National outbreak response staff and other Department of Health authorities frequently ask what type of dengue tests should be used when confronted by a dengue-like febrile outbreak or a potentially high-risk dengue outbreak situation. This is especially true in many areas of the Western Pacific Region where prompt actions are necessary to minimize the impact of outbreaks. While rapid tests can be a useful tool in such situations, their advantages and disadvantages should be well understood.

The WHO Special Programme for Research and Training in Tropical Diseases (TDR) has taken the lead in an evaluation process of commercially available dengue rapid tests. Information and recommendations on the usefulness of dengue rapid tests will be incorporated in a guidebook. However, given the current need to support Member States, an update on the principles and use of rapid tests in dengue is needed to help health practitioners and programme managers decide on when to use these tests and how many individuals should be tested to confirm if an outbreak can be attributed to a dengue virus or not. The Regional Office for the Western Pacific has reviewed currently available evaluation results and knowledge on dengue diagnostic issues to develop this document. It is by no means exhaustive and will be replaced when a full version of recommendations becomes available.

### 1. What is dengue?

Dengue is a viral infection transmitted by a mosquito (vector), the most important of which is *Aedes aegypti*. The disease is characterized by a wide spectrum of clinical manifestations ranging from asymptomatic infection to undifferentiated fever, and life-threatening forms of dengue characterized by severe plasma leakage, bleeding and organ involvement. The incubation period is 4 - 6 days. The clinical presentation of dengue often includes fever, headache, retro-orbital pain, myalgia, arthralgia and rash. In more severe forms, leakage of blood from capillaries can occur and manifest as shock characterized by circulatory failure (weak and rapid pulse, hypotension or narrowing of the pulse pressure, cold and clammy skin and restlessness), petechial rash and internal bleeding.\(^{(1,2)}\)

### 2. What is the dengue virus?

The dengue virus belongs to the family of flavviruses. There are four distinct serotypes (DENV 1-4) that co-circulate in many of the dengue endemic countries of the Asia-Pacific Region. A person can be infected by any one of all four serotypes. A second infection with a different dengue serotype is thought to be associated with increased risk of developing severe manifestations of dengue.

### 3. When do dengue antigens and antibodies appear in blood?

Following the bite of an *Aedes* mosquito, dengue virus will replicate quickly before the development of any signs or symptoms. Viraemia peaks with the onset of fever. The presence of circulating non-structural glycoprotein (NS1) indicates viraemia. If sufficient virus is present, NS1 can be detectable in a patient's blood from day 0 to day 5 following disease onset.\(^{(3)}\) The detection of NS1 antigen is therefore useful as a test of early acute infection.
The body produces antibodies in response to dengue infection. Anti-dengue immunoglobulin M (IgM) and anti-dengue immunoglobulin G (IgG) are the two antibodies that can be targeted serologically by antibody detection assays. Timing is critical when this is done as the curve shows in Figures 1, 2.

In a first infection (primary infection) with the dengue virus, IgM antibody (often not specific to any one of the four serotypes), becomes detectable about five days after disease onset, when circulating virus declines in the blood. IgM level rises quickly to peak at about 2 weeks and declines to undetectable level after 2 - 3 months.\(^{(1,4)}\) IgG antibody appears a few days after IgM. In primary infections, IgG antibodies are produced at a lower level compared to IgM but will persist for many years after infection (Figure 1).

![Figure 1: Antigen and antibody responses in primary dengue infection](image)

In subsequent (secondary) infections, IgM response is typically at a lower level compared to that in a primary infection. IgG response may rise quickly before or simultaneously with an IgM response and will become the predominant immunoglobulin isotype in secondary infections (Figure 2).\(^{(4)}\)

![Figure 2: Antigen and antibody responses in secondary dengue infection](image)

4. Some facts about dengue diagnosis

Dengue diagnosis usually relies on clinical assessment, ideally confirmed by laboratory tests such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and viral culture. Rapid tests detecting anti-dengue antibodies (antibody detection assays) or dengue viral protein (antigen detection assays) can be a useful for surveillance and support diagnosis of dengue infection in conjunction with clinical symptoms, medical history and other epidemiologic information. Clinical diagnosis remains however the mainstay of dengue case identification and management.

5. What is a dengue rapid test?

There are currently two types of rapid tests used in dengue: antigen-detection rapid tests and antibody-detection rapid tests. Dengue antibody-detection rapid tests usually employ an immuno-chromatographic test (ICT) format to detect IgM or IgG or IgM/IgG antibodies. Dengue antigen-detection rapid tests also employ an ICT format and are based on the detection of NS1. Some tests incorporate both antigen and antibody in the same test kit. This combined antigen/antibody test aims to detect dengue infection at both the early stage (when virus is circulating) and the later stage (when antibodies appear).
Many dengue rapid tests are commercially available. Rapid tests are easy to use, providing results in less than an hour. They do not require high level of laboratory infrastructure and thus have wide application and importance in identifying dengue outbreaks. However, care is needed in interpreting both positive and negative results. When performing rapid tests, manufacturers’ instructions enclosed in the test kits must be followed.

Good quality dengue rapid tests are:

1. relatively easy to use, providing results in 30 minutes or less; and
2. useful in outbreak identifications.

However, dengue rapid tests are not recommended to be used as absolute confirmatory assays because:

1. antibody-detection rapid tests cannot confirm the current infection since dengue antibodies may have persisted from an infection in the past; and
2. performance of antigen-detection rapid tests or combined antigen/antibody rapid tests is yet to be fully evaluated.

6. How to read a rapid test result

Figure 3 is an example on how to read the result from an IgM/IgG rapid test. Based on occurrence of test bands in the control (C) and test (M, G) parts, it can be read as any one of the five results: (1) IgM positive; (2) IgG positive; (3) IgM and IgG positive; (4) negative; and (5) invalid. Different products may have different orders of test bands.

7. How effective is a dengue rapid test?

In general, NS1 antigen-detection assays have higher sensitivity during the first five days after onset of fever, decreasing considerably thereafter. Antibody-detection tests, on the other hand, have a higher sensitivity after day five of disease following onset.

Several evaluation studies on performance of commercially available antibody rapid tests have been conducted. Results of these studies show that antibody rapid tests are generally neither highly sensitive nor highly specific to dengue infection.

Sensitivity and specificity were found to vary widely among different rapid tests. In a recent TDR study evaluating four IgM rapid tests, sensitivity ranged from 21% to 99%, specificity from 77% to 98%. Previously, another comparative study on eight commercially available IgM rapid tests showed that sensitivity of these tests ranged from 6% to 65%, specificity from 69% to 100%. Therefore, selection of rapid tests should be based on evidence from evaluation studies, considering optimum values of sensitivity and specificity. Frequently, a test with higher sensitivity may tend to have lower specificity and vice versa. High specificity is important if tests are being used to confirm that dengue is the cause of outbreaks. High sensitivity is very important if
tests are to be used to support clinical diagnosis and for case management. However, no current rapid antibody detection tests appear to reliably achieve this level of sensitivity.

Little information on the performance of NS1 antigen rapid tests or combined antigen/antibody rapid tests is available at present. An evaluation of the performance of an ELISA-based NS1 detection assay showed that sensitivity ranged from 23% to 90%, specificity from 89% to 100%. Sensitivity of a NS1 rapid test is expected to be lower than the above figures because a rapid test usually has lower sensitivity than an ELISA test designed for the same targets. A combined antigen/antibody rapid test, e.g. NS1/IgM test, should expand the time span during which the rapid test could detect a dengue infection. However, the test will be subject to the limitations discussed above, and will require careful interpretation. More information on the performance of antigen and combined antigen/antibody rapid tests will be available as soon as TDR completes its second round of evaluations of rapid dengue tests.

As shown in the evaluation studies, false positives, that is, those instances in which a non-dengue infected person is diagnosed as positive, ranges from 0% to 31%. Rapid tests can yield false positive results due to cross-reactions with other flaviviruses (including Japanese encephalitis, yellow fever, West Nile virus), other pathogens causing fever (e.g. malaria, leptospirosis) and immune disorders such as rheumatoid arthritis and lupus. A positive result produced by a rapid test thus should be confirmed by another more reliable testing technique such as MAC-ELISA in a quality assured laboratory.

False negatives, that is, those cases in which a dengue infected person is falsely diagnosed as negative, ranges from 1% to 94% in rapid tests. As a significant number of dengue infections can be missed by a rapid test, absence of a positive result does not mean the absence of a dengue case.

### Problems to be aware of upon monitoring fever outbreaks using dengue rapid tests*

<table>
<thead>
<tr>
<th>Deficiency in rapid test function</th>
<th>Potential problem in field</th>
<th>Interpretation of a low positivity rate in the field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor specificity, e.g. below 85%</td>
<td>A low rate of positive results might be recorded, even in a population where dengue does not exist.</td>
<td>Results inconclusive. Is this a dengue outbreak, or just the result of background false positive rate of the test?</td>
</tr>
<tr>
<td>Poor sensitivity</td>
<td>The test might indicate a much lower rate of dengue cases than actually exists.</td>
<td>Results inconclusive. Is the outbreak of fever predominantly due to dengue, or is it due to another disease?</td>
</tr>
</tbody>
</table>

*In general, a significant proportion of rapid test results should be confirmed in a reference laboratory*

Interpretation of rapid test results should take into account the patient’s clinical presentation, his/her travel history relative to dengue outbreak locations and the current epidemic situation of the place where his/her exposure to dengue virus may have occurred.

Because IgM antibodies are detectable in blood about five days after disease onset, IgM antibody-detection rapid tests are often not useful for case management. Combined antigen/antibody rapid tests are theoretically able to detect dengue infection across a wider period of time. The performance of these tests, however, has not yet been properly validated. Nevertheless, rapidity and ease of use are advantages of rapid tests, making them widely utilized to screen for potential dengue outbreaks especially in locations where good laboratory infrastructure is not available to conduct other more reliable dengue tests.

Conditions such as climatic events can favour breeding of *Aedes* mosquitoes and thus increase the risk of dengue outbreaks. A dengue outbreak should be suspected if an unusually high number of people with dengue-like febrile illnesses occurs in a relatively short period of time (e.g. few weeks following a flood) in a defined...
community. In such scenarios, a rapid test can be used to screen for a dengue outbreak.

For the purpose of identifying a dengue outbreak, it is not recommended to screen all people with febrile illness with dengue rapid tests. The number of people to be checked by dengue rapid tests would vary depending on the size of the outbreak. The sample size should be large enough to be reliable, but also small enough to be cost effective. There is yet no consensus among leading experts regarding the actual sample sizes.

If the positive rate among tested samples is significantly high, e.g. more than 50%, dengue virus is highly suggestive as the cause of the febrile outbreak. Dengue outbreak responses should immediately aim for the containment of infection. To avoid falsely alarming the public of a dengue outbreak, and to minimize the social and economic consequences due to this false conclusion, positive specimens identified by rapid tests should be verified in a laboratory.

A positive rate among tested samples, e.g. 20% - 50%, is suggestive of a dengue outbreak, but a search for other pathogens should be conducted while carefully considering the clinical dengue case definition criteria.

As it is important to detect the cause of a febrile outbreak early in an epidemic, it is advisable to maintain a suitable stock of dengue rapid tests in dengue-prone countries or areas where rapid access to laboratory test is not otherwise available. Countries are not recommended, however, to test all patients suspected of having dengue infection by rapid test.

### 10. What to consider in purchasing dengue rapid tests

Following are WHO/TDR's recommendations for selecting a dengue rapid test. The importance of these factors may vary according to whether the test is to be used to support surveillance, dengue case detection or case management.

1. **Type of dengue rapid test.** Antigen-detection rapid tests are good to use for screening people in the early stage of illness. Antibody-detection rapid tests are good to use for screening people in the later stage of illness or convalescence.

2. **Performance.** A highly sensitive test is required to support case detection. The specificity of the test is of lesser importance as there are few adverse effects from over treatment of dengue fever (since no specific anti-dengue drugs are available). Cross-reactivity to another disease may be acceptable if the prevalence of the disease in the setting in which the test will be used is low.

3. **Ease of use** is important when a test is used in field settings with no, or variable, access to electricity. Personnel training and the workload of the clinic should also be considered.

4. **Stability and shelf-life.** Stability testing can be crucial in settings with extreme temperatures. High temperatures during storage can directly influence test performance. Even if good storage conditions are available in the laboratory, tests may be exposed to high temperatures during transportation if no proper cold chain is in place. This too affects the test's performance.

5. **Price** often has a strong impact on test selection.

### 11. How to evaluate the quality of your testing program

1. **Quality assurance on validity of test kits.** Temperatures during transport may affect sensitivity of the tests. It is recommended that performance of the rapid tests be checked at a central laboratory.

2. **Proficiency of users.** Adequate training and supervision of end-users of rapid tests should be integrated as far as possible into existing health worker training and quality assurance schemes. Concise, clear standard operating procedures (SOPs) should be prepared in local languages for health workers trained to perform the test.
### Training

Health workers using the test should be trained and assessed and systematically monitored on test processing and interpretation. They must also be trained in clinical assessment.

### References

8. Janisch T, Gaczkowski R, Guzman G, Simmons C. Validation of NS1 ELISA, a presentation in the context of the EU-TDR-Wellcome supported DENO study, 2008.

### Acknowledgement

We wish to thank David Bell, John Ehrenberg, Axel Kroege, Rosanna Peeling, Carl-Michael Nathanson and Duane Gubler for their input and constructive comments in the development of this update.