Use of

Influenza Rapid Diagnostic Tests

World Health Organization

TDR For research on diseases of poverty

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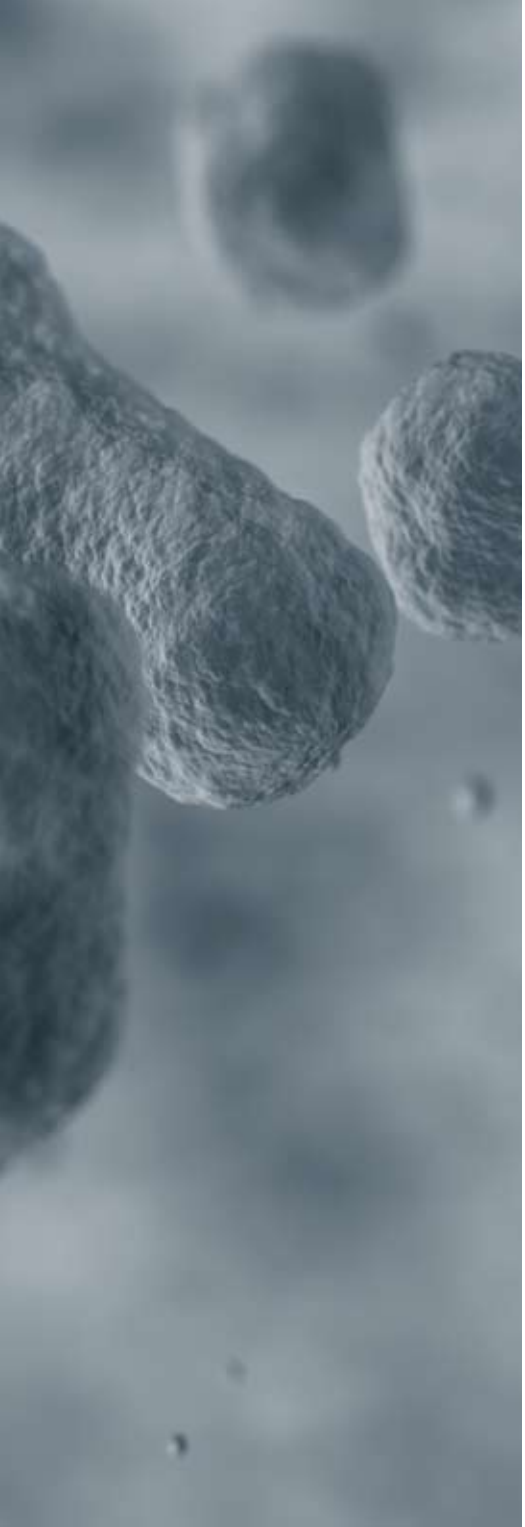
To request copies and address queries, please contact:

Dr Jane Cunningham, WHO/TDR
E-mail: cunninghamj@who.int



Background

Seasonal and pandemic influenza pose ongoing risks to global human public health. The emergence of the novel influenza A(H1N1) 2009 virus in the human population has led to a global influenza pandemic. Influenza diagnostic tests which aid in case management and outbreak control and permit monitoring of disease spread and viral evolution are critical to patient care and public health efforts. As a result, laboratory testing strategies must be developed and optimized in each country. While sophisticated influenza tests are available in specialized laboratories their capacity may be overwhelmed during a pandemic. Point-of-care tests (POCT) or rapid influenza diagnostic tests (RIDT) are available and can be used in remote settings and in physician's offices or clinics without laboratory services. They do not require laboratory equipment and can be performed and interpreted by health-care providers within 5-15 minutes.

This user's guide provides general information on RIDTs and possible applications according to the availability of local epidemiology and influenza laboratory services. In particular, it highlights the limitations of these tests. The aim of this document is to ensure appropriate use and interpretation of the results of RIDTs combined with guidance on good quality planning prior to test deployment so that case management and disease control efforts are optimized.

I. What is influenza?

Influenza is an acute respiratory disease caused by infection with influenza type A and influenza type B viruses. All influenza A viruses are subgrouped on the basis of their surface haemagglutinin (H) and neuraminidase (N) glycoproteins, of which there are 16 known types of H and 9 types of N. The current subtypes of influenza A viruses circulating most widely among humans are A (H1N1) and A(H3N2). Aquatic birds are the primary reservoir for influenza A viruses but they also circulate among other animals including pigs, horses and seals. Humans are the primary reservoir for influenza B viruses.

In humans, both influenza A and B viruses result in seasonal epidemics with winter peaks in temperate zones and year-round circulation in the tropics with rainy season and dry season peaks in activity. Both viruses continually evolve through the accumulation of mutations leading to antigenic drift of the H and N glycoproteins. Influenza A viruses can also evolve through additional mechanisms that allow the emergence of a novel influenza A virus. These can potentially cause a rare influenza pandemic if the novel virus spreads in a sustained manner through largely susceptible populations.

The spectrum of influenza virus infection is wide, ranging from non-febrile, mild upper respiratory tract illness, febrile influenza-like illness (ILI) to severe or even fatal complications. The greatest burden of illness usually occurs among children, while the greatest severe disease burden in terms of hospitalization and death occurs in those with underlying medical conditions, infants and young children, and the elderly.

Uncomplicated seasonal influenza is associated with an incubation period of 1-4 days, followed by the acute onset of signs and symptoms including a fever ≥38°C, myalgia, headache, sore throat and a protracted cough. Children may present with gastrointestinal symptoms, while influenza in the elderly may present as lethargy without an elevated temperature. In adults, viral replication and probable communicability is greatest in the first 3-5 days of illness. In young children and immunocompromised persons, it can be longer (eg. 7-10 days in the former and weeks to months in the latter.

The emergence and rapid worldwide spread of a novel influenza A (H1N1) virus in the spring 2009 has resulted in the first influenza pandemic since 1968. While the majority of illnesses caused by pandemic influenza A (H1N1) 2009 virus infection have been self-limited mild-to-moderate uncomplicated disease, severe complications including fatal outcomes have been reported and the highest attack rates have been in children and young adults. Risk factors for severe disease from pandemic (H1N1) 2009 virus infection reported to date are considered similar to those risk factors identified for complications from seasonal influenza. Those at greatest risk include: infants and young children, in particular <2 years and adults >65 years; pregnant women and persons of any age with chronic pulmonary, cardiac, renal or hepatic disease or metabolic disorders.

Detailed recommendations for the prevention and control of annual influenza epidemics are issued annually by national health agencies and the World Health Organization (WHO). WHO and countries also have pandemic plans. Immunization and antiviral drugs play key roles. Antiviral medications are available for treatment. Further information regarding vaccination and use of antivirals against seasonal and pandemic influenza can be found in *WHO recommendations on pandemic (H1N1) 2009 vaccines*[1] and *WHO Guidelines for Pharmacological Management of Pandemic (H1N1)*

1 http://www.who.int/csr/disease/swineflu/notes/h1n1_vaccine_20090713/en/index

II. How is influenza diagnosed?

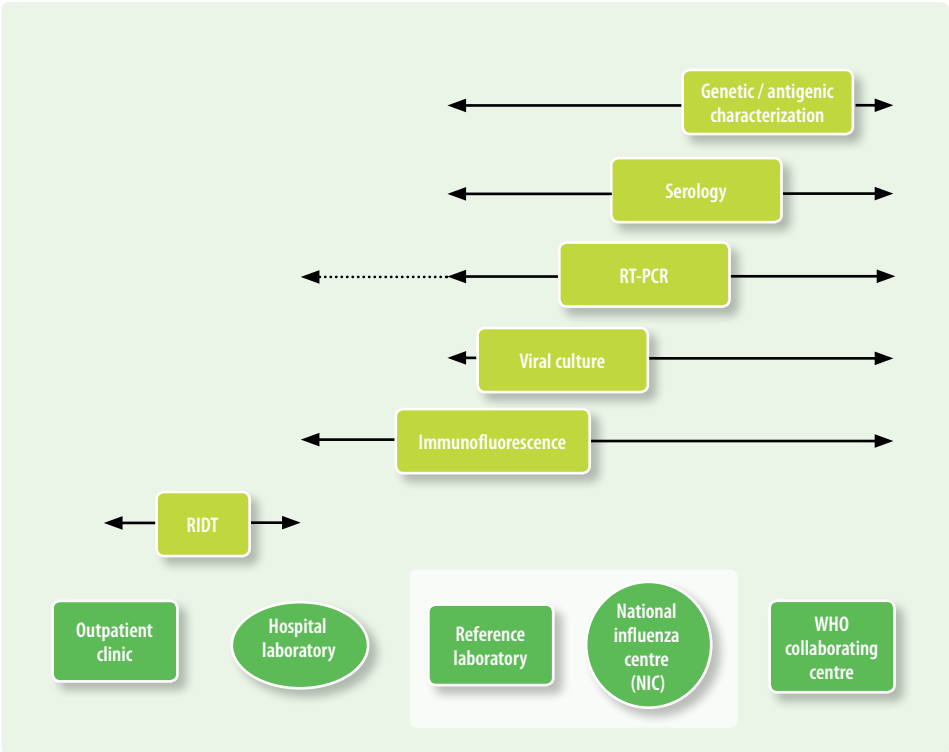
The majority of cases of human influenza are clinically diagnosed. However, during periods of low influenza activity and outside of outbreak situations, the circulation of other respiratory viruses which can present as ILI makes the clinical diagnosis of influenza difficult. Influenza illness may be clinically indistinguishable from infection with rhinovirus, respiratory synctial virus, parainfluenza, adenovirus and others. Therefore, collection of appropriate respiratory samples and the application of a laboratory diagnostic test is required to establish a definitive diagnosis.

Proper collection, storage and transport of respiratory specimens is the essential first step for laboratory detection of influenza virus infections. Laboratory confirmation of influenza virus from throat, nasal and nasopharyngeal secretions or tracheal aspirate or washings is commonly performed using direct antigen detection, virus isolation, or detection of influenza-specific RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). For a summary of methods for the proper collection, storage and transport of clinical specimens and of laboratory methodologies for influenza diagnosis, refer to the documents below:

1. *Instructions for storage and transport of suspected or confirmed human and animal specimens and virus isolates of pandemic (H1N1) 2009.* [2]
2. *WHO Information for Laboratory Diagnosis of New Influenza A (H1N1) Virus.*[3]

3 http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/index.html
4 http://www.who.int/csr/resources/publications/swineflu/diagnostic_recommendations/en/index.html

Figure 1: Diagnostic assays available in different laboratory settings



Available diagnostic methods have important differences in terms of performance, ease and timeliness, equipment and reagent costs, and availability. With the exception of direct antigen detection via rapid diagnostic tests which can be performed at the point of care and in remote settings, diagnosis requires sophisticated laboratory capacity and highly skilled laboratory staff (Figure 1).

III. Why and how is influenza activity monitored?

Surveillance and monitoring of influenza activity is crucial for the management of a pandemic at global and national levels. At different stages of the pandemic, monitoring may be carried out differently depending on the country and it's laboratory capacity.[5]
To facilitate global influenza surveillance and monitoring efforts, WHO has coordinated a Global Influenza Surveillance Network (GISN), comprised mainly of 5 Collaborating Centres and more than 130 National Influenza Centres (NIC) around the world. As a result, there is a mechanism in most countries to move clinical samples up the chain of laboratories to monitor geographical spread and permit evaluation of the virus for changes in antigenicity, antiviral sensitivity and other viral properties. This, in turn, influences guidance on clinical management of influenza and public health efforts including identification of candidate viruses for vaccine selection.

5 Human infection with pandemic (H1N1) 2009 virus: updated interim WHO guidance on global surveillance 10 July 2009, **http://www.who.int/csr/disease/swineflu/WHO_case_definition_swine_flu_2009_04_29.pdf**

IV. Potential roles of rapid influenza diagnostic tests (RIDTs)

Individual patient management

Rapid influenza diagnostic tests (RIDTs) can help in the diagnosis and management of patients who present with ILI particularly if performed within the first 4 days of the onset of symptoms. However, they have serious limitations in that most commercially available RIDTs cannot specifically confirm pandemic influenza A (H1N1) 2009 virus infection and a positive test result cannot differentiate between pandemic (H1N1) 2009 virus or exclude infection with seasonal influenza A viruses. Also, because of low test sensitivity, a negative result cannot exclude pandemic or seasonal influenza virus infection. To this end, other information, including surveillance data on circulating influenza viruses; symptoms and clinical findings; travel history and/or exposure to confirmed or probable influenza cases, is required to aid interpretation of results if they are to optimally inform patient management decisions. In general, the use of RIDTs in hospitalized patients should not be encouraged where RT-PCR or immunofluorescence assays for influenza are available. See the section on 'How effectively do rapid tests detect influenza?' for further information.

Institutional and semi-closed community outbreak management

RIDTs can help to quickly identify influenza A in institutions, schools and/or communities with increasing reports of ILI and can help facilitate timely implementation of control interventions for institutional control outbreaks and inform public health guidance. Semi-closed communities may also benefit from testing including testing passengers on cruiseships where outbreaks may occur at any time of the year. Despite reduced sensitivity relative to other tests like viral culture and RT-PCR, rapid antigen detection in a proportion of ILI cases in an outbreak setting is suggestive that influenza is involved. Whenever possible, at least some positive specimens should be confirmed by one of these more sensitive and specific methods.

Box 1

Commercially available RIDTs advantages and disadvantages

Advantages:

- **Many distinguish between influenza A and B**
- **Are unlikely to produce false positive results**

Disadvantages:

- **Cannot rule out infection with influenza**
- **Majority cannot provide subtype information**
- **Cannot provide information regarding antiviral drug susceptibility**

Travellers

International travellers with suspected influenza should be treated as per the national guidelines regarding clinical management of acute respiratory infections.

Surveillance

As highlighted above, confirmation of influenza in the community can aid interpretation of RIDT results and enhance the accuracy of clinical diagnoses. RIDTs have been used to identify foci of influenza activity and guide targeted sample collection for viral culture and characterization but should **not** be used alone for surveillance purposes. However, RIDT results might be useful in detecting some positives during the introduction and transmission of pandemic (H1N1) 2009 virus into a community/region and may be used to prioritize patient specimens for collection and transport to laboratories offering confirmatory testing, as well as with capacity to characterize the virus and monitor viral evolution through antigenic analysis.

In all cases the accuracy of an RIDT is dependent on the quality of the specimen and the care and expertise with which the RIDT is prepared and interpreted. There are several items to remember when using an RIDT (Box 2).

Box 2

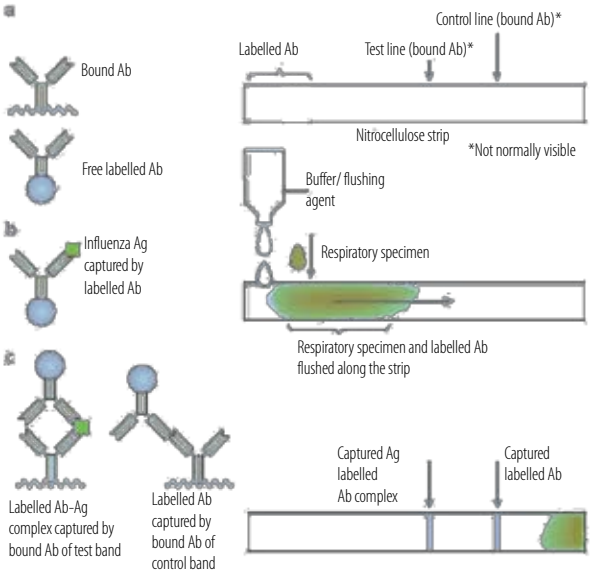
Items to remember when using an RIDT:

- **Training on the use and interpretation of results using a RIDT is essential.**
- **A management plan for results must be in place i.e. what will happen with a positive or negative or indeterminate result?**
- **Product instructions should be strictly followed, including specified reading times.**
- **Biosafety precautions should be followed.**
- **If the envelope is punctured or badly damaged, the RIDT should be discarded.**
- **The test envelope should only be opened when it has reached ambient temperature and the RIDT should be used immediately after opening.**
- **An RIDT can not be re-used.**

V. How do rapid influenza diagnostic tests work?

Influenza rapid diagnostic tests (RIDTs) otherwise known as point-of-care tests (POCT) or 'dipsticks', detect specific influenza viral antigens (proteins) in respiratory specimens of infected people. The RIDT signals the presence of viral antigens by a colour change or other optical signal (Figure 2). The most common antigen target in commercially available pan-influenza[6], influenza A, influenza B, or combination influenza A and B tests is nucleoprotein (NP). These tests do not differentiate between influenza A or B subtypes.

Figure 2: Mode of action of common RIDT format



Mode of action of common RIDT format: (a) Dye-labelled antibody (Ab), specific for target antigen, is present on the lower end of the nitrocellulose strip or in a well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line and either antibody specific for the labelled antibody, or antigen, is bound at the control line, (b) Respiratory specimen and buffer, which have been placed on the strip or in the well, are mixed with the labelled antibody and are drawn up the strip across the lines of bound antibody. (c) If antigen is present, some labelled antibody will be trapped on the test line. Other labelled antibody is trapped on the control line.

6 Tests which detect influenza but do not distinguish between A or B.

Formats

RIDTs commonly come in three different formats — dipsticks, cassettes or cards. Dipsticks are placed directly in wells or tubes containing the respiratory specimen and test kit extraction agent. Alternatively, the nitrocellulose strip can be placed inside a plastic housing (cassette) or bound to thick paper (card).

Specimen collection

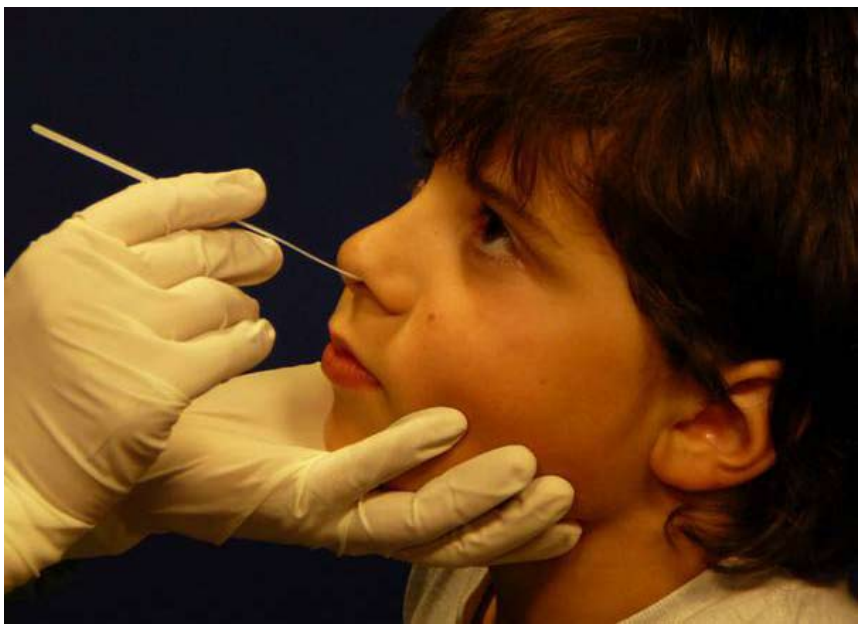
Tests accept one or more types of respiratory specimen types such as nasal aspirates, nasopharyngeal aspirates or swabs, nasal washes or swabs and throat swabs. For severely ill patients, endotracheal aspirates and bronchoalveolar lavage (BAL) may also be used with some test kits but not all kits have been evaluated for all sample types. In some cases the swabs may be included with the test kits and in others they must be purchased separately. Package inserts usually indicate compatible swabs but in general swabs made of calcium alginate are not acceptable. If specimens must be transported to the testing facility and if maintaining viability of the virus is important (for culture), then they must be placed in a sterile viral transport media (VTM) compatible with the test kit and transported at 4°C within 24 hours. Compatible VTM are usually listed by the manufacturer in the package instructions.

To optimize RIDT performance, specimen collection should be performed by a trained health worker and during the window of time in which the patient is likely to be replicating virus to sufficient titers that antigen can be detected. Guidance on specimen collection can be found in Instructions for storage and transport of suspected or confirmed human and animal specimens and virus isolates of pandemic (H1N1) 2009.[7]

7 http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/index.htm

The magnitude and duration of replication of pandemic (H1N1) 2009 virus are incompletely characterized and likely to vary with the severity of illness. The current approach is to base estimates upon seasonal influenza virus infection, until specific A H1N1 information becomes available.

Ideally, respiratory specimens should be collected as early in the illness as possible. Virus replication starts to wane by the third day of symptoms and in adults virus is often not detectable after 5 days. This period will be extended; however, for children, immunocompromised or immunosuppressed persons and it appears to be the case in patients with severe lower respiratory tract illness due to pandemic H1N1 infection.



Complexity

Commercially available RDTs for influenza vary in their requirements for specimen type, approved settings in which they can be used, number of processing steps, need for accurate timing and, interpretation of results, which will influence the extent of training and supervision required. To this end, an ease-of-use assessment is an important consideration along with test performance.

Speed

The time-to-results varies between tests but the majority of influenza A/B RDTs can be done in 5-15 minutes. In some cases, manufacturers specify the maximum reading time and/or provide a stopping solution that can be added to permit a reliable delayed reading.

Equipment and training needs

Standard kit contents do not necessarily include everything required to perform the test and this must be verified prior to purchasing RIDTs. At least one commercially available RIDT utilizes a reading instrument.

Training in performance of RIDTs is highly recommended for all RIDTs and should be tailored to tests given their varying complexity and acceptable specimen type.

VI. How effectively do rapid tests detect influenza?

Commercially available RIDTs are reactive with the nucleoprotein of pandemic (H1N1) 2009 virus (Hurt et al 2009; Chan et al 2009); however, data are very limited regarding their sensitivity[8] compared with real-time RT-PCR are very limited. Based on preliminary results using clinical specimens and compared with real-time RT-PCR, sensitivity ranges from 10-69% (Faix et al 2009; Ginocchio et al 2009; MMWR 2009; Vasoo et al 2009; Dexler et al 2009). For seasonal influenza, sensitivity ranges from 10-96% in selected reports compared with RT-PCR or viral culture (Uyeki 2003; Hurt et al 2007; Uyeki 2009; Rouleau 2009). In all cases, it is important to consider that test sensitivity can be affected by several factors (see Box 3). Overall, the data indicate that RIDTs may miss many infections with pandemic (H1N1) 2009 virus (false negative results may be common).

Limited data suggest that the specificity of RIDTs[9] for pandemic (H1N1) 2009 virus is high compared with viral culture or RT-PCR (93.3% compared with culture [Ginocchio et al 2009] 100% compared with RT-PCR [Vasoo et al]). For seasonal influenza, specificity ranges from 90-100% in a selection of published reports. Therefore, while false positive results can occur, they are uncommon when influenza viruses are circulating locally.

In order to correctly interpret the results of RIDTs and determine their appropriateness for use, the prevalence of circulating influenza virus strains in the community must also be considered, as this affects the positive[10] and negative predictive value (PPV and NPV) of the tests. If the prevalence is unknown, RIDT results become difficult to interpret and are of limited use in informing case management decisions.

Box 3

Factors influencing RIDT sensitivity:

- **Influenza type and subtype (variation in antigen structure and expression) .**
- **Number of virus present in the specimen. This is dependent on the timing of collection during the clinical course, patient age and immune status.**
- **Condition of the RIDT.**
- **Quality and source of the sample NP vs. nasal vs. throat swab.**
- **Correctness of the technique to perform the test and interpret the results (by the reader).**
- **Use of transport medium: possible dilution effect vs. growth/viral replication.**

8 The proportion of people with pandemic H1N1that have a positive test result

9 The proportion of people without influenza H1N1 that have a negative test result.

10 Positive predictive value (PPV) is the probability that a positive result accurately indicates the presence of infection. Negative predictive value (NPV) is the probability that a negative result accurately indicates the absence of infection.

Annex 1 gives an example of how these various parameters interact. In general, the following applies:

- During peak influenza activity: a positive result has the highest probability of indicating the presence of infection and the lowest probability that a negative result indicates the absence of infection.
- During low influenza activity: a positive result has the lowest probability of indicating the presence of infection (false-positives more likely) and the highest probability that a negative result is true/accurate.
- Influenza activity unknown: the probability of results indicating the correct presence or absence of infection is unknown. RIDT results cannot be interpreted with confidence.

VII. Deciding whether to use RIDTs and understanding what rapid test results mean

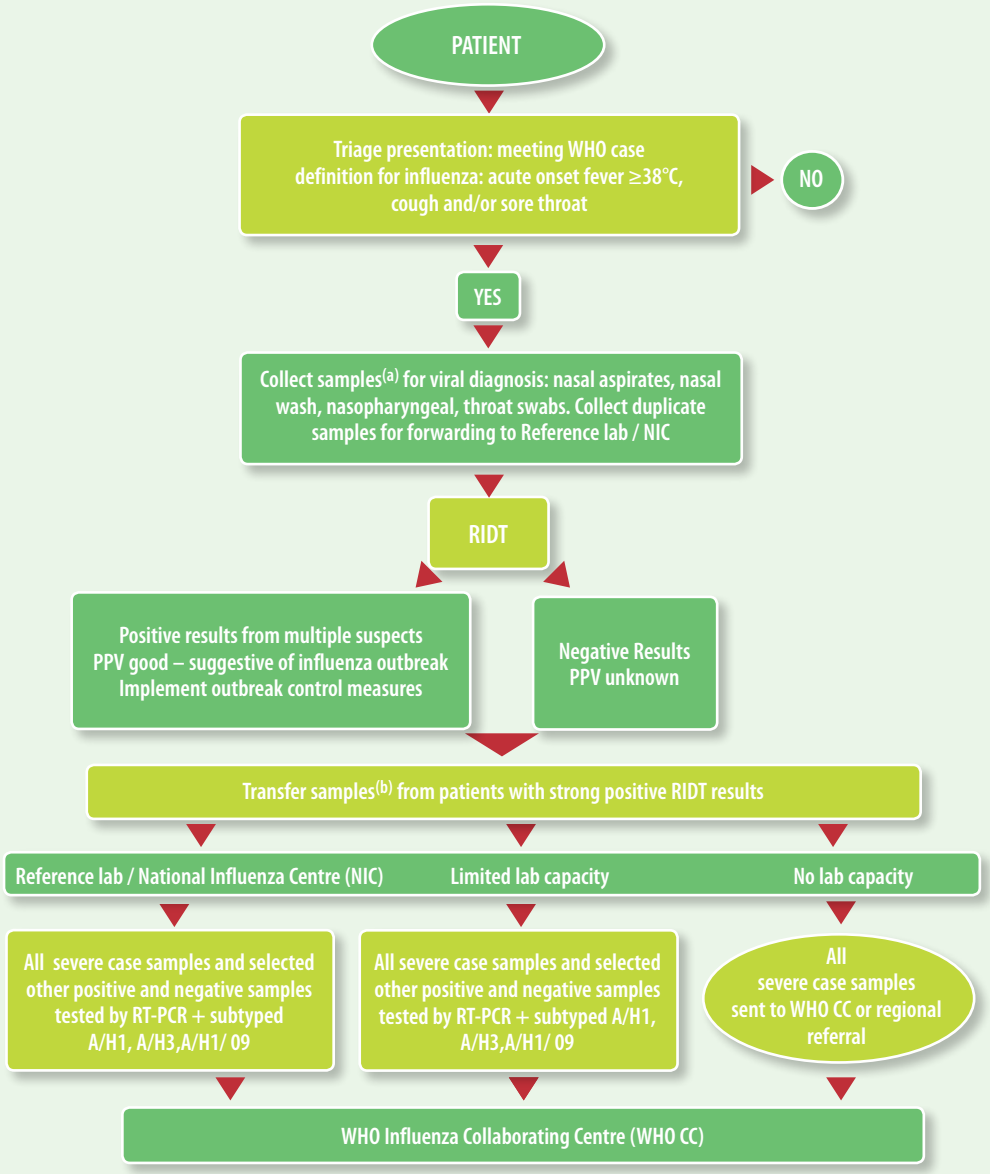
Despite evidence of limitations in test performance, rapid influenza diagnostic tests (RIDTs), if correctly interpreted, can play a role in guiding patient management, public health decision making and assisting influenza surveillance efforts. Table 1 summarizes the interpretation of RIDT results. In the following flow charts (1-3), RIDT testing and interpretation is presented as part of overall influenza diagnostic testing algorithms in settings with varying influenza activity and surveillance practices.

Table 1: Summary of interpretation of RIDT results

RIDT Result	Possible interpretation	Most likely if:
Positive flu A	Pandemic (H1N1) 2009 virus infection	Widespread community transmission of pandemic (H1N1) 2009 virus - peak of the outbreak
	Seasonal influenza A virus infection	Widespread community transmission of seasonal influenza A
	False positive result	When influenza is uncommon in the community (at the beginning and end of an outbreak)
Positive flu B	Seasonal influenza B virus infection	Widespread community transmission of seasonal influenza B
	False positive result	When influenza is uncommon in the community (at the beginning and end of an outbreak)
Negative results[(a)]	True negative	When influenza is uncommon in the community (at the beginning and end of an outbreak)
	False negative	When influenza is common in the community - at the peak of an outbreak
		Period of viral replication has passed and/or insufficient antigen to register a positive result
		RIDT has been damaged, reducing its sensitivity

(a) *All negative results require appropriate further investigation.*

Flowchart 1: Suspected outbreak of influenza in closed community / institution

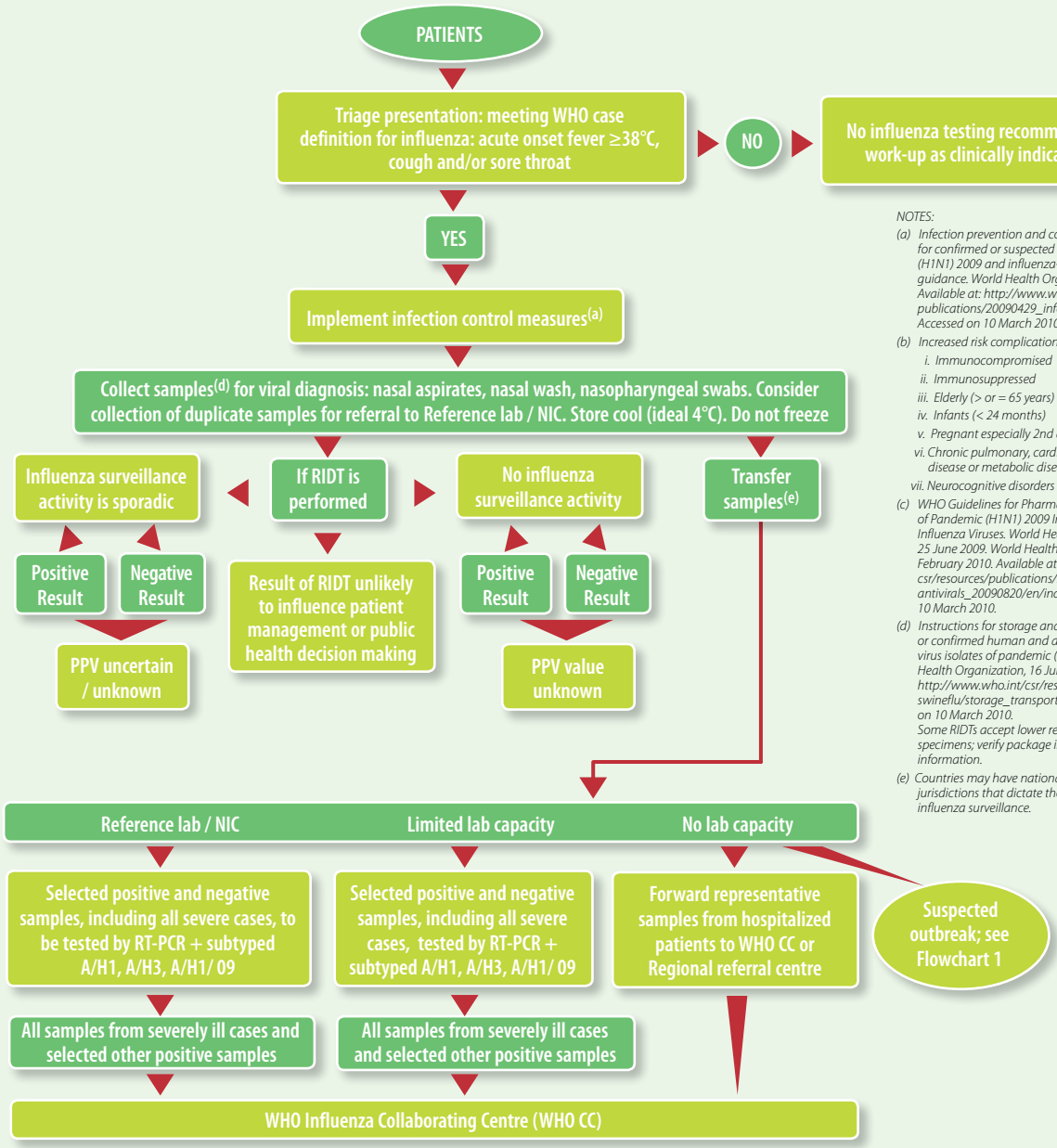


NOTES:

(a) Instructions for storage and transport of suspected or confirmed human and animal specimens and virus isolates of pandemic (H1N1) 2009 http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/index.html
Some RIDTs accept lower respiratory tract specimens; verify package insert/ product information.

(b) Countries may have national, regional or local jurisdictions that dictate the requirements for influenza surveillance.

Flowchart 2: Transition from sporadic cases to widespread community transmission of pandemic (H1N1) 2009 virus



NOTES:

(a) Infection prevention and control in health care for confirmed or suspected cases of pandemic (H1N1) 2009 and influenza-like illnesses Interim guidance. World Health Organization, 25 June 2009. Available at: http://www.who.int/csr/resources/publications/20090429_infection_control_en.pdf. Accessed on 10 March 2010.

(b) Increased risk complications

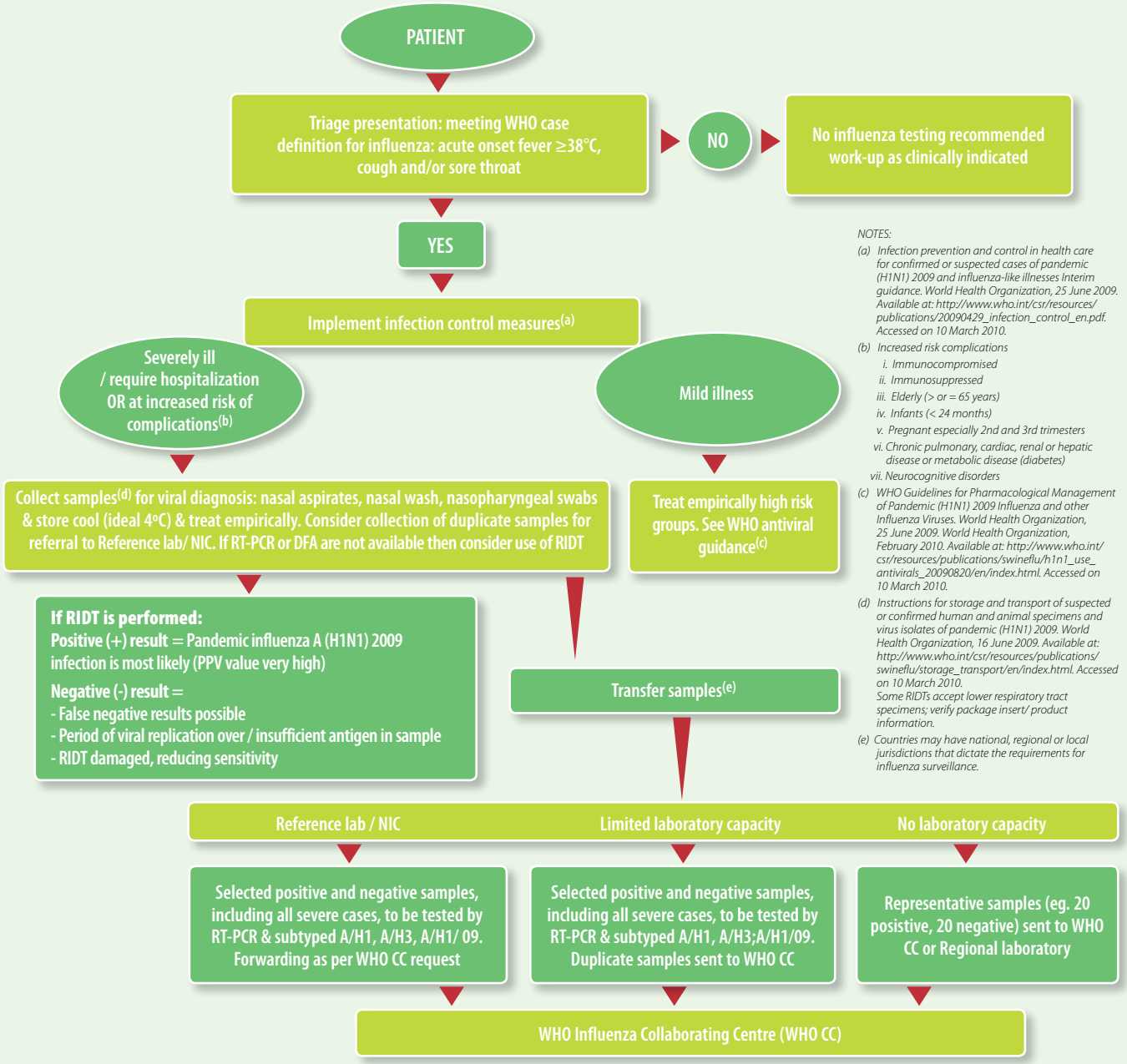
- *i. Immunocompromised*
- *ii. Immunosuppressed*
- *iii. Elderly (> or = 65 years)*
- *iv. Infants (< 24 months)*
- *v. Pregnant especially 2nd and 3rd trimesters*
- *vi. Chronic pulmonary, cardiac, renal or hepatic disease or metabolic disease (diabetes)*
- *vii. Neurocognitive disorders*

(c) WHO Guidelines for Pharmacological Management of Pandemic (H1N1) 2009 Influenza and other Influenza Viruses. World Health Organization, 25 June 2009. World Health Organization, February 2010. Available at: http://www.who.int/csr/resources/publications/swineflu/h1n1_use_antivirals_20090820/en/index.html. Accessed on 10 March 2010.

(d) Instructions for storage and transport of suspected or confirmed human and animal specimens and virus isolates of pandemic (H1N1) 2009. World Health Organization, 16 June 2009. Available at: http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/index.html. Accessed on 10 March 2010.
Some RIDTs accept lower respiratory tract specimens; verify package insert/ product information.

(e) Countries may have national, regional or local jurisdictions that dictate the requirements for influenza surveillance.

Flowchart 3: Widespread community activity of pandemic (H1N1) 2009 virus



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(a) Infection prevention and control in health care for confirmed or suspected cases of pandemic (H1N1) 2009 and influenza-like illnesses Interim guidance. World Health Organization, 25 June 2009. Available at: http://www.who.int/csr/resources/publications/20090429_infection_control_en.pdf. Accessed on 10 March 2010.

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(e) Countries may have national, regional or local jurisdictions that dictate the requirements for influenza surveillance.

VIII. What to consider before selecting an RIDT for purchase

Currently there are many commercially available RIDTs on the market from which to choose. Selection of RIDTs demands consideration of several test characteristics and requirements, as well as price. Each is considered in detail below.

Influenza virus targeted: Influenza RDTs detect only influenza A, detect A and B, or detect and distinguish between influenza A and B.

Test performance: Relative sensitivities of commercially available RIDT products are difficult to assess from the published literature and few comparative studies exist on RIDT clinical sensitivity and specificity for pandemic influenza (H1N1) 2009. In all cases, test performance is influenced by patient age, duration of illness before specimen collection, specimen type, transport and storage conditions, and, potentially, the type of influenza virus (although nucleoprotein antigens are highly conserved across influenza A viruses).

In general, test sensitivity will be inadequate to rule out influenza infection in those testing negative, but false positive results tend to be relatively uncommon when there is widespread disease and therefore, positive results can direct treatment.

Ease of use: The amount of training and supervision required is an important consideration and will be influenced by the test format (dipstick, cassette, card), number of processing steps, need for precise timing and/or measurements and/or additional equipment, ease of interpretation, time to results and biosafety risks.

Conditions of use: Most RIDTs can be stored between 15-30°C. If the temperature is above 30°C, then a 'cool chain' for transport and storage may need to be established and periodic quality control checks are required to ensure the validity of tests results.

Shelf life: Most influenza RDTs have a shelf life between 12-24 months from date of manufacture. A shelf life of 18 months is generally recommended if tests will be deployed to remote, resource-poor locations. A longer shelf life reduces pressure on the supply chain and avoids wastage of expired tests.

Costs: The cost of RIDTs will vary according to the test and volume to be purchased. 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 RIDTs must also be considered.

Tendering and the availability of product information

Together with considerations of the sensitivity and cost of a product, it is useful to know the quality of manufacturing processes, the long term viability of a company, and the consistency of production. This will influence the ability of a company to replace a product, should the received lot fail the quality control process, and will ensure long-term supply of a product to minimize the need for re-training. Purchasers should request information from manufacturers during the tendering process (see Box 4). Point 4 implies that the place of manufacture of rapid tests should be disclosed to the purchaser, if rapid tests are re-labeled. Clarity of packaging of the end product is essential to allow identification of product type, production lots, and expiry date.

Box 4

Information to request from RIDT manufacturers:

1. **Real-time temperature stability data on the product and accelerated data on the purchased lot;**
2. **Evidence of good manufacturing practice (e.g. GMP or ISO certification; ISO13485:2003 is a standard specific for medical devices);**
3. **Evidence of successful operational use or good quality field data on the product;**
4. **Long-term viability of manufacturer (to ensure continuity of supply);**
5. **Availability of product support;**
6. **Provision of sample products for assessment and testing for ease of use;**
7. **Agreement for replacement of products that fail agreed-upon quality control procedures; and**
8. **Box sizes appropriate to the rate of use of tests in the intended area, to minimize storage time in poor conditions and limit the need to split boxes.**

IX. Transporting and storing RIDTs

Exposure to high temperatures is often a major contributor to poor performance, particularly during transport and storage. Transfer from the manufacturer, together with road transport within a country, are particularly vulnerable times. High humidity can rapidly degrade rapid tests, including prolonged exposure to humidity after removal from the envelope or if the envelope is damaged. Most manufacturers recommend that rapid tests are stored between 4°C and 30°C. Expiry dates are generally set according to these conditions. If kits are stored at temperatures exceeding the recommended limits, it is likely that the shelf life of the rapid tests will be reduced and sensitivity lost prior to the expiry date. The maintenance of temperatures between 4°C and 30°C for shipment of rapid tests is essential. Transport of rapid tests from manufacturers and within countries should be monitored as follows:

Shipping from manufacturers

1. Before shipping, the manufacturer contacts consignees with details of air waybill numbers, airline carrier, flight number, numbers of containers, and expected arrival time. These details should be sent by e-mail and followed up by facsimile.
2. The shipper (air carrier) is notified of temperature storage requirements by the manufacturer in writing and by clear markings on cartons and related documents (stowage of the shipment close to the walls of some aircraft may result in freezing.)
3. The manufacturer initiates shipment only when the consignee confirms the shipping notification is received.
4. Consignees then arrange to have customs agents or other personnel on site to receive materials: shipments should be moved immediately to moderate temperature storage (<30°C, if possible). Avoid leaving materials on airport tarmacs, in customs sheds or in vehicles.

Ground transportation

Ground transportation during any stage of delivery should be carried out without delay and with attention to ambient temperature both while the vehicle is moving and while it is parked. Avoid leaving rapid tests in vehicles parked in the sun.

Storage

1. Storage at central and final field facilities should be within the manufacturer's specifications.
2. Maximize the time rapid tests are stored in centralized, controlled conditions; minimize uncontrolled storage in remote areas. Smaller box sizes may help achieve this.
3. Select a cool peripheral storage location; thatch roofing may be cooler than iron, maximize shade, and/or consider evaporative cooling cabinets. Transport and storage at temperatures >30ºC is sometimes unavoidable, as in many remote locations where rapid tests are intended for use. Monitoring of sensitivity of rapid tests at appropriate intervals is therefore essential. WHO is developing recommendation materials for quality assurance to address these issues.

X. Evaluating the quality of the testing programme

In between receipt of RIDTs from the manufacturer and field deployment, test sensitivity should be evaluated at a central laboratory. Additional testing should be performed periodically throughout the recommended shelf-life and a plan should be in place to recall the RIDTs, if necessary. WHO will soon make available to centralized laboratories panels of inactivated viruses including pandemic (H1N1) 2009 virus and seasonal influenza A (H1N1, H3N2) and negative controls, which can be used to screen RIDTs prior to release and for training end-users.

Health-care workers using the tests should be trained and monitored for proficiency in test preparation and interpretation. Ideally, this should be performed in real-time in the field using real patient samples, as the stability of RIDT results varies, making post-test assessment difficult.

Quality assurance (QA) should be an integral part of RIDT budgets and implementation plans.

Annex 1

Test sensitivity and specificity and the importance of prevalence in the estimation of predictive value of diagnostic tests

Sensitivity and specificity of a test are not the only parameters of importance in evaluating its appropriateness in a given population. The prevalence of the disease is important in assessing its positive and negative predictive value. The predictive value of the same test can differ between countries and between different populations in the same country as follows:

Given a population of 10 000 with the prevalence of influenza estimated at 1, 5, 10, and 15% and at a test sensitivity of 85, 90 and 95% and specificity of 95%, the positive predictive value (PPV) and negative predictive value (NPV) and the number of false negative and false positive tests are shown in the table below

Prevalence	Sensitivity	Specificity	PPV	NPV	#False negatives	#False positives
1%	85%	95%	15	100	15	495
1%	90%	95%	15	100	10	495
1%	95%	95%	16	100	5	495
5%	85%	95%	47	99	75	475
5%	90%	95%	49	99	50	475
5%	95%	95%	50	100	25	475
10%	85%	95%	65	98	150	450
10%	90%	95%	67	99	100	450
10%	95%	95%	68	99	50	450
15%	85%	95%	75	98	225	425
15%	90%	95%	76	98	150	425
15%	95%	95%	77	99	75	425

The results presented in the preceding table are not specific to rapid tests but can be applied to all diagnostic tests.

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UNICEF • UNDP • World Bank • WHO

TDR/World Health Organization
20, Avenue Appia
1211 Geneva 27
Switzerland

Fax: (+41) 22 791-4854
tdr@who.int
www.who.int/tdr

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