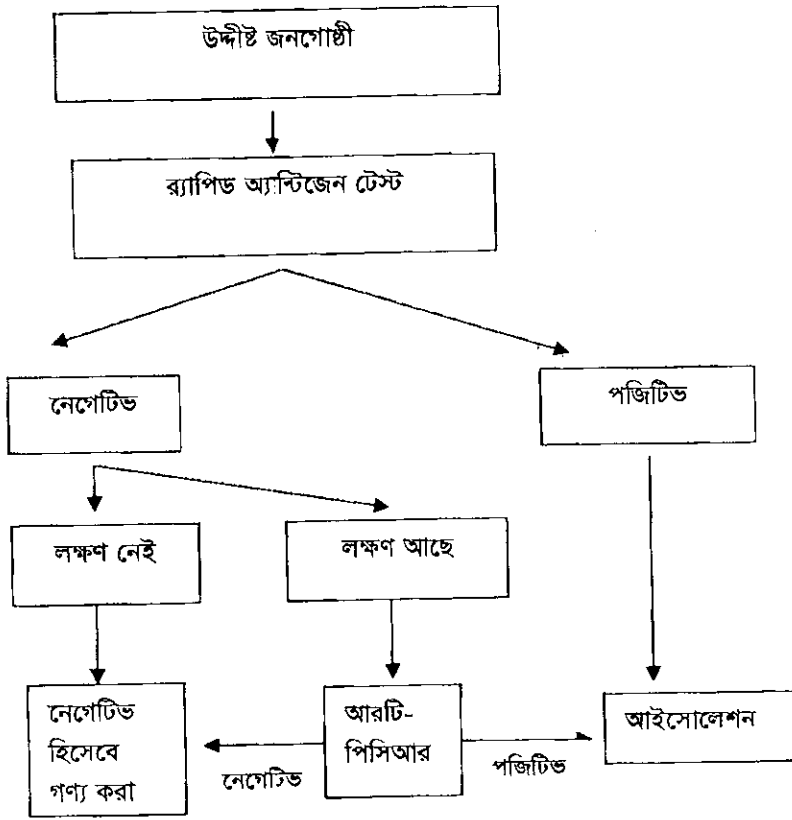
জেলা সদর হাসপাতালে অ্যান্টিজেন পরীক্ষা

জেলা পর্যায়ে যে সব হাসপাতালে কোভিড-১৯ রোগী চিকিৎসা দেয়া হয় এবং আরটি-পিসিআর ল্যাবরেটরির সুবিধা নেই সে ধরনের জেলা হাসপাতালে অ্যান্টিজেন পরীক্ষা করা যেতে পারে। বর্তমানে এমন জেলার সংখ্যা ৩৯টি। এইসব জেলার সকল নমুনা অন্যান্য জেলার আরটি-পিসিআর ল্যাবরেটরিতে প্রেরণ করা হয়। এতে


২৭/৭/১৯
অধ্যাপক ডাঃ আবুল বাসীর মোহাম্মদ কুশীদ আলম
মহা-পরিচালক
ঢাকা।

করে রিপোর্ট আসতে দেরী হয়, ল্যাবরেটরীগুলোতেও নমুনা জমে থাকে। জেলা হাসপাতালে অ্যান্টিজেন পরীক্ষার ব্যবস্থা করা হলে কেবলমাত্র নেগেটিভ নমুনা আরটি-পিসিআর পরীক্ষার জন্য প্রেরণ করতে হবে। যেখানে কোভিড-১৯ রোগীর সংখ্যা বেশী সে ধরনের জেলা হাসপাতালে (বিশেষ করে যেখানে তাৎক্ষণিকভাবে আরআরটি-পিসিআর ল্যাবরেটরী সুবিধা পাওয়া যায় না) প্রাথমিক পর্যায়ে পরীক্ষামূলকভাবে এই টেস্ট আরম্ভ করা যেতে পারে। যেমন: রাজবাড়ি, গোপালগঞ্জ, শরিয়তপুর, বিনাইদহ, মেহেরপুর, চুয়াডাঙ্গা, নড়াইল, মাগুরা, ঠাকুরগাঁও, লক্ষ্মীপুর।

চিত্র ১ঃ চিকিৎসা কেন্দ্রে পরীক্ষার পর্যায়

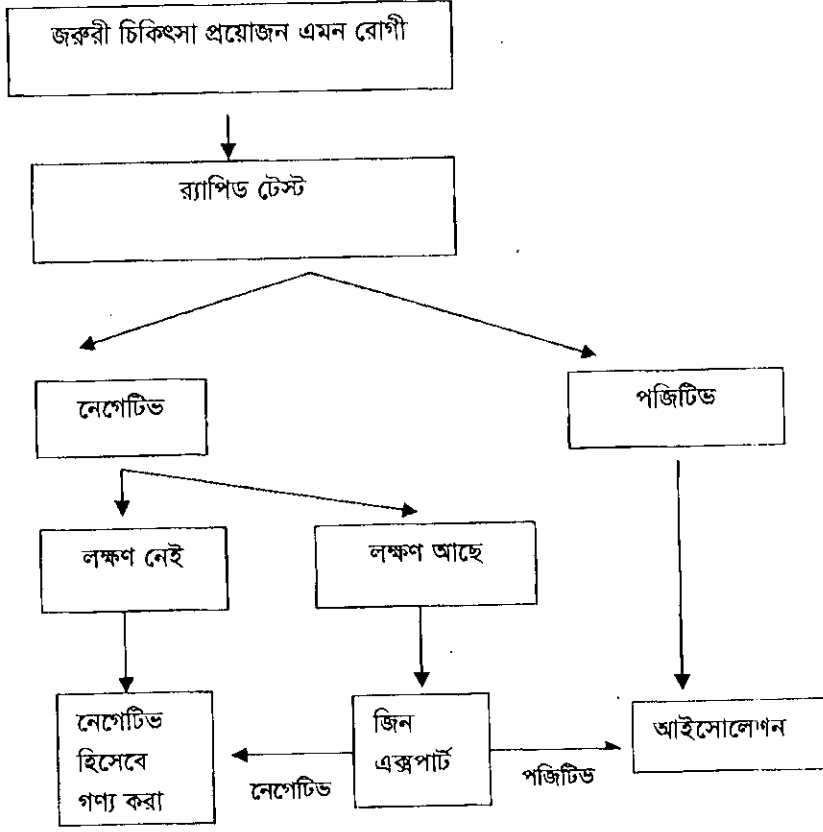


বিশেষায়িত হাসপাতালে অ্যান্টিজেন পরীক্ষা

বিশেষায়িত হাসপাতালে অন্যান্য রোগের জরুরী চিকিৎসার জন্য তাৎক্ষণিকভাবে কোভিড পরীক্ষার প্রয়োজন হয়। জাতীয় হৃদরোগ ইনস্টিটিউট, শেখ হাসিনা বার্ন ও প্লাস্টিক সার্জারী ইনস্টিটিউট, জাতীয় কিডনি ইনস্টিটিউট, জাতীয় ক্যান্সার ইনস্টিটিউট, নিটোর, বারডেম, ন্যাশনাল হার্ট ফাউন্ডেশন ইত্যাদি হাসপাতালে অ্যান্টিজেন পরীক্ষার প্রচলন করা যেতে পারে। তবে অ্যান্টিজেন টেস্টের ফলাফল নেগেটিভ হলে নিশ্চিত হওয়ার জন্য পিসিআর পরীক্ষার পরিবর্তে জিনএক্সপার্ট পদ্ধতিতে দ্বিতীয়বার পরীক্ষা করা যেতে পারে। এতে করে দ্রুত ফলাফল পাওয়া যেতে পারে এবং অপচয় কম হবে।

(Handwritten signature)

চিত্র ২ঃ বিশেষায়িত হাসপাতালে পরীক্ষার পর্যায়



কিটের পরিমাণ নির্ধারণ

প্রাথমিক পর্যায়ে ১০টি হাসপাতালে দৈনিক গড়ে প্রয়োজন = ৩০০ পরীক্ষা X ১০ টি হাসপাতাল
 = ৩০০০টি

পরবর্তীতে ৩৯টি জেলা হাসপাতাল ও বিশেষায়িত হাসপাতালে দৈনিক গড়ে প্রয়োজন
 = ২০০ পরীক্ষা X ৫০টি হাসপাতাল
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প্রয়োজনীয় পদক্ষেপ

- স্বাস্থ্য মন্ত্রণালয় থেকে স্বাস্থ্য ও ঔষধ অধিদপ্তরকে অ্যান্টিজেন পরীক্ষা বিষয়ে প্রয়োজনীয় নির্দেশনা প্রদান।
- অ্যান্টিজেন পরীক্ষা কিট ক্রয়ের জন্য অর্থ বরাদ্দ।
- বিশেষায়িত হাসপাতালে জিন এক্সপার্ট পরীক্ষার ব্যবস্থা নিশ্চিত করা।
- জেলা সদর হাসপাতালে পরীক্ষার প্রস্তুতি গ্রহণ।

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 সন্দ্বীপা, ঢাকা।

Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays

Interim guidance

11 September 2020



Background

Since the beginning of the COVID-19 pandemic, laboratories have been using nucleic acid amplification tests (NAATs), such as real time reverse transcription polymerase chain reaction (rRT-PCR) assays, to detect SARS-CoV-2, the virus that causes the disease. In many countries, access to this form of testing has been challenging. The search is on to develop reliable but less expensive and faster diagnostic tests that detect antigens specific for SARS-CoV-2 infection. Antigen-detection diagnostic tests are designed to directly detect SARS-CoV-2 proteins produced by replicating virus in respiratory secretions and have been developed as both laboratory-based tests, and for near-patient use, so-called rapid diagnostic tests, or RDTs. The diagnostic development landscape is dynamic, with nearly a hundred companies developing or manufacturing rapid tests for SARS-CoV-2 antigen detection (1).

This document offers advice on the potential role of antigen-detecting RDTs (Ag-RDT) in the diagnosis of COVID-19 and the need for careful test selection. The information on Ag-RDTs in this document updates guidance that was included in the Scientific Brief entitled *WHO Advice on use of point of care immunodiagnostic test for COVID-19* published on 8 April 2020. Guidance on the use of Ag-RDTs will be regularly updated as new evidence becomes available.

Most Ag-RDTs for COVID-19 use a sandwich immunodetection method employing a simple-to-use lateral flow test format commonly employed for HIV, malaria and influenza testing. Ag-RDTs are usually comprised of a plastic cassette with sample and buffer wells, a nitrocellulose matrix strip, with a test line with bound antibody specific for conjugated target antigen-antibody complexes and a control line with bound antibody specific for conjugated-antibody. In the case of SARS-CoV-2 RDTs the target analyte is often the virus' nucleocapsid protein, preferred because of its relative abundance. Typically, all materials that are required to perform the test, including sample collection materials, are provided in the commercial kit, with the exception of a timer.

After collecting the respiratory specimen and applying it to the test strip, results are read by the operator within 10 to 30 minutes with or without the aid of a reader instrument. The use of a reader standardizes interpretation of test results, reducing variance in assay interpretation by different operators, but requires ancillary equipment. Most of the currently manufactured tests require nasal or nasopharyngeal swab samples, but companies are carrying out studies to assess the performance of their tests using alternative sample types such as saliva, oral fluid and sample collection systems to potentially expand options for use and to facilitate safe and efficient testing. Generally, the ease-of-use and rapid turnaround time of Ag-RDTs offers the potential to expand access to testing and decrease delays in diagnosis by shifting to decentralized testing of patients with early symptoms. The trade-off for simplicity of operation of Ag-RDTs is a decrease in sensitivity compared to NAAT. Very few of the SARS-CoV-2 Ag-RDTs have undergone stringent regulatory review. Only four tests have received United States Food and Drug Administration (FDA) Emergency Use Authorization (EUA), and another two tests have been approved by Japan's Pharmaceutical and Medical Devices Agency. Only three companies have submitted documents toward WHO's Emergency Use Listing (EUL) procedure (2, 3).

Data on the sensitivity and specificity of currently available Ag-RDTs for SARS-CoV-2 have been derived from studies that vary in design and in the test brands being evaluated. They have shown that sensitivity compared to NAAT in samples from upper respiratory tract (nasal or nasopharyngeal swabs) appears to be highly variable, ranging from 0-94% (4-13) but specificity is consistently reported to be high (>97%). Although more evidence is needed on real-world performance and operational aspects, Ag-RDTs are most likely to perform well in patients with high viral loads (Ct values ≤ 25 or $> 10^6$ genomic virus copies/mL) which usually appear in the pre-symptomatic (1-3 days before symptom onset) and early symptomatic phases of the illness (within the first 5-7 days of illness) (14, 15). This offers the opportunity for early diagnosis and interruption of transmission through targeted isolation

and cohorting of the most infectious cases and their close contacts (16). Patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher.

Despite these expected limitations in performance, if correctly performed and interpreted, Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. At minimum, Ag-RDTs would need to correctly identify significantly more cases than they would miss (sensitivity $\geq 80\%$) and have very high specificity ($\geq 97-100\%$). Based on these performance parameters, this interim guidance proposes several potential roles for Ag-RDT and offers general recommendations for selection of tests and key considerations for their implementation.

General recommendations for the use of SARS-CoV-2 Ag-RDTs

1. SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of $\geq 80\%$ sensitivity and $\geq 97\%$ specificity compared to a NAAT reference assay¹ can be used to diagnose SARS-CoV-2 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility.

To optimize performance, testing with Ag-RDTs should be conducted by trained operators in strict accordance with the manufacturer's instructions and within the first 5-7 days following the onset of symptoms.

2. Appropriate scenarios for use of COVID-19 Ag-RDTs include the following:

i) To respond to suspected outbreaks of COVID-19 in remote settings, institutions and semi-closed communities where NAAT is not immediately available. Positive Ag-RDT results from multiple suspects is highly suggestive of a COVID-19 outbreak and would allow for early implementation of infection control measures. Where possible, all samples giving positive Ag-RDT results (or at least a subset) should be transported to laboratories with NAAT capability for confirmatory testing.

ii) To support outbreak investigations (e.g. in closed or semi-closed groups including schools, care-homes, cruise ships, prisons, work-places and dormitories, etc.) In NAAT-confirmed COVID-19 outbreaks, Ag-RDTs could be used to screen at-risk individuals and rapidly isolate positive cases (and initiate other

contact tracing efforts) and prioritize sample collection from RDT-negative individuals for NAAT.

iii) To monitor trends in disease incidence in communities, and particularly among essential workers and health workers during outbreaks or in regions of widespread community transmission where the positive predictive value and negative predictive value of an Ag-RDT result is sufficient to enable effective infection control.²

iv) Where there is widespread community transmission, RDTs may be used for early detection and isolation of positive cases in health facilities, COVID-19 testing centres/sites, care homes, prisons, schools, front-line and health-care workers and for contact tracing. Note that the safe management of patients with RDT-negative samples will depend on the RDT performance and the community prevalence of COVID-19 (see Annex). A negative Ag-RDT result cannot completely exclude an active COVID-19 infection, and, therefore, repeat testing or preferably confirmatory testing (NAAT) should be performed whenever possible (Figure 1), particularly in symptomatic patients.

v) Testing of asymptomatic contacts of cases may be considered even if the Ag-RDT is not specifically authorized for this use, since asymptomatic cases have been demonstrated to have viral loads similar to symptomatic cases (17), though in that situation, a negative Ag-RDT should not remove a contact from quarantine requirements.

3. For initial introduction of Ag-RDTs into clinical use, countries should consider selecting some settings where NAAT confirmatory testing is currently available so that staff can gain confidence in the assays, confirm performance of the selected RDT, and troubleshoot any implementation issues encountered. Wherever NAAT will be used for confirmatory testing in patients screened using an Ag-RDT, the samples for the two tests should be collected at roughly the same time, or at most within a period of less than 2 days.

4. In situations where confirmatory testing with NAAT is not feasible, any indications that results may be incorrect should raise suspicions about validity. Examples would include patients who are test-positive but have a clinical syndrome not consistent with COVID-19, or patients with a positive test detected in a low-prevalence setting (where the predictive value of a positive test is low and the risk of false-positives high).

¹ Based on well-designed and executed evaluations in representative populations

² Risk of false positive results is high in low prevalence settings; positive predictive value is 78% if prevalence is 10% and

minimum performance criteria met; increases to 93% if prevalence is 20%

Other warning signals might include patients who are test-negative but have a classical syndrome, are close contacts of a case or are tested in a high-prevalence setting. In such situations, considerations should be given to repeating the test, especially if there is also any uncertainty about the visual result (faint bands) or adequacy of sampling.

5. Use of Ag-RDTs is not recommended in settings or populations with low expected prevalence of disease (e.g. screening at points of entry, blood donation, elective surgery), especially where confirmatory testing by NAAT is not readily available. Such use will not be possible until there are more data from high-quality studies confirming high specificity (>99%) of one or more of the commercialized Ag-RDT test kits.

Selection of tests for procurement and implementation:

Though there are a limited number of SARS-CoV-2 Ag-RDTs currently available commercially, multiple products, of variable quality and performance, are expected to enter the marketplace soon. As noted in the Introduction, most commercial SARS-CoV-2 Ag-RDTs use a conventional lateral flow format with colloidal gold or other visible dye as indicators. Several systems, including some with US FDA approval under EUA, use alternative indicators that may lead to enhanced sensitivity but require a specific device to read and interpret the test results.

There are a number of factors to consider when selecting Ag-RDTs for use in the scenarios presented above, in the recommendations section. These include:

1. **Quality of available data used to validate the test.** The source of data should be considered (independent vs. internal/corporate sponsorship) as should study design (e.g. the reference standard used, the type of specimen, the delay between sample collection and test execution and the number of days since symptom onset), the number of subjects enrolled, and the setting of enrolment. As the concentration of virus in specimens is the greatest predictor of test sensitivity, the selection of patients and study sites is of critical importance. Prospective clinical studies are generally superior to retrospective studies. Data from studies independent of corporate sponsorship have particular value if the studies are well-performed.
2. **Reported performance.** Data demonstrating the performance of an RDT should be carefully reviewed before procurement is initiated. Given the relatively low prevalence of active SARS-CoV-2 infections even in settings with community transmission, high specificity (minimum $\geq 97\%$ and ideally $\geq 99\%$) is

necessary to avoid many false-positive results. Sensitivity will depend on the status of patients studied (degree of illness, days since onset of symptoms, etc.) as well as the product quality, but should reach a minimum of $\geq 80\%$. A useful assessment is the sensitivity of the test in patients with a rRT-PCR cycle threshold (Ct) below a specific value (e.g. 28 or 30), because the virus is expected to be abundant in respiratory samples when the test is in this range, and test sensitivity correspondingly high (exceeding 90% in some published and unpublished studies) (4,11). It is important to note, however, that Ct values at a given input concentration of target RNA vary between rRT-PCR assays and are not strictly quantitative.

3. **Manufacturing quality and regulatory status.** Tests should be procured from manufacturers who work under a quality management system (e.g. ISO 13485) and with at least local regulatory approval or right of free sale granted by the country of manufacture. RDTs, as all *in vitro* diagnostics intended for clinical use, should undergo a rigorous and transparent regulatory review. Approval or authorization by a stringent regulatory body and/or Emergency Use Listing by WHO should be available at the time of procurement.
4. **Manufacturing capacity and further evidence of quality.** Many new companies without a history of success in the manufacture, sales and support of *in vitro* diagnostics are entering the market with SARS-CoV-2 Ag-RDTs. Procurers should consider the range of other products offered by the company (especially lateral flow tests), what regulatory approvals they have for non-emergency diagnostic products, and their manufacturing and post-market surveillance capacity. Many companies are able to manufacture high-quality prototypes or completed tests at low volume but may have difficulty when scaling up manufacturing to meet global needs.
5. **Distribution and technical support.** Consideration should be given to a supplier's distribution and product support capacity, especially in low and middle-income countries. This is particularly true for tests that require additional equipment like readers.
6. **Shipping and storage conditions and shelf-life.** The capacity to withstand temperature stress and having an extended shelf-life are critical to the ease-of-use of Ag-RDTs. With new products, shelf-life must be estimated based on accelerated stability studies (usually at higher temperatures), but target shelf-life should be at least 12-18 months at 30°C and

ideally 40°C. A cold chain requirement for shipping and/or storage would significantly increase the cost and complexity of procurement and distribution.

7. **Specimen collection requirements.** SARS-CoV-2 Ag-RDTs vary in their requirements for specimen type, number of processing steps, need for accurate timing, instrumentation and interpretation of results, which will influence the extent of training and supervision required. For this reason, an ease-of-use assessment is an important consideration along with test performance.
8. **Contents of test kit.** Standard kit contents do not necessarily include everything required to perform and quality control the test, and this must be verified prior to purchase. Several commercially available Ag-RDTs for SARS-CoV-2 utilize a reading instrument.
9. **The cost of the test.** The cost of tests will vary according to the test and the volume to be purchased. In general, they should be less expensive than PCR tests. The cost of transportation, import tariffs, storage, end-user training (and supervision) and post-purchase quality control testing activities required to support quality implementation of RDTs must also be considered.
10. **Availability, completeness and clarity of instructions for use.** These should be clear, contain illustrations and be user-friendly for a non-laboratory specialist.

Implementation considerations:

1. Even though Ag-RDTs may be considerably easier to perform than NAAT, they still require that supplier-recommended procedures be strictly followed with due attention to documentation, execution of time-dependent or volume-dependent steps, storage conditions and shelf-life and equipment and stock management. All test operators must have training in sample collection, relevant biosafety, performance of the test and interpretation and reporting of results as well as in waste management. Quality control measures also need to be put in place.
2. Post-market surveillance, with regulatory oversight, is critical to discover defects in product performance and is an important requirement for the manufacturer. The health system should ensure there is monitoring and evaluation of COVID-19 diagnostic testing activities and clear mechanisms for reporting problems (18).

3. Use of instrumented detection systems demands additional training requirements (instrument use, calibration as required, service requirements, operating conditions) and sufficient infrastructure, such as a reliable source of electricity.
4. Sample collection is one of the most critical factors affecting performance of Ag-RDTs. Instructions for use should be carefully followed, and any staff collecting samples should be trained in the methodology.
5. Each of these tests has a specifically indicated method for sample processing after collection. Instructions should be followed precisely, and no alternative reagents used (e.g. water or other liquid instead of dilution/mixing buffer).
6. Biosafety requirements for operators must be in place – personal protective equipment, biohazard waste bag and good ventilation are essential (19).

Methods

This Interim Guidance document outlines potential use and non-use case scenarios for SARS-CoV-2 antigen-detecting RDTs based on minimum performance criteria. Minimum performance requirements for Ag-RDTs were established through a formal process of target product profile (TPPs) development for priority SARS-CoV-2 diagnostics (20). They were informed by an evolving understanding of the temporal dynamics of viral shedding and transmissibility and the anticipated benefits of earlier and expanded testing. PubMed and medRxiv databases were searched for both peer-reviewed and published, pre-print reports of test accuracy of point of care/near patient rapid antigen-detecting SARS-CoV-2 tests. One systematic review of diagnostic SARS test accuracy was identified (21). Additionally, unpublished independent reports on the performance of two SARS-CoV-2 Ag-RDTs were shared confidentially with WHO. Interim guidance was reviewed by members of the WHO Reference Laboratory Network for COVID-19 and members of the WHO COVID-19 Diagnostics Target Product Profile Review Group, as well as other outside experts.

We recognize the shortcomings of the available evidence. They include small sample sizes, skewed sampling based on expected presence or absence of SARS-CoV-2 infection, and lack of details in studies aimed at validating tests regarding symptom status or time from symptom onset. In addition, the lack of data from asymptomatic cases, use of tests outside of manufacturers' instructions for use and performance of tests in laboratories as opposed to point of care/near-patient settings limit the generalizability of recommendations. Nonetheless, it was concluded that some Ag-RDTs are likely to at least meet and likely exceed minimum performance requirements in the early

phase of the illness (within the first 5-7 days, when viral loads and risk of transmission are highest). Expanding testing to potentially interrupt transmission through the use of antigen RDTs is considered more beneficial than not testing or performing tests that fail to inform infection control measures due to very long turnaround times or the risk of false negatives in patients with low viral loads.

Test performance

The performance of an Ag-RDT is determined by the sensitivity and specificity of the test to detect a SARS-CoV-2 infection compared with a reference standard, NAAT (generally rRT-PCR).

Sensitivity is the percentage of cases positive by a NAAT reference standard that are detected as positive by the Ag-RDT under evaluation.

Specificity is the percentage of cases negative by a NAAT reference standard that are detected as negative by the Ag-RDT under evaluation. The prevalence of disease in the community being tested strongly affects the predictive value of a positive or negative result (see Annex). Thus, the clinical value of a positive or negative test result will depend on what action is taken on the basis of the test result when interpreted in the context of local prevalence.

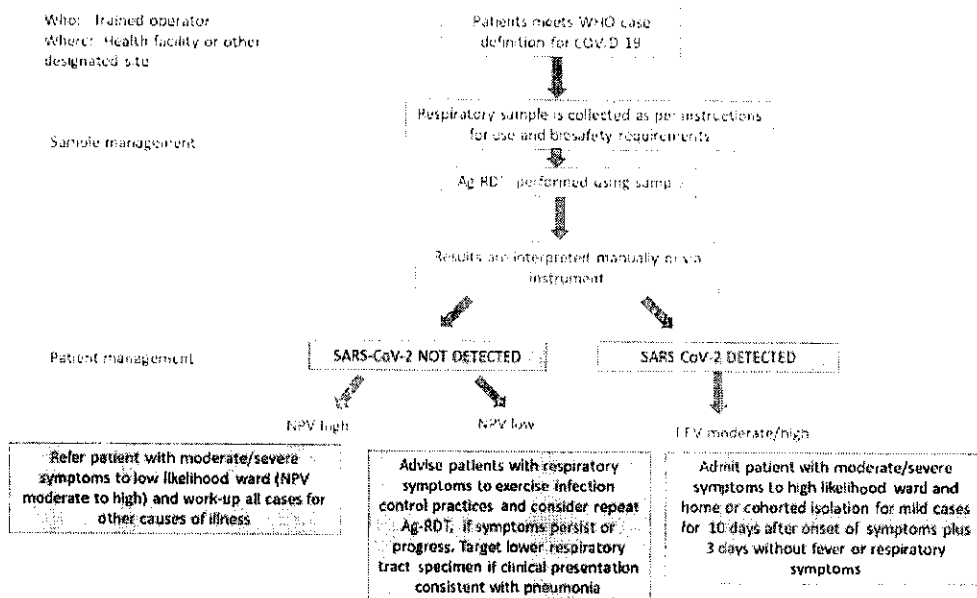
In general, the higher the prevalence of SARS-CoV-2 infection in the tested population, the more likely a person who tests positive is to have COVID-19. The lower the prevalence in the community, the more likely

a test-negative patient is not to have the disease, see Annex. For example, when the prevalence of active SARS-CoV-2 infection in a community is 1%, even a test that is 99% specific would have a poor positive predictive value, since one-half of all positive results would be false positive.

Roles for antigen detecting RDTs for case management and surveillance for COVID-19

Use of Ag-RDTs can be considered in countries or areas that are experiencing widespread community transmission, where the health system may be overburdened and where it may not be possible to test all or any suspect cases by NAAT. As with all diagnostic tests, but especially those with sub-optimal sensitivity and/or specificity, to correctly interpret and act on the results of the RDT, the **prevalence of disease (according to the reference standard) must be estimated based on surveillance**, since this determines the positive and negative predictive values (PPV and NPV, respectively) of the RDTs (see Annex). The proposed process for utilizing an Ag-RDT for COVID-19 case management when there is widespread community transmission is shown in Figure 1. In such a setting, the pre-test probability of COVID-19 disease (the likelihood that the patient has COVID-19 before their results are known, based on epidemiologic and clinical factors) is relatively high, and positive test results have a high predictive value. Likewise, in a setting of community transmission, the predictive value of a negative RDT result may be low, even when there are strong epidemiologic or clinical indicators of COVID-19 exposure or disease.

Figure 1. Flowchart demonstrating the potential use of antigen-based RDTs (that meet minimum performance criteria) in settings of widespread community transmission and where there is no NAAT capacity.



NPV- negative predictive value; PPV – positive predictive value

Table 1. Situations where SARS-CoV-2 Ag-RDTs should not be used, based on currently available information

Do not use SARS-CoV-2 Ag-RDTs:	Explanation
In individuals without symptoms unless the person is a contact of a confirmed case	Pre-test probability (the likelihood, before testing, that the patient has the disease based on epidemiology, case contact, clinical findings) is low.
Where there are zero or only sporadic cases	Ag-RDTs are not recommended for routine surveillance purposes or case management in this setting. Positive test results would likely be false positives. Molecular testing is preferred.
Appropriate biosafety and infection prevention and control measures (IPC) are lacking	To safeguard health workers, respiratory sample collection for any test from patients with suspected COVID-19 requires that operators wear gloves, gown, mask and face shield or goggles (19, 22, 23).
Management of the patient does not change based on the result of the test	If test-positive and test-negative patients will be treated the same way because of unknown or low PPV and/or NPV, then there is no benefit to testing.
For airport or border screening at points of entry	Prevalence of COVID-19 will be highly variable among travellers, and it is therefore not possible to determine PPV and NPV of test results. Positive and negative tests would require confirmatory testing to increase PPV and NPV for decision making.
In screening prior to blood donation	A positive RDT result would not necessarily correlate with presence of viremia. Asymptomatic blood donors do not meet the definition of a suspect case (24).

Factors influencing test performance

- As mentioned above, many factors may affect the performance of antigen-detecting RDTs. Consequently, findings in clinical settings may be variable. The following should be taken into account:
 - patient factors such as the time from illness onset and immune status sample type (upper or lower respiratory tract), quality and processing, including storage conditions and dilution in viral transport medium
 - viral factors including the concentration and duration of viral antigen shedding and structural variation in the target antigen, cross reactivity with other viruses
 - specific protein target, as some antigens are produced in higher concentrations than others, e.g. nucleocapsid versus spike proteins
 - product design or quality issues including:
 - insufficient antibody quantity or affinity for the target antigen(s)
 - poor packaging and exposure to heat and humidity during improper transport and/or storage, which can degrade antibodies in the test

- unclear or incorrect instructions that can affect test performance
- inadequate training or competency of the test operator, which may lead to error in preparing the antigen-detecting RDT, performing the test or interpreting the result, with erroneous conclusions.

Future updates and product specific recommendations

WHO is working closely with groups evaluating the performance and operational characteristics of commercialized SARS-CoV-2 antigen detecting RDTs to systematically compile the evidence as it emerges and coordinate updates. Currently, there is insufficient evidence on performance and operational use to recommend specific commercial products.

References

1. Foundation for Innovative New Diagnostics. SARS-CoV-2 Diagnostic Pipeline 2020 [Available from: <https://www.fnddx.org/covid-19/pipeline/>].
2. U.S. Food & Drug Administration. In Vitro Diagnostics EUAs 2020 [Available from: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>].
3. Agency PaMD. PMDA's Efforts to Combat COVID-19 2020 [Available from: <https://www.pmda.go.jp/english/about-pmda/0002.html>].
4. Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, et al. Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis.* 2020;S1201-9712(20):30405-7.
5. Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, et al. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. *medRxiv.* 2020:2020.03.07.20032524.
6. Lambert-Niclot S, Cuffel A, Le Pape S, Vauloup-Fellous C, Morand-Joubert L, Roque-Afonso AM, et al. Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. *J Clin Microbiol.* 2020;58(8).
7. Mertens P, De Vos N, Martiny D, Jassoy C, Mirazimi A, Cuyppers L, et al. Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. *Front Med (Lausanne).* 2020;7:225-.
8. Blairon L, Mokrane S, Wilmet A, Dessilly G, Kabamba-Mukadi B, Beukinga I, et al. Large-scale, molecular and serological SARS-CoV-2 screening of healthcare workers in a 4-site public hospital in Belgium after COVID-19 outbreak. *J Infect.* 2020;S0163-4453(20)30514-4.
9. Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2020;129:104500-.
10. Nagura-Ikeda M, Imai K, Tabata S, Miyoshi K, Murahara N, Mizuno T, et al. Clinical evaluation of self-collected saliva by RT-qPCR, direct RT-qPCR, RT-LAMP, and a rapid antigen test to diagnose COVID-19. *J Clin Microbiol.* 2020;JCM.01438-20.
11. Omi K, Takeda Y, Mori M. SARS-CoV-2 qRT-PCR Ct value distribution in Japan and possible utility of rapid antigen testing kit. *medRxiv.* 2020:2020.06.16.20131243.
12. Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol.* 2020;129:104455.
13. Weitzel T, Legarraga P, Iruretagoyena M, Pizarro G, Vollrath V, Araos R, et al. Head-to-head comparison of four antigen-based rapid detection tests for the diagnosis of SARS-CoV-2 in respiratory samples. *bioRxiv.* 2020:2020.05.27.119255.
14. Weiss A, Jellingsø M, Sommer MOA. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: A systematic review and meta-analysis. *EBioMedicine.* 2020;58.
15. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. *New England Journal of Medicine.* 2020;382(22):2081-90.
16. Bulard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2020;eiv: a638.
17. Lee S, Kim T, Lee E, Lee C, Kim H, Rhee H, et al. Clinical Course and Molecular Viral Shedding Among Asymptomatic and Symptomatic Patients With SARS-CoV-2 Infection in a Community Treatment Center in the Republic of Korea. *JAMA Internal Medicine.* 2020.
18. World Health Organization. Post-market surveillance of in-vitro diagnostics. 2015.
19. World Health Organization. Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19) 2020 [updated 13 May 2020. Available from: [https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-\(covid-19\)](https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19))].
20. World Health Organization. COVID-19 Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.0.1 2020 [updated 31 July 2020. Available from: <https://www.who.int/publications/m/item/covid-19-target-product-profiles-for-priority-diagnostics-to-support-response-to-the-covid-19-pandemic-v.0.1>].
21. Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database of Systematic Reviews.* 2020(8).
22. World Health Organization. Infection prevention and control during health care when COVID-19 is suspected 2020 [updated 19 March 2020. Available from: [https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-\(covid-19\)](https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19))].
23. World Health Organization. Rational use of personal protective equipment (PPE) for coronavirus disease (COVID-19) 2020 [updated 4 April 2020. Available from: [https://www.who.int/publications/i/item/rational-use-of-personal-protective-equipment-for-coronavirus-disease-\(covid-19\)-and-considerations-during-severe-shortages](https://www.who.int/publications/i/item/rational-use-of-personal-protective-equipment-for-coronavirus-disease-(covid-19)-and-considerations-during-severe-shortages)].
24. World Health Organization. WHO COVID-19 Case definition 2020 [updated 7 August 2020. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.1].

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Declaration of interests

Declarations of interests were collected from all external contributors and assessed for any conflicts of interest. There were no significant conflicts of interest declared.

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire 2 years after the date of publication.

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Annex

Annex : Positive predictive value (PPV) and negative predictive value (NPV) and the number of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) tests in a population of 10 000 with the prevalence of COVID-19 estimated at 5, 10, 20, 30% prevalence and based on recommended performance criteria: sensitivity of 70, 80%, 90% and specificity of 98% and 100%.

Example prevalence target populations	Prevalence (%)	Sensitivity	Specificity	NPV	PPV	TP	FP	TN	FN	No. with disease	No. positive tests	Total
Symptomatic general population; contacts of index case	5	70	98	98	60	350	238	9263	150	500	588	10000
		70	100	98	88	350	48	9453	150	500	398	10000
		80	98	99	63	400	238	9263	100	500	638	10000
		80	100	99	89	400	48	9453	100	500	448	10000
		90	98	99	65	450	238	9263	50	500	688	10000
		90	100	99	90	450	48	9453	50	500	498	10000
		70	98	97	76	700	225	8775	300	1000	925	10000
		70	100	97	94	700	45	8955	300	1000	745	10000
		80	98	98	78	800	225	8775	200	1000	1025	10000
		80	100	98	95	800	45	8955	200	1000	845	10000
Community transmission: Symptomatic patients presenting to health care facilities; contacts of index cases; institutions & closed communities with confirmed outbreaks	10	90	98	99	80	900	225	8775	100	1000	1125	10000
		90	100	99	95	900	45	8955	100	1000	945	10000
		70	98	93	88	1400	200	7800	600	2000	1600945	10000
		70	100	93	91	1400	40	7960	600	2000	1440	10000
Symptomatic at referral centre; Symptomatic or screening of health care workers; care homes	20	80	98	95	89	1600	200	7800	400	2000	1800	10000
		80	100	95	98	1600	40	7960	400	2000	1640	10000
		90	98	98	90	1800	200	7800	200	2000	2000	10000
		90	100	98	98	1800	40	7960	200	2000	1840	10000
		70	98	88	92	2100	175	6825	900	3000	2275	10000
		70	100	89	98	2100	35	6965	900	3000	2135	10000
Symptomatic health care worker/cleaners; care home residents	30	80	98	92	93	2400	175	6825	600	3000	2575	10000
		80	100	92	99	2400	35	6965	600	3000	2435	10000
		90	98	96	94	2700	175	6825	300	3000	2875	10000
		90	100	96	99	2700	35	6965	300	3000	2735	10000
		70	98	88	92	2100	175	6825	900	3000	2275	10000
		70	100	89	98	2100	35	6965	900	3000	2135	10000