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

INTERIM GUIDANCE

World Health Organization

HEALTH EMERGENCIES programme

Diagnostic testing for SARS-CoV-2: updated guidance

https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

World Health Organization

Diagnostic testing for SARS-CoV-2

Interim guidance

11 September 2020

World Health Organization

Introduction

This document provides interim guidance to laboratories and other stakeholders involved in diagnostics for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It covers the main considerations for specimen collection, nucleic acid amplification testing (NAAT), antigen (Ag), antibody (Ab) detection and quality assurance. This document will be updated as new information becomes available. Feedback can be sent to WHElab@who.int.

Changes from the previous version

The title of this interim guidance has changed from "Laboratory testing for COVID-19 in suspected human cases" to "Diagnostic testing for SARS-CoV-2". Additional relevant background information and a clinical diagnostic algorithm has been added to the document. Furthermore, the guidance has been updated with new findings from the literature and best practices.

Relevant WHO documents

WHO has developed interim guidance and technical briefs to assist policy-makers and laboratories on testing for SARS-CoV-2. These documents cover laboratory testing strategy [1], laboratory assessment tool [2], laboratory biosafety [3], advice on the use of point-of-care immunodiagnostic tests [4], antigen detection in diagnosis of SARS-CoV-2 infection using rapid immunoassays [5], guidance for the investigations of clusters [6], public health surveillance [7] and operational considerations for surveillance using GISRS [8]. In addition, early investigation protocols [9] can be used by countries to implement epidemiological studies and enhance understanding of transmission patterns, disease severity and prevalence, clinical features and risk factors of SARS-CoV-2 infection.

Background on SARS-CoV-2

WHO was first alerted to a cluster of pneumonia of unknown etiology in Wuhan, People's Republic of China on 31 December 2019. The virus was initially tentatively named 2019 novel coronavirus (2019-nCoV).

Subsequently the International Committee of Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2 [10]. COVID-19 is the name of the illness caused by SARS-CoV-2.

SARS-CoV-2 is classified within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family *Coronaviridae* [11]. It is an enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome [10]. The virus has an RNA proofreading mechanism keeping the mutation rate relatively low. The genome encodes for non-structural proteins (some of these are essential in forming the replicase transcriptase complex), four structural proteins (spike (S), envelope (E), membrane (M), nucleocapsid (N)) and putative accessory proteins [12-14]. The virus binds to an angiotensin-converting enzyme 2 (ACE2) receptor for cell entry [15-17].

SARS-CoV-2 is the seventh coronavirus identified that is known to infect humans (HCoV). Four of these viruses, HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43, are endemic, seasonal and tend to cause mild respiratory disease. The other two viruses are the more virulent zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1). SARS-CoV-2 is most genetically similar to SARS-CoV-1, and both of these viruses belong to the subgenus *Sarbecovirus* within the genus *Betacoronavirus* [11]. However, SARS-CoV-1 is currently not known to circulate in the human population.

The clinical presentation of SARS-CoV-2 infection can range from asymptomatic infection to severe disease [18-27]. Mortality rates differ per country [28]. Early laboratory diagnosis of a SARS-CoV-2 infection can aid clinical management and outbreak control. Diagnostic testing can involve detecting the virus itself (viral RNA or antigen) or detecting the human immune response to infection (antibodies or other biomarkers).

While our understanding of SARS-CoV-2 has rapidly expanded, there are still many outstanding questions that need to be addressed. WHO encourages research and the sharing of results that may contribute toward an improved characterization of SARS-CoV-2 [29, 30].

Ag-based RDT updated guidance – 11 Sept 2020

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Interim guidance

11 September 2020

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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 immunodiagnostics 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

https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays

HEALTH EMERGENCIES programme

Work in progress – Updated 19 August 2020

CORONAVIRUS GLOBAL RESPONSE

- SRA/PQ either completed or underway by late Aug
- Evaluation by FIND

There are many Ag RDTs in the pipeline

	Early development	Unknown development stage	Late development	Research Use Only[1]	Validation	Regulatory approved[2]/launch
Visually-read RDT 61 mfrs 38 mfrs	1. Amasu tech. / DATA Aminotek, ZA 2. Beijing Wantai, CN 3. Biosynex, FR 4. Core Diag. corp., US 5. InTec Products, CN 6. Lumos/Kestrel, US 7. Mantle Biotech., US 8. Pace diagnostics, US 9. Qingdao Hightop Biotech, CN 10. Redcell Biotech./Univ. of Pretoria, TR 11. Schweitzer Biotech, TW 12. Skin Reju. Tech., ZA 13. SRI International, US 14. Zumutor Bio., IN	1. Aptamer Group / Cytiva, UK 2. Assure Tech., CN 3. Chembio, US 4. Core Tech., CN 5. DCN, US 6. Dynamiker, CN 7. Humasis / Celltrion, KR 8. Jinis Diagnostics, CN 9. Kephera, US 10. KH medicals, KR 11. Lionex, DE 12. PRIME4DIA, KR 13. SD Biosensor, KR 14. XING Group Holdings / Lumos, AU 15. Zalgen Labs, US	1. Avacta/Cytiva, UK/US 2. Cupid limited, IN 3. Green Cross Medical Science, KR 4. Hunan Lituo Biotech., CN 5. Roche, CH 6. BD, US	1. Leadgene Biomedical, TW	1. AllTest, CN 2. ARD, US 3. Bioperfectus, CN 4. Denka Seiken, JP (finalized validation, preparing PMDA) 5. Edinburg Genetics, UK 6. Decheng Biotech./Co-Inn. Bio., CN 7. Invex Health, IN 8. Joysbio Biotech., CN 9. Maxim biomedical, US 10. Medsource Ozone Biomed., IN 11. Mologic, UK 12. NG Biotech, FR 13. OraSure, US 14. Sona Nanotech, CN 15. Tigsun Diagnostics, CN 16. VivaCheck, CN 17. Wondfo, CN	1. Bionote, KR 2. Coris BioConcept, BE 3. Fujirebio/Miraca, JP 4. GenBody, KR 5. Liming Bio-Products, CN 6. Lomina, CH 7. Mylab Discovery Solutions, IN 8. New Gene BioEng., CN 9. RapiGen, KR 10. SD Biosensor, KR 11. Abbott Laboratories, GE
RDT + reader or visualizer 15 mfrs 9 mfrs	1. MAVEN Diagnostic, US 2. Sathguru Management Consultants, IN	1. AnteoTech, AU 2. Luminostics, US	1. NBPostech, KR (validation in Sept., launch in Oct.) 2. PMC, IN			1. BD, US 2. Bioeasy, CN 3. Boditech Med, KR 4. Decheng Biotech, CN 5. Kewei Clinical Diagnostic Reagent, CN 6. PCL, KR 7. Quidel, US 8. Savant Biotech, CN 9. LumiraDx, De
POC immunoassays[3] 7 mfrs 6 mfrs	1. Achira Labs, IN 2. Novosens, AR 3. Qorvo Biotech., US 4. Ricovr Healthcare, US		1. Pinpoint science, US (validation in Sept.)			1. BBB/Celltrion, KR 2. Medisys Intl, CH

1. Some companies' products are RUO while still working on validation to achieve regulatory approval. 2. Obtained CE-IVD / USA FDA EUA / BR, IN, JP authorizations.
3. Small POC instrument (portable in most cases) where the consumables are not a lateral flow assay, but more a cartridge based on different technologies (e.g., microfluidics)

Source: FIND

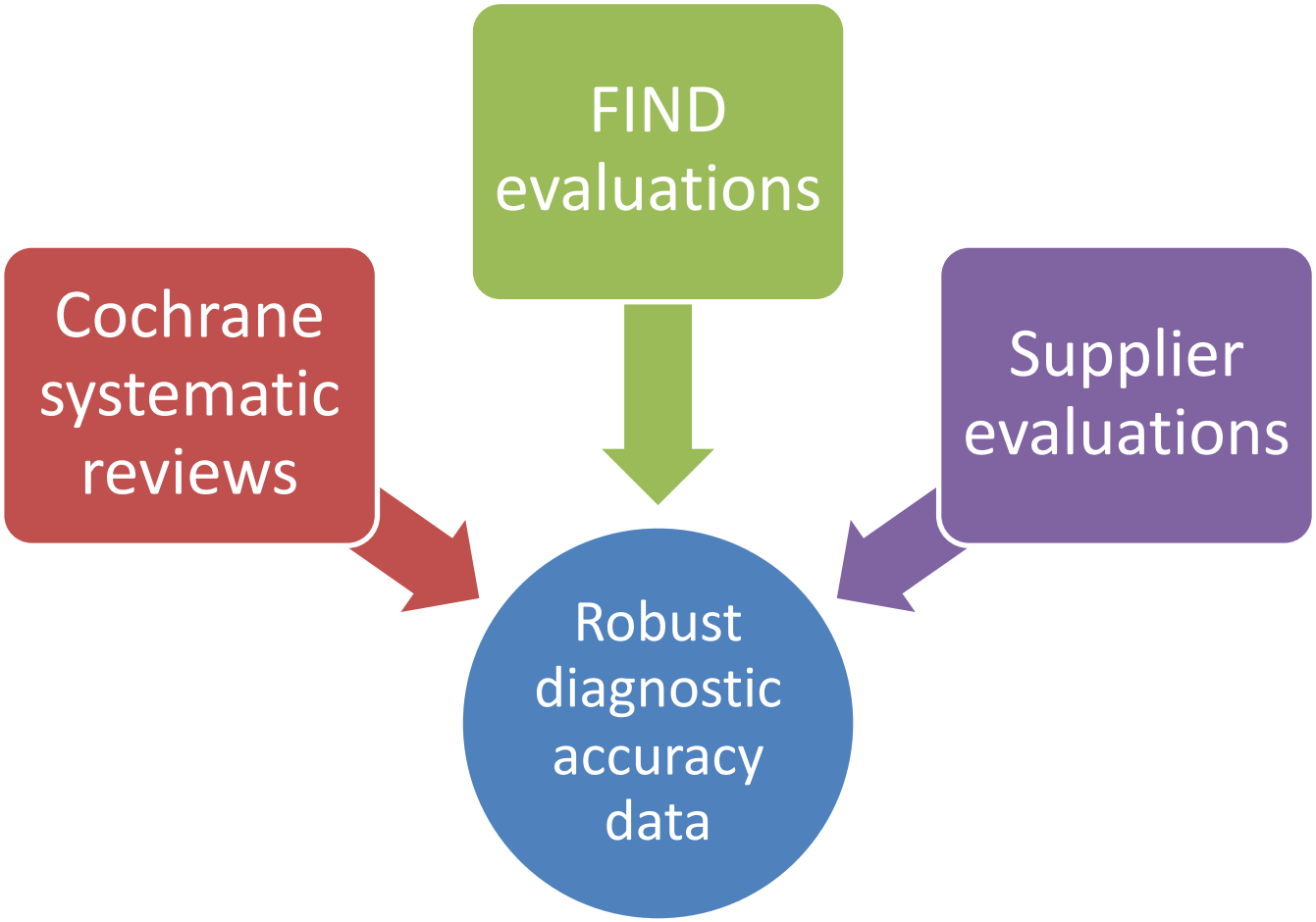
WHO Emergency Use Listing

SARS-CoV-2 Rapid Antigen Tests: progress of the active applications in the emergency use listing assessment pipeline

Product name	Product code(s)	Manufacturer name	Dossier review	QMS Desk Assessment
Panbio COVID-19 Ag Rapid Test Device (Nasopharyngeal)	41FK10	Abbott Rapid Diagnostics Jena GmbH	🗁	R
STANDARD Q COVID-19 Ag Test	09COV30D and 10COVC10	SD Biosensor Inc.	R	🗁
ESPLINE SARS-CoV-2	231906	Fujirebio, Inc	🗁	🗁
BIOEASY Diagnostic kit for SARS-CoV-2 Ag (Fluorescence Immunochromatographic Assay)	YRLF04401025, YRLF04401050 and YRLF04401100	Shenzhen Bioeasy Biotechnology Co., Ltd	awaiting submission	awaiting submission

Last updated: 15 Sept 2020; https://www.who.int/diagnostics_laboratory/EUL/en/

Diagnostic Accuracy of Ag-based RDTs

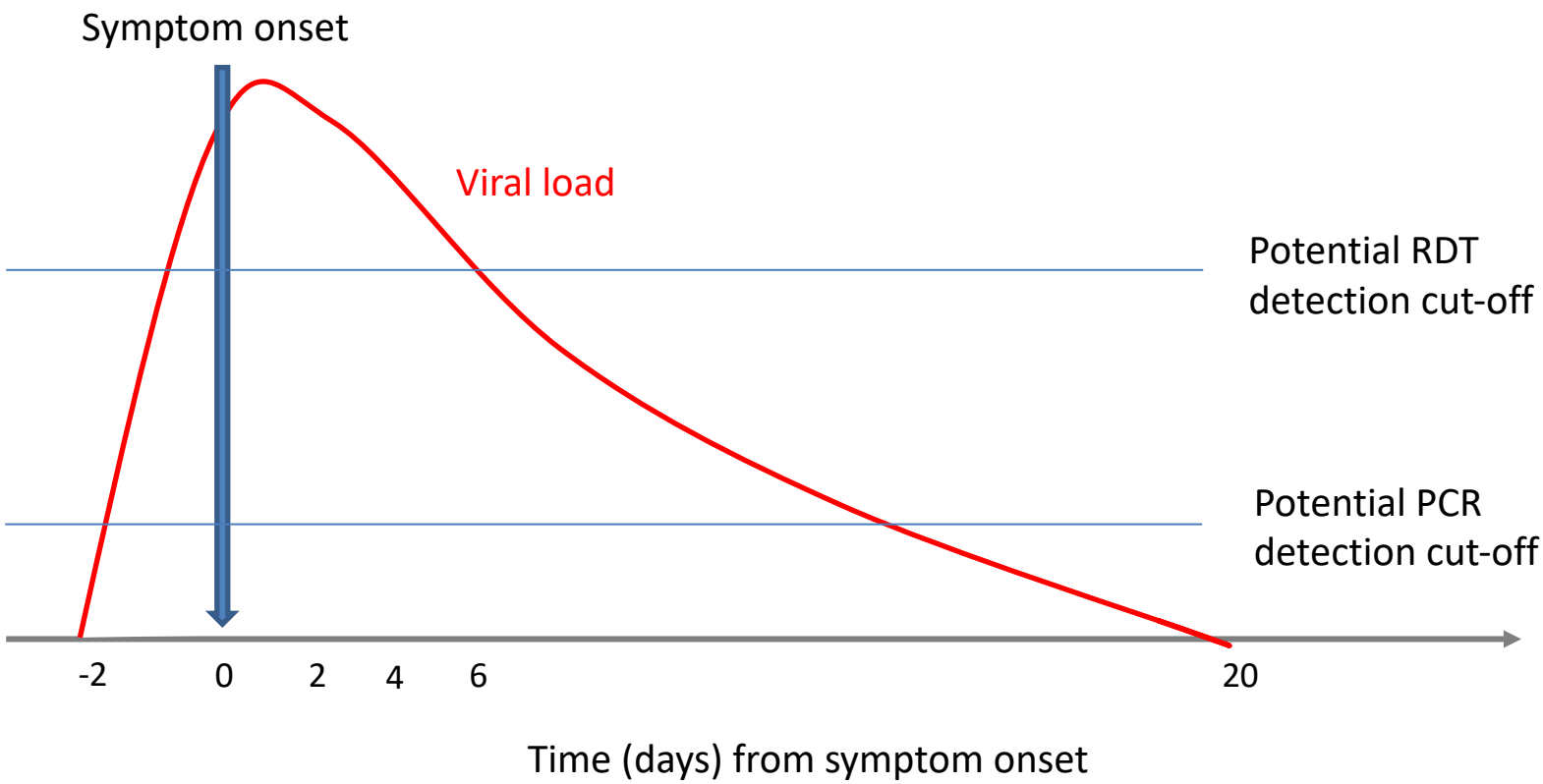


General recommendations for the use of SARS-CoV-2 Ag-RDTs

SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay **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.**

Who can be detected with an Ag-based RDT?



The link between performance and prevalence = predictive value

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
Community transmission: Symptomatic patients presenting to health care facilities; contacts of index cases; institutions & closed communities with confirmed outbreaks	10	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
		90	98	99	80	900	225	8775	100	1000	1125	10000
		90	100	99	95	900	45	8955	100	1000	945	10000

⬇ sensitivity = ⬆ false negatives → potential ⬆ transmissions

⬇ specificity = ⬆ false positives → suggestive (false) outbreaks

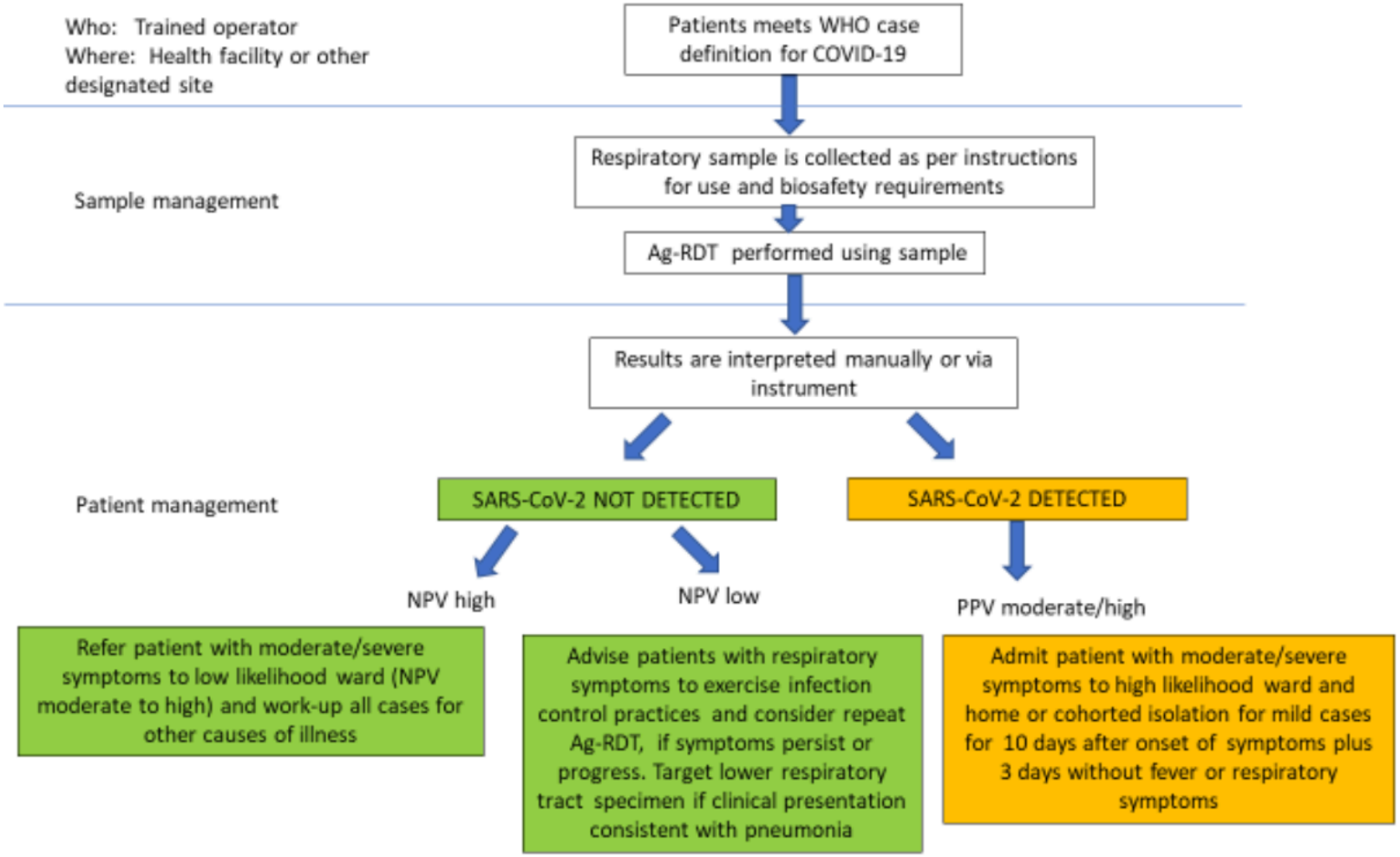
Situations where SARS-CoV-2 Ag-RDTs should not currently be used

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).

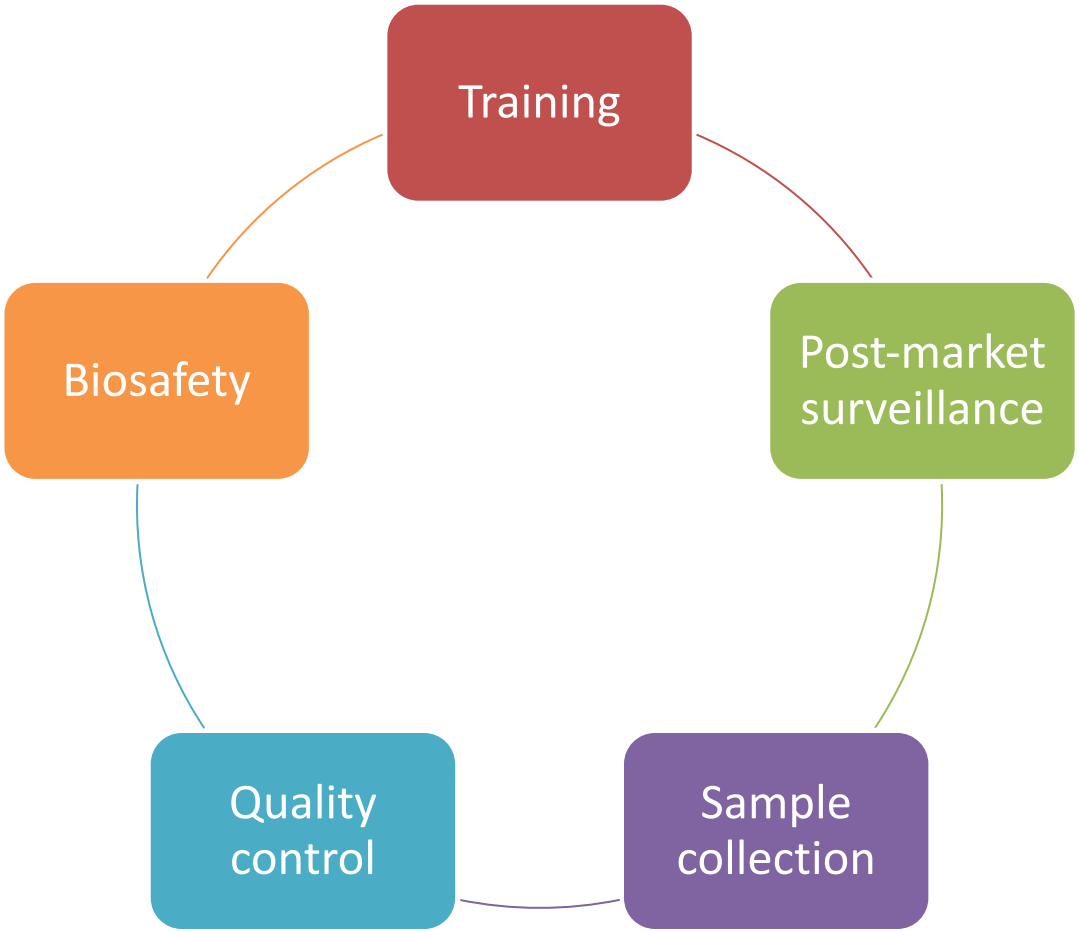
Scenarios for use of COVID-19 Ag-RDTs

	Population recommended to be screened
Outbreak response	*To respond to suspected outbreaks of COVID-19 in remote settings, institutions and semi-closed communities where NAAT is not immediately available.*
Outbreak investigation/Contact tracing	*To support outbreak investigations (e.g. in closed or semi-closed groups including schools, care-homes, cruise ships, prisons, work-places and dormitories, etc.) and to screen at-risk individuals*
Monitor trends in disease incidence	*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.*
Community Transmission Screening	*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.*
Testing of Asymptomatic contacts	*Testing of asymptomatic contacts of cases may be considered even if the Ag-RDT is not specifically authorized for this use*

The potential use of antigen-based RDTs



Key implementation considerations



Thank you!