Laboratory testing for dengue virus

Interim guidance
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World Health Organization

Key points

- This guidance synthesizes current evidence on dengue virus (DENV) laboratory testing and diagnostics and provides practical recommendations for laboratories, public health officials, clinicians, and stakeholders implementing dengue diagnostic testing, in the context of the global emergency.
- DENV infection presents with a broad range of clinical manifestations, from mild to severe illness. Patients with suspected dengue should receive appropriate clinical management without waiting for diagnostic test results. Priority testing should be given to those at increased risk of severe disease, such as infants, young children, pregnant women, individuals with pre-existing medical conditions, and travelers from endemic areas.
- DENV can be detected in plasma, whole blood, capillary blood, urine, cerebrospinal fluid (CSF), and tissue samples, with serum being the preferred sample type.
- Proper handling and storage of samples during transportation are critical for accurate results, and laboratories must comply with national and international regulations. Dengue samples fall under Category B – UN3373 "Biological substance", while DENV cultures are classified as Category A – UN2814 "Infectious substance affecting humans".
- Dengue diagnosis requires selecting appropriate laboratory methods based on the testing indication with their accuracy influenced by sample type, stage of infection, prevalence of dengue in the region, co-infections, prior DENV or orthoflavivirus infections, and vaccination history.
    - In the acute phase of infection (0–7 days post-symptom onset), direct virus detection of DENV RNA is possible using nucleic acid amplification tests (NAATs) that are highly sensitive and specific, with many RT-PCR assays capable of detecting and differentiating all four serotypes.
    - During the acute phase, NS1 antigen can be detected by NS1 ELISA or rapid diagnostic tests (RDTs) in serum up to nine days after symptom onset in primary infections and up to 6 days in secondary infections.
    - In the later phase (>7 days post-symptom onset), serological tests (ELISA and RDT) detecting IgM and IgG antibodies, play a key role in confirming acute or recent infections, with paired serum samples (acute and convalescent, collected 10–14 days apart) improving diagnostic accuracy.
    - Further characterization of DENV for surveillance and outbreak investigation can be performed using plaque reduction neutralization tests (PRNT) to quantify serotype-specific neutralizing antibodies, multiplex real-time PCR for serotype differentiation, and next-generation sequencing for genomic characterization.
- Primary and secondary dengue infections exhibit distinct diagnostic patterns. In primary infections, viral RNA and NS1 antigen are detectable in the acute phase, while IgM peaks around two weeks and subsequently declines, and IgG appears later but persists longer. In secondary infections, viral RNA remains detectable, but NS1 antigen detection is reduced due to the formation of antigen-antibody complexes with pre-existing antibodies. IgM is reduced or absent, whereas IgG rises earlier and dominates the immune response. High IgG

levels combined with viral RNA, NS1 antigen, or IgM suggest secondary infection. IgM/IgG ratios may aid in the differentiation of primary and secondary infections.

- There is no universal consensus on the optimal diagnostic algorithm, and algorithms should be adapted to suit the context, considering the endemicity of DENV and other orthoflaviviruses co-circulating in the country, the presence/absence of programmatic orthoflavivirus vaccination programmes, and the testing capacity of the national laboratory system. This guidance outlines a proposed diagnostic algorithm for suspected dengue cases, specifying the appropriate diagnostic methods based on days post symptom onset:
    - In the acute phase (0–7 days), NAAT targeting viral RNA, NS1 antigen detection by ELISA or RDT, or a combination test (NS1 + IgM) are recommended for early confirmation of dengue infection.
    - Testing in the convalescent phase (>7 days) involves IgM serology, followed by paired sera testing at a 10–14 day interval or the use of combination tests (NS1 + IgM) to enhance detection of primary and secondary infections. However, IgM serology may cross-react with other orthoflaviviruses, and a negative result does not exclude dengue. Further testing, such as seroconversion with convalescent serum or differential diagnosis, may be required for confirmation.
- DENV testing of suspected or confirmed cases should be handled using a risk-based approach in line with the WHO Laboratory biosafety manual, fourth edition *(1)*.
- Dengue testing in endemic countries should be integrated across all levels of the national laboratory system, according to infrastructure and biosafety requirements.
- Genomic sequencing is an important tool for analyzing and characterizing the DENV genome, with 19 identified genotypes across the four serotypes.
- Dengue diagnostic tests are not prequalified by WHO. Procurement of DENV tests should be guided by evidence of good quality and satisfactory performance from independent evaluations.

Purpose of this document and updates from previous guidance

This interim guidance synthesizes current evidence on DENV laboratory testing and diagnostics. It is intended to provide practical and useful guidance for laboratories, countries and stakeholders implementing dengue diagnostics in the context of the global emergency.

Relationship to existing WHO publications:

This document updates and supersedes Chapter 4 "Laboratory diagnosis and diagnostic tests" of the WHO *Dengue guidelines for diagnosis, treatment, prevention and control (2)* reflecting critical advances including the following:

- presents updated case definitions based on recent WHO publications;
- illustrates the progression of diagnostic markers for primary and secondary DENV infections in relation to days from symptom onset following the bite of an infected mosquito;
- includes a proposed diagnostic algorithm for a suspected dengue case which details the available diagnostic methods and the appropriate timing for their use based on days post symptom onset;
- describes the comprehensive range of diagnostic techniques and methodologies available for dengue including combination tests and rapid diagnostic tests;
- includes a table presenting the range of analytes and corresponding test methodologies for dengue diagnosis, testing indications, and the advantages and limitations of each dengue diagnostic test type;
- includes a section on genomic surveillance which describes the utility of next generation sequencing in outbreak investigation and DENV genomic surveillance;
- describes DENV testing across the tiers of the laboratory network proposing diagnostic options for each level of the laboratory network including at the point of care;
- includes a table on the considerations for selecting dengue diagnostics which presents essential considerations for selecting dengue diagnostics including test characteristics and practical requirements.

Other sections of the 2009 guidelines (treatment, prevention) remain valid and should be read in conjunction with this update.

The interim guidance also updates and complements the following WHO publications:

- *Laboratory testing for Zika virus and dengue virus infections (3)*, by expanding guidance on detecting DENV;
- *Recommendations for laboratory detection and diagnosis of arbovirus infections in the Region of the Americas (3)*, by focusing on dengue and refining the DENV-specific recommendations;
- *Technical note: algorithm for laboratory confirmation of dengue cases (5)*, by incorporating RDTs into the diagnostic algorithm and including combination test (NS1 and IgM);
- *Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever (6)*, by introducing a new diagnostic algorithm and modern technologies.

Target audience

This document provides guidance for laboratory professionals, public health officials, clinicians and other stakeholders involved in dengue testing, surveillance and disease control programme management.

Introduction

Dengue is a growing global public health concern, as global transmission continues to strain health care systems and impede economic stability *(7)*. Over the past two decades, the global incidence of dengue has increased significantly, with reported cases approximately doubling each year since 2021, and expanding into previously unaffected regions. In December 2023, the World Health Organization (WHO) declared a global emergency and launched a global surveillance system, the global dengue surveillance dashboard *(8, 9)*. Throughout 2024, a total of 14,284,310 cases and 10,554 deaths were reported to WHO from 107 countries, territories, and areas, with data consolidated from 182 countries and territories, including 75 that reported zero cases *(9)*. All six WHO regions were affected in 2024, with the Americas experiencing the highest burden, reporting 13,017,112 cases and 8,152 deaths across 48 countries. However, variations in surveillance capacities and methodologies across countries and regions limit direct comparisons.

A combination of environmental, social and climatic factors has contributed to the heightened transmission of dengue virus (DENV) and the global emergency. Rapid and unplanned urbanization, population mobility, densely populated cities with inadequate public health infrastructure, and the resurgence of global travel following the coronavirus disease (COVID-19) pandemic have further exacerbated the situation. Climate change, along with the El Niño phenomenon, has resulted in altered precipitation patterns, increased humidity and drought, and elevated temperatures, all of which favour the breeding and propagation of mosquitoes *(10)*. The expanding range of the mosquito vector poses an increased risk of DENV transmission in new geographical areas *(7)*. Furthermore, complex humanitarian crises amid political and financial instability have weakened health systems, destroyed health care facilities and delayed access to medical care, amplifying the impact of dengue *(11)*. In addition, the co-circulation of multiple DENV serotypes and inadequate vector control have raised the likelihood of severe dengue cases and increased mortality rates associated with secondary infections.

The co-circulation of DENV and other arboviruses further complicates the situation in many countries. DENV, chikungunya virus and Zika virus, all transmitted by *Aedes* mosquitoes, have overlapping geographical distributions and clinical manifestations, which makes it difficult to detect and control outbreaks *(11)*. Furthermore, antibodies induced after exposure to one orthoflavivirus may cross-react with antigens of other members of the genus, affecting the diagnostic interpretation of serological tests *(13)*. In addition, various non-orthoflaviviruses, such as Oropouche virus (a member of the *Orthobunyavirus* genus within the *Peribunyaviridae* family), exhibit dengue-like symptoms. Oropouche virus was responsible for sporadic outbreaks across the Americas in 2024 *(14–16)*. Given the similarities in clinical manifestation, it is essential to establish testing strategies and diagnostic capacity to differentiate DENV from other co-circulating viruses.

Laboratory-based and point of care diagnostics are critical to control and manage dengue, yet global disparities in laboratory capabilities present significant challenges. The diagnostic algorithms, testing strategies and test methodologies employed vary, depending on the capabilities of national laboratory systems. Furthermore, dengue diagnostic tests are not prequalified by WHO, and the wide range of available tests – including nucleic acid amplification tests (NAATs), enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDTs) – vary significantly in quality and performance. This variability makes it challenging to select and implement accurate diagnostic assays, particularly in resource-limited settings, which further complicates the detection and diagnosis of dengue.

Dengue virus and disease

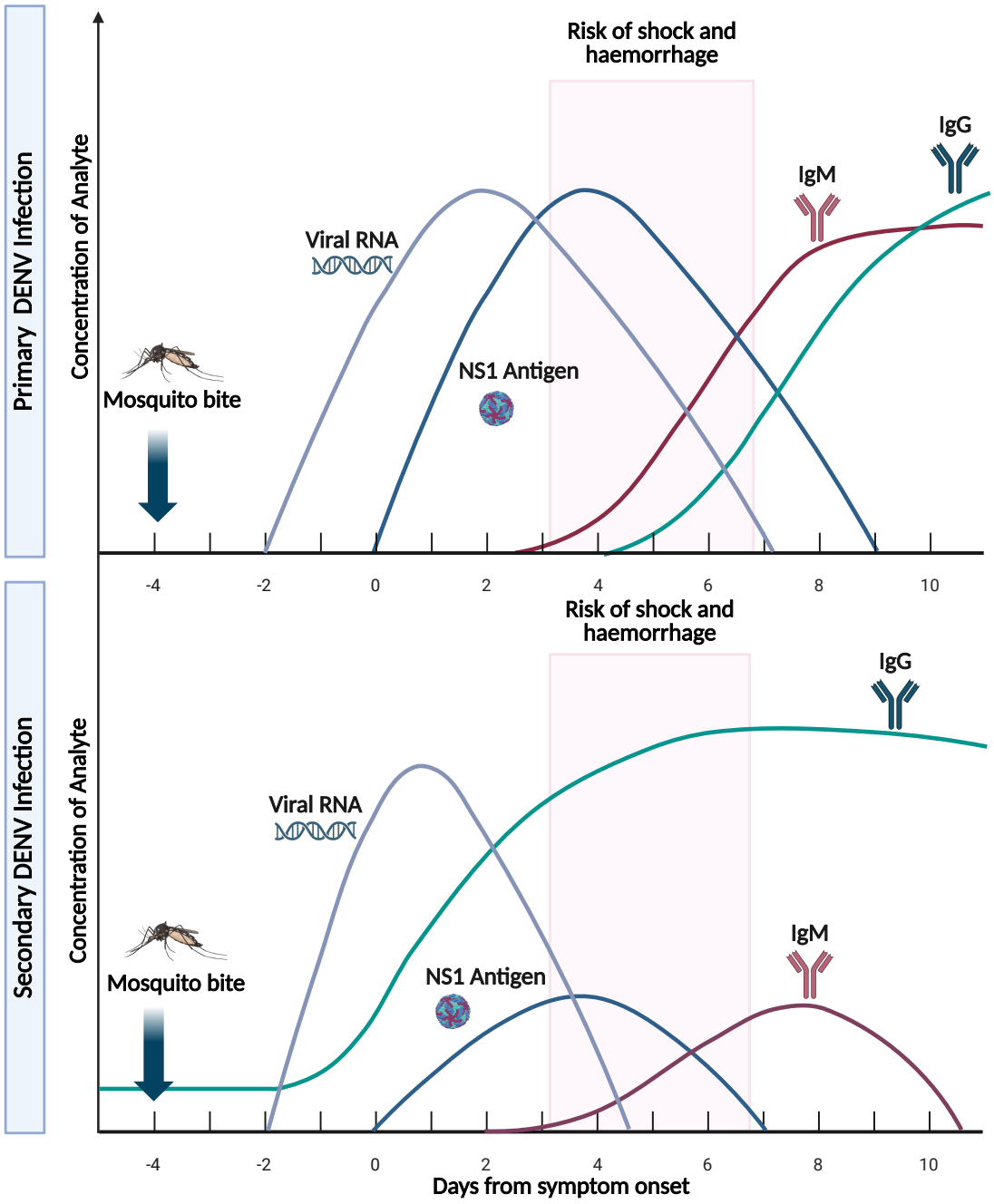
DENV, a member of the *Orthoflavivirus* genus in the *Flaviviridae* family, is the causative agent of dengue. Its genome is a single-stranded, positive-sense RNA that encodes three structural (capsid [C], membrane [M] and envelope [E]) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins *(17, 18)*. The primary mode of DENV transmission is through the bites of infected female *Aedes aegypti* and *Aedes albopictus* mosquitoes, which thrive in tropical and subtropical environments *(19)*. DENV is classified into four distinct serotypes (DENV-1, -2, -3 and -4), which share approximately 65% of their genome and each serotype incorporates several genotypes *(20)*. Infection with one DENV serotype is thought to provide lifelong immunity against that serotype, but only short-term cross-protection against the other serotypes (approximately 8–12 months) *(21, 22)*.

Dengue is a self-limiting febrile illness with symptoms ranging from asymptomatic to severe multi-organ dysfunction *(2)*. It is estimated that 40% to 80% of infections caused by DENV are asymptomatic, and fewer than 5% of all dengue patients develop severe, life-threatening conditions *(23, 24)*. Severe dengue can manifest in both primary and secondary infections. However, the likelihood of severe disease may be elevated in secondary infections due to antibody-dependent enhancement *(25, 26)*. The accurate prediction of severe disease development in patients remains elusive. However, certain risk factors for progression to severe disease have been identified, including age (primarily children), pre-existing chronic diseases and ethnic background *(27)*. Epidemiological and phylogenetic studies indicate that particular DENV genotypes and lineages are more virulent and associated with outbreaks *(28–30)*.

Due to the lack of specificity of clinical diagnosis, laboratory confirmation is necessary to definitively diagnose DENV infection. Currently, there are several conventional methods used to directly detect the virus and its components or indirectly detect virus-specific antibodies by serology. The accuracy of the various diagnostic tools may be influenced by the type of sample, timing of testing during the course of the patient's illness, presence of coinfections, history of previous DENV or other orthoflavivirus infections, and orthoflavivirus vaccination status *(31)*. From zero to seven days after the onset of symptoms, the virus or viral components can be detected in the blood, urine and infected tissues *(2, 31)*. Accordingly, direct detection (such as virus isolation or NAAT) or antigen-based assays (such as ELISA or RDT) can be used to diagnose the infection in the early phase. As the acute phase of infection subsides beyond the seventh day after symptom onset, the immune response becomes more prominent and serological tests are increasingly reliable *(2, 5)*. During this phase, the patient produces immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against DENV.

Fig. 1 illustrates the progression of diagnostic analytes for primary and secondary DENV infections in relation to days from symptom onset following the bite of an infected mosquito. In primary infections (top panel), viral RNA and NS1 antigens are detectable early, with RNA persisting for up to seven days and NS1 up to nine days, or longer in some cases. IgM antibodies emerge around days 3–5, while IgG appears later. In secondary infections (bottom panel), IgG levels rise rapidly, NS1 and viral RNA are cleared more quickly, and IgM is either absent or present at lower levels. The figure also highlights the period when the risk of shock and haemorrhage is highest, typically as fever subsides. Proper interpretation of diagnostic results requires consideration of symptom onset and sample timing, as early serological tests may yield false-negative results. Potential cross-reactivity with other orthoflavivirus antibodies further underscores the need for differential testing *(32).*

Fig. 1. Progression of diagnostic markers in primary and secondary DENV infections in relation to days from symptom onset



Note: Although the shaded areas indicating the *risk of shock and haemorrhage* appear similar for visual consistency, the actual risk is substantially higher during secondary dengue virus (DENV) infection than in primary infection.

Case definitions and clinical presentation

Due to limited access to testing services and the lack of specific therapeutics to treat dengue, many countries rely on clinical diagnosis using agreed upon case definitions. Box 1 presents the WHO case definitions for suspected, probable and confirmed cases of dengue *(33)*, and Box 2 outlines the clinical manifestations of dengue *(33)*. DENV infection can manifest across a wide spectrum of clinical presentations, ranging from mild to severe disease. The following groups should be prioritized for testing, as some may be at increased risk of progressing to severe disease: young children and infants, pregnant women, people with underlying medical conditions, and travelers returning from orthoflavivirus-endemic regions.

Box 1. WHO case definitions for a suspected or clinically compatible case of dengue (adapted from *(33)*) *5*

Case	Definition
Suspected case	A person who lives in or has travelled in the previous 14 days to areas with dengue transmission, and presents with acute fever that has typically lasted two to seven days along with two or more of the following clinical manifestations: nausea or vomiting, exanthema, headache or retro-orbital pain, myalgia or arthralgia, petechiae or positive tourniquet test (+), or leukopenia, with or without any warning sign or sign of severity A suspected case is also considered to be any child who resides in or has travelled in the previous 14 days to an area with dengue transmission and presents with acute febrile symptoms, usually for two to seven days, without an apparent focus
Probable case	Suspected case of dengue that also has: • IgM detected in a single serum sample (collected during the acute or convalescent phase); or • epidemiological link to a confirmed case.
Confirmed case	Suspected case of dengue that also has any of the following: • DENV detected by NAAT; • DENV isolated by culture; • detection of NS1 antigen by ELISA or RDT; • seroconversion or four-fold increase in antibody titre, excluding other orthoflaviviruses; or • in deceased patients, detection of the viral genome or NS1 antigen from autopsy tissue by molecular, histopathological and/or immunohistochemical study.

Box 2. Dengue clinical manifestations *(33)*

Severity classification	Definition
Dengue without warning signs	A person who lives in or has travelled in the previous 14 days to areas with dengue transmission, and has fever usually for two to seven days and two or more of the following clinical manifestations: • nausea or vomiting • exanthema • headache or retro-orbital pain • myalgia or arthralgia • petechiae or positive tourniquet test • leukopenia.
Dengue with warning signs	Any case of dengue that presents with one or more of the following signs as, or preferably after, fever drops: • intense and sustained abdominal pain, or tenderness of the abdomen • persistent vomiting • fluid accumulation • mucosal bleed • lethargy or restlessness • postural hypotension (lipothymia) • liver enlargement > 2 cm below the costal margin • progressive increase in haematocrit.
Severe dengue	Any case of dengue that has one or more of the following clinical manifestations: • shock or respiratory distress due to severe plasma leakage • severe bleeding: as assessed by the attending physician • severe organ involvement (liver damage, myocarditis, etc.).

Perinatal transmission can occur, and peripartum maternal infection may elevate the likelihood of symptomatic infection in newborns. In addition, placental transfer of maternal IgG antibodies against DENV from a previous infection might heighten the risk of severe dengue among infants infected at 6–12 months of age, when the protective effect of the antibodies diminishes *(24)*. Symptoms of dengue in perinatally infected neonates include ascites or pleural effusions, fever, haemorrhagic manifestations, hypotension and thrombocytopenia.

Box 3 outlines the differential diagnoses of dengue, highlighting a wide range of potentially co-circulating diseases.

Box 3. List of pathogens to be considered in the differential diagnosis of patients presenting with acute febrile illness *(2-6)*

Pathogen classification	List of pathogens
Arthropod-borne viruses	DENV, Chikungunya virus, West Nile virus, Yellow fever virus, Zika virus, Japanese encephalitis virus, Tick-borne encephalitis virus, Oropouche virus, Crimean-Congo haemorrhagic fever virus, St. Louis encephalitis virus, Mayaro virus
Other viral pathogens	Measles virus, Rubella virus, Epstein-Barr virus, Enteroviruses, Influenza virus, Hepatitis A virus, Hantavirus, Arenavirus, SARS-CoV-2
Bacterial pathogens	*Neisseria meningitidis, Leptospira* spp.*, Salmonella typhi, Burkholderia pseudomallei, Rickettsial* species*, Streptococcus pyogenes*
Parasitic pathogens	*Plasmodium* spp.

Note: Arthropod-borne viruses (arboviruses) are viruses transmitted by arthropods (e.g. mosquitoes, ticks) and have similar clinical presentation to dengue. In Box 3, non-arboviral pathogens are grouped by types of pathogens.

Sample types, collection, transportation and storage considerations

DENV can be detected in a range of patient samples, including plasma, whole blood, capillary blood, urine, cerebrospinal fluid (CSF) and tissue samples, but the preferred sample type is serum *(31, 34)*. Table 1 presents sample types used for dengue diagnosis, recommended quantity and collection, handling and transport conditions. The choice of sample depends on the specific test being conducted and the stage of infection (see Table 2). When handling and storing dengue samples, it is essential to adhere to appropriate standard operating procedures to ensure quality and safety.

Serum samples are commonly used in dengue testing because they are reliable for detecting DENV RNA, NS1 and IgM/IgG antibodies. If the testing is conducted within 48 hours, serum samples should be refrigerated at 2–8°C. If testing is delayed, storage at -20°C or lower is recommended *(35)*.

Whole blood samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA) can be used to detect DENV. If the sample is to be used within 24 hours, it should be stored at 4–8°C. Freezing should be avoided, as it could compromise the sample's integrity *(35)*. For molecular methods, heparin should be avoided as an anticoagulant because it interferes with reverse transcription polymerase chain reaction (RT-PCR) and other molecular tests.

Plasma samples can also be used to detect DENV. However, the detection rate might be lower than with whole blood and serum samples *(36, 37)*.

Capillary blood is often used for DENV detection in resource-limited settings, for testing young children or during extensive dengue outbreaks. Capillary blood is usually collected from the fingertip, the lower section of the earlobe, or the lateral area of the heel in infants. This sample type is traditionally stored as dried blood spots on filter paper and, more recently, in Microtainers® at room temperature for short-term storage *(38, 39)*.

Urine samples (midstream), collected in sterile containers, can also be used to detect DENV. If the sample cannot be tested immediately, it should be refrigerated at 2–8°C for up to 24–48 hours *(4)*.

CSF can be used to detect DENV or DENV-IgM antibodies in encephalitic cases only. Experienced personnel should be involved in the collection of sterile CSF samples via lumbar puncture. Due to the fragile nature of CSF samples, CSF should be analysed immediately following collection. Should testing be delayed by more than a few hours, the CSF sample should be stored at 2–8°C to preserve its integrity. For long-term preservation, CSF samples should be stored at -70°C or lower to ensure the integrity of the RNA. It is important to note that freezing at -70°C or lower may affect the stability of antibodies. In addition, freeze–thaw cycles of CSF samples should be avoided *(4, 40)*.

Tissue samples, such as fixed liver, kidney, spleen and lung tissue, are optimal for DENV tissue testing *(41)*. Samples should be processed immediately. For long-term storage, samples should be maintained at -80°C. In the case of histological examination, tissue may be preserved in 10% formalin solution and subsequently processed for paraffin embedding *(4)*.

RNA extracted from patient samples is prone to degradation by ribonucleases. Therefore, proper processing, handling and storage are essential to preserve RNA quality and ensure reproducible results. For optimal stability and long-term storage, RNA should be stored in ribonuclease-free tubes or cryovials and kept at -70°C or lower. If the RNA will be stored for an extended period, it is advisable to store it in aliquots to avoid freeze–thaw cycles.

Table 1. Sample types used for dengue diagnosis, recommended quantity and collection, handling and transport conditions

Sample type	Recommended quantity	Minimum quantity	Sample collection and transport	Handling and transport temperature (°C)
Serum	2.5 mL	0.5 mL	No additives	≤ 2–8
Plasma	2.5 mL	0.5 mL	No additives	≤ 2–8
Whole blood without anticoagulant	5 mL	1 mL	Tubes without anticoagulant	2–8
Whole blood with anticoagulant	5 mL	1 mL	Tubes with EDTA or other anticoagulant	2–8
Capillary blood	100 µL	20 µL	Capillary tube	≤ 20–22
Urine	5 mL	1 mL	No additives	2–8
CSF	1 mL	0.5 mL	No additives	≤ 2–8

Correct handling and storage of samples during transportation is essential for accurate diagnostic testing. Laboratories should comply with national and/or international regulations *(42)*. Where national regulations do not exist, international recommendations should be followed. Dengue samples are categorized as Category B - UN3373 "Biological substance", including human or animal materials intended for diagnosis or investigation *(1)*. DENV cultures are classified as Category A – UN2814 “Infectious substance affecting humans” due to the higher risk of infection associated with cultures. All DENV samples transported should have appropriate triple packaging, labelling and documentation. For international sample referral, it is essential that laboratory personnel be certified through infectious substances shipping training. For additional information on the shipping requirements for infectious substances, please see the WHO publication *Guidance on regulations for the transport of infectious substances, 2023–2024 (42)*.

Laboratory testing methods

The diagnosis of dengue can involve various methodologies and potential testing algorithms that can be employed differently across countries depending on contextual factors. Diagnostic tools should be selected and used appropriately, considering the testing indication, the stage of infection of the patient, the prevalence of dengue in the country or region, and the capacity of national laboratory systems. Testing for the presence of the dengue virus (DENV) should be conducted in well-equipped laboratories by trained personnel following the necessary technical and safety protocols. The different laboratory diagnostic methods used in dengue diagnosis are described in detail below.

RNA detection by nucleic acid amplification tests

RT-PCR is the most common NAAT used to detect DENV RNA extracted from patient sample at early stage of infection *(43)*. It offers a rapid, sensitive and specific method of confirming DENV infection *(3)*. The sensitivity and specificity of commercial RT-PCR-based diagnostics are high (83.9–90.3% and 100%, respectively, reported by Mat Jusoh & Shueb *(44)*). When using NAATs, it is critical to use standardized protocols, including internal process controls and validated primers and probes, to avoid cross-contamination and false-negative results *(31, 45)*.

Most of the recently developed RT-PCR test target all DENV serotypes, and some can differentiate the four serotypes in a single assay *(43)*. In regions where orthoflaviviruses co-circulate, the differentiation or concurrent detection of Zika virus, DENV and chikungunya virus RNA through a multiplex approach can be considered.

While RT-PCR methods require well equipped laboratories and skilled personnel, alternative rapid, simple and adaptable NAAT formats have been developed for detecting dengue in resource-limited settings. These include reverse transcription loop-mediated isothermal amplification (RT-LAMP) and reverse transcription insulated isothermal PCR *(46)*. Some multiplex molecular diagnostic assays are commercially available for near point-of-care testing. These are sample-in-answer-out assays that automate nucleic acid extraction, amplification and detection. They require only a few minutes of operator hands-on time and give the results in an hour or less *(47, 48)*. Some multiplex fever panels include the detection of DENV, Zika, chikungunya, yellow fever and West Nile viruses from whole blood, serum or plasma. These may be useful to support outbreak investigation and surveillance.

Several research studies have identified an extended detection window for DENV RNA in whole blood *(49–51)*. These findings suggest that the presence of virus-infected cells within whole-blood samples increases the amount of viral genetic material. Consequently, even cases with low levels of viraemia may be detected using whole blood *(49)*. In some studies, the presence of viral RNA in urine was detectable between 14 and 32 days following the onset of fever, offering a potential tool for detecting dengue in the later phases of infection and when a cold chain is not assured *(52)*. Nevertheless, the testing of serum or plasma may be more practical due to logistical considerations related to sample processing, storage and RNA extraction. In addition, most RNA extraction kits demonstrate better performance and reliability with serum specimens. DENV infection, when present with severe neurological complications such as encephalitis, meningitis and Guillain-Barré syndrome, can be confirmed by the detection of viral antigens and DENV RNA in CSF samples. Combining both CSF and serum specimens allows for a more accurate diagnosis for clinical management *(53)*.

NS1 antigen detection by ELISA or RDT

NS1 is a highly conserved glycoprotein detectable in serum for up to nine days after symptom onset in primary infections *(54)*, whereas in secondary infections, detection declines by days 6-7 *(55)*. High NS1 levels have been associated with severe disease outcome *(56, 57)*. Laboratory-based ELISA or point-of-care RDTs are widely used for NS1 detection *(31)*.

NS1 ELISA is highly sensitive (100%) and specific (99.2%) for DENV *(58)* and has been reported to be as specific as conventional molecular diagnostic tests *(59)*.
RDTs provide rapid results and are important tests for supporting dengue diagnosis in resource-limited settings. Several studies have reported high sensitivity (68.5%-87.0%) and specificity (100%) for DENV *(60–63)*. However, the different genotypes of the four DENV serotypes circulating in different geographical regions influence the sensitivity

of NS1 RDT detection *(64)*. Therefore, it is crucial to monitor the circulating serotypes in a specific geographical area and during a particular outbreak in order to effectively inform diagnostic decisions.

In secondary infections, the sensitivity of NS1 declines (67.1%-77.3%, compared to 94.7–98.3% for primary infections, reported for ELISA and RDT tests *(65)*), due to pre-existing IgG antibodies forming antigen–antibody complexes *(65–67)*. Therefore, it is recommended that combination testing methods be included, such as the combination of NS1 along with a method for detecting IgM +/- IgG, as this will maximize the chances of capturing both primary and secondary DENV infections *(68, 69)*.

RDTs that combine NS1 and IgM (+/- IgG) provide rapid results, and the positive predictive value can be higher when both analytes are considered together than when relying on a single analyte (NS1 or IgM alone). However, the positive predictive value may still vary based on disease prevalence, and using a single analyte is more prone to false-positive results in low prevalence settings. One study showed that concurrent testing for both NS1 and IgM analytes with RDTs improves the sensitivities of the single analytes (NS1 68.5–87.0%, and IgM 58.3–84.3%) to 92.6–99.1% *(63)*. In addition, a systematic review reported that combined NS1/IgM/IgG RDT demonstrate pooled sensitivity and specificity of 91% and 96%, respectively, when validated against reference standards PCR, NS1 or IgM ELISA, virus isolation, or their combination *(70)*. These findings indicate that combined NS1 and IgM RDT can be used in outbreak settings to confirm dengue *(71)*.

Serological testing by ELISA or RDT

Serological tests are designed to detect IgM, IgG or a combination of the two and are available in ELISA and RDT formats. In primary DENV infections, IgM and IgG are not detectable within the first few days of symptom onset *(2)*. IgM becomes detectable in 50% of patients by days 3–5, reaching its peak approximately two weeks after symptom onset and subsequently declining to undetectable levels over a period of two to three months or longer (Fig. 1) *(2, 72, 73)*. Conversely, secondary infections are distinguished by elevated IgG levels, which typically peak 6–15 days after symptom onset (Fig. 1). In secondary infections, IgM is detected in limited quantities and, in some instances, may not be detected at all *(72)*. High levels of IgG antibodies early in the illness, along with positive NAAT, NS1 or IgM, suggest a secondary infection.

ELISA-based IgM detection tests offer higher sensitivity (61.5–99.0%) and specificity (79.9–97.8%) than RDT (20.5–97.7% and 76.6–90.6%, respectively, reported by Tang & Ooi *(65)*). The various steps and long incubation time of ELISA allow for greater molecular binding, which increases the sensitivity of the method *(74)*. Furthermore, some manufacturers have noted that DENV IgG RDTs have purposefully been designed to detect only higher levels of IgG present after the onset of primary and secondary DENV infections *(75)*.

Antibody detection plays a major role in confirming acute or recent DENV infection more than seven days after symptom onset. Laboratory confirmation of DENV infection requires paired serum samples, acute and convalescent samples, collected at a 10–14 day interval *(6)*. If the IgM levels show at least a four-fold increase in the second sample, it suggests seroconversion. Seroconversion of IgM (e.g. negative acute and positive convalescent sample) confirms the infection *(4, 16, 31)*. IgG seroconversion, or a four-fold or greater increase in IgG titre between the acute sample and the convalescent sample, suggests secondary dengue infection, which may increase the risk of severe dengue. The presence of IgG very early after symptom onset and discrete IgM levels may suggest a secondary infection in the absence of previous orthoflavivirus vaccination. False-positives have been reported among patients with *Plasmodium* spp. or *Leptospirosis* infections *(76–78)*. Therefore, the final interpretation of a positive IgM or IgG finding should be accompanied by a clinical history and epidemiological information.

Distinguishing between primary and secondary DENV infections can be attempted using IgM/IgG ratios. Some laboratories and commercial assays classify primary infections using thresholds such as an IgM/IgG optical density (OD) ratio > 1.2 (with patient sera diluted at 1:100) or > 1.4 (with sera diluted at 1:20). By contrast, ratios below this cut-off suggest a secondary infection. However, these thresholds are assay- and dilution-dependent, vary across laboratories and manufacturers, and lack universal standardization *(2)*.

Obtaining acute and convalescent paired samples is challenging in many countries due to the loss to follow-up in outpatient clinics and short hospital stays. This complicates both paired sampling and consequently the use of IgG as

an accurate testing analyte. Serological tests for IgM and IgG are influenced by cross-reactivity with other orthoflaviviruses, necessitating careful interpretation of test results. IgG exhibits a high degree of cross-reactivity, whereas IgM is less affected.

Plaque reduction neutralization tests

PRNT is currently considered the gold standard for characterizing and quantifying circulating levels of anti-DENV neutralizing antibodies. PRNT is based on the ability of serum antibodies from infected persons to neutralize virus infection in cell cultures *(4)*. This method has been used to evaluate vaccine efficacy and protection and to differentiate infections caused by the four distinct DENV serotypes *(79)*. Due to the cross-reactivity of orthoflaviviruses and the four dengue serotypes, neutralizing assays also facilitate the differential diagnosis of closely related orthoflaviviruses and distinguish serotype-specific immunity. However, PRNT is time-consuming and labour-intensive, and is typically executed in reference laboratories *(80, 81)*.

Virus isolation

Virus isolation is used to characterize viruses at the phenotypic and genotypic levels, including cell tropism, protein expression, infectivity, transmissibility and antigenicity. This method requires the inoculation of diagnostic samples into mammalian or mosquito cell cultures *(2, 3)*. While considered the gold standard for direct identification of the virus, it is time-consuming and labour-intensive, requiring specific biosafety and biosecurity precautions that are often lacking in low-resource settings. Therefore, this method is seldom used for diagnostic purposes *(82)*. Nevertheless, arbovirus reference laboratories should maintain the capacity for virus isolation to further characterize the virus.

Testing indications

Accurate laboratory-based and point-of-care diagnostics are necessary for patient management, surveillance and outbreak investigation of DENV *(56)*. Case-based information can in turn support the implementation of vector control programmes and dengue prevention measures.

For patient management, the testing method should be rapid, sensitive and specific. Methods include:

- NAAT for RNA detection, including near-patient molecular tests
- ELISA or RDT for NS1 antigen detection
- ELISA or RDT combination test for NS1 and IgM
- testing of other pathogens for differential diagnosis.

Patient management should not be delayed while awaiting diagnostic test results. Individuals at risk of developing severe disease should be monitored and receive appropriate clinical care while testing is conducted to confirm DENV infection.

For outbreak investigation, in countries with a high burden of dengue where outbreaks are frequent, the testing method used for dengue diagnosis should be rapid, sensitive and specific. Methods include:

- NAAT for RNA detection, including near point of care molecular tests
- ELISA or RDT for NS1 antigen detection
- ELISA or RDT combination test for NS1 and IgM.

Outbreak definitions based on epidemiological thresholds (e.g. number of cases or incidence rate) rely on the timely analysis of local surveillance data to establish if cases are above a predefined threshold, which varies according to the season. The reliance on surveillance data to detect an outbreak at an early stage is challenging when there are inadequate data to determine a reference or baseline value. Thresholds, such as an excess of reported dengue cases above a defined level (for example, z times the standard deviation above the “moving mean” of cases in the previous five years) in low- or high-transmission seasons, have been considered useful *(83)*. In some non-endemic settings, one case could be considered an outbreak. Once the etiology of an outbreak has been established, testing requirements may differ (as may the need for testing).

For surveillance, a sensitive, specific test methodology that is adaptable for high-throughput is preferred, but turnaround time is generally less of a priority. Methods include:

- ELISA for IgM/IgG antibodies

- NAAT for RNA detection, including multiplex RT-PCR for differential testing of multiple pathogens.

For surveillance, there is no fixed sample size. The sample frame depends on the local dengue endemicity and laboratory capacity. During outbreaks, a proportion of suspected cases should be tested (for example 10-30%), taking into consideration laboratory capacity and resources *(83)*.
For further characterization of DENV to support outbreak investigation and surveillance, the following tests can be used:

- multiplex real-time PCR to differentiate DENV serotypes;
- plaque reduction neutralization test (PRNT) to quantify serotype-specific neutralizing antibodies; and
- next-generation sequencing (NGS) for genomic surveillance (see below for further details), with the sampling strategies for sequencing to align with the sequencing objectives and to base on the proportion of positive cases rather than absolute numbers.

Table 2 summarizes the range of analytes and corresponding test methodologies for dengue diagnosis, the testing indications, and the advantages and limitations of each diagnostic method.

Table 2. The range of analytes and corresponding test methodologies for dengue diagnosis, testing indications, and the advantages and limitations of each dengue diagnostic

Analyte	Test	Testing Indication	Advantages	Limitations
RNA	NAAT	Patient management and surveillance	• High sensitivity and specificity • Rapid results, especially near-patient options – turnaround time from a few hours to days, depending on laboratory capacity and specimen referral • Possibility of automation to increase testing throughput • Can identify specific serotypes	• Only detectable in acute phase of illness (0–7 days post symptom onset and longer in some cases) • Requires higher volume of serum for the sample, at least 0.5 mL compared to serological tests • Requires appropriate facilities and trained staff • Potential false-positives due to contamination • Does not distinguish between primary and secondary infections • Affected by inadequate transport and sample storage conditions • Virus evolution may lead to target failure
	NGS	Surveillance and further characterization	• Used for further characterization of dengue including differentiating DENV genotypes • Identifies multiple DENV serotypes, genotypes and virulence genes simultaneously • Monitors molecular changes and mutations over time	• Expensive • Requires appropriate facilities, computational and storage capacity, and technical expertise • Time-consuming and long turnaround time
NS1	ELISA	Patient management and outbreak investigation	• NS1 highly conserved – low risk of virus evolution affecting epitopes • High sensitivity and specificity	• Only detectable in acute phase of illness (0–7 days post symptom onset and up to 9 days in some cases) • Requires venous blood draw and sample transport to laboratory • Potential false-negatives in secondary infections • Requires qualified and skilled personnel for testing and result interpretation • Turnaround time can extend to several days if laboratories have limited testing capacity or when specimens require referral, potentially delaying diagnosis
	RDT	Patient management and outbreak investigation	• NS1 highly conserved – low risk of virus evolution affecting epitopes • Easy to perform and point-of-care testing available • Can use capillary or finger-pricked whole blood • Rapid results (15–30 minutes)	• Only detectable in acute phase of illness (0–7 days post symptom onset and up to 9 days in some cases) • Lower sensitivity compared with NAAT and ELISA • Potential false-negatives with secondary infection and certain DENV serotypes

				• Accuracy, performance and ease of use vary depending on the brand and manufacturer
Combination NS1 & IgM	ELISA	Patient management and outbreak investigation	• Improved sensitivity compared with single analyte tests • Reduced risk of false-negatives	• Potential false-positives due to cross-reactivity with other orthoflaviviruses, complicating the interpretation of results • Turnaround time can extend to several days if laboratories have limited testing capacity or when specimens require referral, potentially delaying diagnosis
	RDT	Patient management and outbreak investigation	• Improved sensitivity compared with single analyte tests • Easy to perform and point-of-care testing available • Can use capillary or finger-pricked whole blood • Rapid results (15–30 minutes)	• Performance has not been sufficiently evaluated and may vary across different epidemiological contexts • Potential false-positives due to cross-reactivity with other orthoflaviviruses, complicating the interpretation of results
IgM/IgG	ELISA	Patient management, outbreak investigation and surveillance	• Identifies probable dengue cases by detecting IgM in a single sample • Quantitative tests distinguish between primary and secondary infections	• Lower specificity compared with NAAT • Persistence of IgM can cause false-positives in some patients • False-positives/-negatives (IgM levels can be low in secondary infections and high cross-reactivity with other orthoflavivirus antigens/antibodies) • Confirmation requires two or more serum samples taken 10–14 days apart, which reduces the utility in patient management • Requires qualified personnel for testing and result interpretation • Turnaround time can extend to several days if laboratories have limited testing capacity or when specimens require referral, potentially delaying diagnosis
	RDT	Patient management, outbreak investigation and surveillance	• Easy to perform and point-of-care testing available • Can use capillary or finger-pricked whole blood • Rapid results (15–30 minutes)	• Provides a qualitative, not quantitative result • False-positives/-negatives (IgM levels can be low in secondary infections and high cross-reactivity with other orthoflavivirus antigens/antibodies)
	PRNT	Outbreak investigation and surveillance	• Characterizes and quantifies serotype-specific neutralizing antibodies • Facilitates differentiation of cross-reactive samples through determination of quantitative neutralizing titres	• Requires convalescent sample • Laborious and time-consuming protocol • Requires expertise in handing live virus culture

Proposed algorithm for dengue diagnosis in suspected cases

Dengue diagnostic algorithms should be adapted to suit the context, depending on the endemicity of DENV and other orthoflaviviruses co-circulating in the country, the presence/absence of programmatic orthoflavivirus vaccination programmes, and the testing capacity of the national laboratory system. There is no clear consensus in the literature on the optimal diagnostic testing algorithm. However, the national diagnostic algorithm should be standardized and consistently implemented according to the testing menus at the different levels of the national laboratory system (see below for further details)and the testing indications.

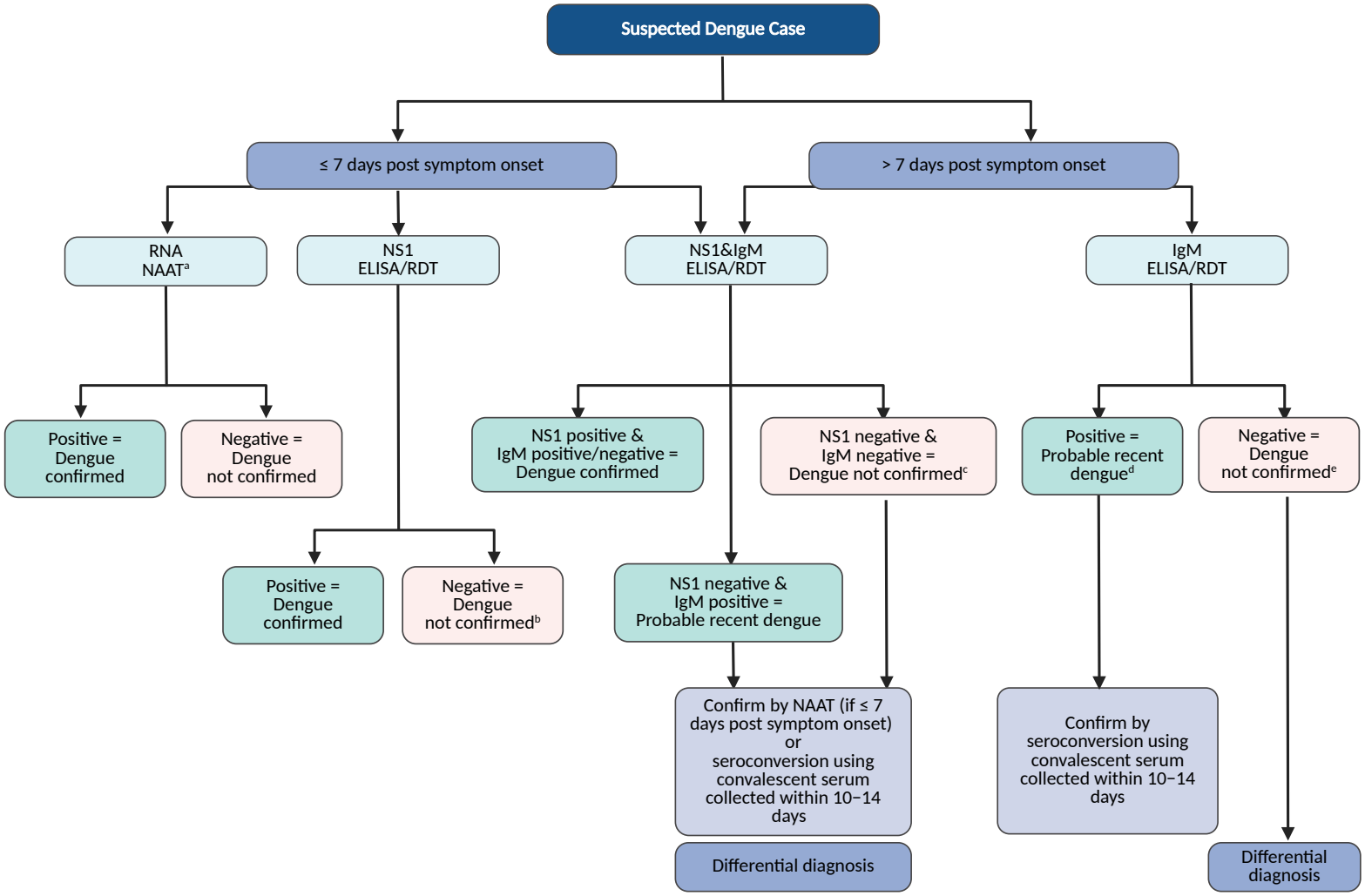
Fig. 2 illustrates a proposed diagnostic algorithm for suspected dengue cases, detailing the available diagnostic methods and the appropriate timing of their use based on days post symptom onset. NAAT targeting viral RNA and the detection of NS1 antigens by ELISA or RDT are recommended for early detection in the first seven days after symptom onset, although RNA may occasionally remain detectable beyond this period. The combined use of NS1 and IgM tests at appropriate time points aids in confirming DENV infection. Serological testing for IgM after seven days is recommended, but there may be cross-reactivity with other orthoflaviviruses. Negative results do not exclude dengue, and further testing, such as seroconversion with convalescent serum, may be necessary. Negative test results require differential diagnosis to confirm cause of infection. Interpretation of results requires consideration of clinical presentation, epidemiological context and local dengue transmission patterns. The prevalence of dengue in the population also influences test selection. In endemic settings, the positive predictive value (PPV) of tests -probability that a positive test result is correct- increases making positive results more likely to reflect true infections. Table 3 presents the different testing methods and interpretation of their results at different stages of dengue infection. Serological methods have limitations, particularly in regions with co-circulating orthoflaviviruses, and it is advisable to include molecular methods (NAAT) whenever possible, considering their higher specificity *(4)*. For samples that yield negative results with RT-PCR, NS1 and IgM/IgG tests, additional testing for other arboviruses or febrile diseases can aid in differential diagnosis (see Box 3 above).

Table 3. Recommended testing methods and interpretation of results at different stages of dengue infection

Stage of infection	Analyte	Testing method	Result	Interpretation
Acute (1–7 days)	RNA	NAAT[a]	+	Confirms acute dengue infection
			-	Dengue not confirmed
	NS1	ELISA/RDT	+	Confirms acute dengue infection[b]
			-	Dengue not confirmed
Acute (1–7 days) and convalescent (> 7 days)	NS1 & IgM	ELISA/RDT	NS1+/IgM+	Confirms acute dengue infection
		ELISA/RDT	NS1+/IgM-	Confirms acute dengue infection
		ELISA/RDT	NS1-/IgM-	Dengue not confirmed
		ELISA/RDT	NS1-/IgM+	Probable recent dengue infection[c]
Convalescent (> 7 days)	IgM	ELISA/RDT	+	Probable recent dengue infection[c]
	IgM	ELISA/RDT	-	Dengue not confirmed [d]

[a] NAAT sensitivity progressively declines after seven days. [b] Depending on the clinical manifestation and dengue prevalence and transmission. NS1+ RDT can be considered a confirmed dengue case in endemic areas with high prevalence. Results should be interpreted considering the test's sensitivity, specificity, and the DENV serotype predominant in the region. Confirmation with NAAT is recommended when there is low dengue circulation or for special cases, such as the first reported dengue case in a geographic area. [c] Consider all clinical and epidemiological factors for accurate case interpretation and conduct additional investigations, such as testing for seroconversion, increase in IgM antibody titre, and differential diagnosis to exclude infection with other orthoflaviviruses. [d] Investigate the case and perform differential diagnosis. For differential diagnosis, consider vaccination status or infection with other orthoflaviviruses, depending on the country's epidemiological situation.

Fig. 2. Proposed algorithm for dengue diagnostics in suspected dengue cases



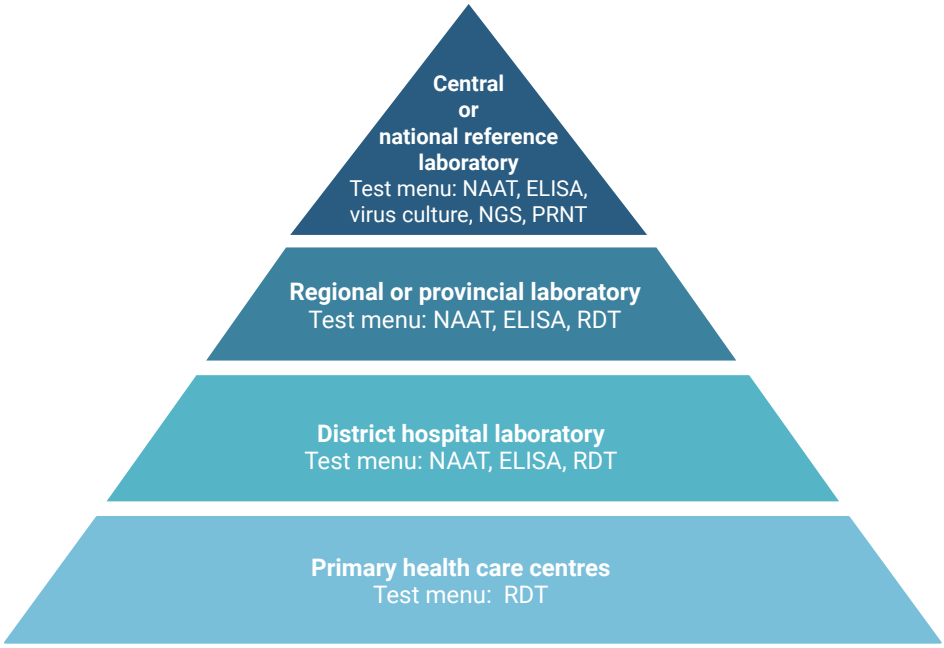
[a] NAAT sensitivity progressively declines after seven days. [b] May be false-negative due to low NS1 detected in secondary infections; confirm by NAAT if high clinical suspicion. [c] May be false-negative in secondary or tertiary dengue infections; confirm by NAAT if ≤ 7 days or by seroconversion if > 7 days, using convalescent serum samples taken after 10–14 days. A four-fold rise in IgG titre confirms recent infection. Consider PRNT if cross-reactivity with other orthoflaviviruses is suspected. [d] May be false-positive due to cross-reactivity with other orthoflaviviruses; confirm by seroconversion in paired samples collected 10–14 days apart. A four-fold rise in IgG titre confirms recent infection. [e] In secondary infections, IgM is stunted or absent. Investigate the case and perform differential diagnosis. For differential diagnosis, consider vaccination status or infection with other orthoflaviviruses, depending on the country's epidemiological situation.

Testing across the tiered laboratory network

In dengue-endemic countries, it is important to integrate dengue testing capacity at different levels of the national laboratory system. The laboratory system should facilitate access to quality testing and be tailored to meet the community's needs and the local dengue epidemiology. In countries where dengue is managed by dengue control programmes, it is important to facilitate strong collaboration between the programme and the national laboratory system to ensure appropriate use of tests at the various levels of the system, and timely data sharing.

Fig. 3 outlines the options for implementing different testing menus at each level of a tiered national laboratory network. At primary health care centres, dengue RDTs are often available outside of laboratory settings and further testing may be conducted by referring samples to higher tiers of the laboratory network. At the intermediate level, in district and peripheral laboratories, testing requires more advanced infrastructure and technical expertise, such as moderate complexity NAATs and ELISA. At the national reference laboratory, testing can entail advanced techniques, infrastructure and biosafety precautions.

Fig. 3 Proposed dengue diagnostic test menu in a tiered laboratory network *(Adapted from (2))*



Genomic surveillance

Genomic sequencing serves as a vital molecular tool for the analysis of the DENV genome and characterization of DENV *(84)*. Each DENV serotype is associated with four to six geographically distinct genotypes, defined as groups of DENV strains that usually exhibit no more than a 7.2% variation in their nucleotide sequences *(85, 86)*. To date, 19 DENV genotypes have been identified, consisting of five in DENV-1 (1I–V), six in DENV-2 (2I–VI), four in DENV-3 (3I, 3II, 3III, 3V) and four in DENV-4 (4I–IV) *(87)*. A recently proposed classification model for DENV lineages introduces two subgenotypic levels: major lineages represent broader evolutionary groupings, and minor lineages reflect finer scale diversity *(88)*. Antigenic drift in structural proteins can drive immune evasion even within genotypes, underscoring the need for genomic surveillance *(89).* Different genotypes within the same serotype may exhibit variation in their capacity to infect host cells, induce severe disease manifestations and influence the efficacy of diagnostic methods, including NAATs and serological tests *(90)*. This observed intra-serotype antigenic variation plays a significant role in determining genotype-specific disease outcomes and patterns of epidemics *(91)*.

Integrating genomic sequencing with geographical, epidemiological and clinical data holds promise for advancing the understanding of DENV epidemiology. DENV sequencing can play an important role in the effective surveillance and

management of dengue outbreaks. When integrated with epidemiological information, it facilitates real-time monitoring of DENV across diverse geographical regions, helps to identify the co-circulation of multiple serotypes and emerging variants, and tracks any changes in transmission *(92–95)*. This is important given the serotype-specific nature of DENV immunity, as detecting substantial shifts in circulating serotypes may indicate the potential for an outbreak within endemic settings *(96)*. Sequencing representative samples can provide insights into pathogen dynamics, evaluate disease severity resulting from the introduction of novel serotypes, and assess the effectiveness of vaccines and diagnostics, identifying possible mutations in NAAT primers *(92–95)*. Proactive monitoring of antiviral resistance markers (e.g. NS5 mutations) is also recommended to safeguard treatment efficacy *(97, 98)*. Regional and country-specific data on the precise genetics of circulating DENV strains and host immune status can also be used to improve control strategies *(99)*. This level of understanding can assist health authorities in efficiently allocating resources and enabling preparedness and timely response to potential outbreaks *(100)*.

Furthermore, integrating genomic surveillance within vector control strategies can yield a more holistic approach to managing dengue outbreaks. This can involve the identification of the mosquito species responsible for transmitting DENV and genetic markers of insecticide resistance in *Aedes* populations *(101)*. These data facilitate timely risk assessment and focused vector control efforts, especially during periods of heightened transmission *(99, 102)*. Coupling these data with environmental data (e.g. climate, urbanization trends) strengthens predictive models for DENV spread.

Different technologies are employed for the genomic sequencing of DENV, ranging from conventional Sanger sequencing to high-throughput NGS. Where possible public health laboratories can leverage their existing networks, expertise and infrastructure to strategically generate genomic data, aligned with their national strategic objectives. To maximize the use of genomic data and reduce duplication, laboratories should adopt harmonized sequencing methods and standardized data reporting. Publicly available protocols can optimize the sequencing processes and are compatible with various sequencing instruments and library preparation kits, enabling methodological consistency *(103)*. Generated sequences should be shared publicly, ensuring that data-sharing policies are adhered to and sensitive information is protected. These practices ensure the generation of high-quality, interoperable data that can be readily used and integrated into global databases. Standardizing methodologies, data collection and visualization can collectively enhance the capacity to predict and mitigate future dengue threats, ultimately safeguarding at-risk populations. For more information on enhancing genomic surveillance capacities, please refer to the WHO publication *Global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022–2032 (104).*

Biological risk management

Laboratories should assess and mitigate biosafety and biosecurity risks associated with dengue testing to ensure a safe work environment for personnel and the wider public. Specimens from suspected or confirmed dengue cases should be handled using a risk-based approach, in line with the WHO *Laboratory biosafety manual, fourth edition (1)*. When dealing with biological specimens, including RNA extraction, adhering to biosafety protocols equivalent to biosafety level 2 is essential. Concurrently, live virus cultures should be handled under heightened control measures equivalent to biosafety level 3.

Quality management

To ensure the accuracy and reliability of laboratory results, laboratories diagnosing dengue should implement robust quality management systems and participate in recognized external quality assessment programmes for dengue and other arboviruses *(3, 105)*. Quality management systems include validation and verification of test kits prior to use, regular calibration and maintenance of equipment, and adherence to established standard operating procedures. In addition, all personnel should be adequately trained and competent to perform the procedures and interpret the test results.

Internal quality control measures should be included for each test. For NAATs, such as RT-PCR, both positive and negative controls should be included in each assay to ensure accurate virus detection, confirm sample quality and monitor potential contamination or non-specific amplification. If any of the assay controls fail, a re-test is

recommended *(106)*. RDTs often have a built-in internal control to confirm their functionality. Some manufacturers may also supply positive control materials for performance verification. The testing frequency of controls should be determined based on the manufacturer's instructions for use. For ELISA, it is essential to use known positive and negative samples to validate or verify the test's performance. It is also important to include calibrators with known antibody concentrations to ensure the assay's quality and accuracy, as well as buffer controls to check for non-specific binding or background signals *(74)*. To guarantee reproducibility, adding samples, standards and controls in duplicates or triplicates is recommended.

Reporting of cases and test results

All countries affected by DENV are advised to establish a dengue surveillance system, as it plays a crucial role in monitoring trends, promptly identifying outbreaks and implementing control measures *(83)*. Dengue cases should be reported as per local public health authority guidelines. Data collected should be standardized, encompassing patient demographics, clinical symptoms, laboratory findings, and key epidemiological factors such as travel history, pregnancy status and vaccination status. Regular collaboration with epidemiologists and public health officials to analyze and disseminate reports can ensure timely and effective dengue responses. The WHO global dengue surveillance dashboard supports these efforts and provides country-level data on confirmed and severe dengue cases, including circulating serotypes *(9)*.

Selection and supply of dengue diagnostics

Dengue in vitro diagnostics are not yet part of the WHO prequalification portfolio. To address this gap and support the procurement of dengue diagnostics, the WHO Department of Regulation and Prequalification, WHO Health Emergencies programme and Global Neglected Tropical Diseases Programme jointly collaborated to establish an Expert Review Panel for Diagnostics *(107)*. This panel was established to evaluate and recommend dengue diagnostics (RDT, NAAT, ELISA) to support countries in identifying quality-assured products.

Functional laboratory services require a sustainable supply of reagents and supplies, well maintained equipment, and a robust procurement and logistics management system. However, in many low- and middle-income settings, several factors, including supply chain disruptions, resource constraints and infrastructure gaps, hinder the continuous supply of commodities.

Prior to the selection of a diagnostic assay, it is important to consider the prevalence and endemicity of dengue, as well as the circulating DENV serotypes within the country. In addition, the interoperability of dengue diagnostic assays with existing instruments is critical. Several commercial assays are designed to be compatible with different platforms, minimizing the need for costly equipment upgrades.

Currently, a variety of kits are commercially available on the market. Table 4 presents essential considerations for selecting dengue diagnostics. These include test characteristics, such as accuracy, sensitivity and specificity, as well as practical requirements, such as ease of use, laboratory capabilities and cost-effectiveness.

Table 4. List of key considerations for selecting dengue diagnostics

Key consideration	Description
Test methodology	This will depend on the analyte targeted and should consider the testing indication, number of days post symptom onset, level of the health system and laboratory capacity.
Test performance	Reviewing the performance data of dengue kits and literature is essential before making procurement decisions. Kits with high specificity and sensitivity should be selected to minimize the risk of false-positive and false-negative results. Kits should also be reviewed for potential cross-reactivity with other orthoflaviviruses circulating in the region of use.

Key consideration	Description
Ease of use	The level of training and supervision needed is an essential factor and depends on the test format, the number of processing steps, the need for additional equipment, ease of interpretation, the time required for results and biosafety concerns.
Storage and shelf-life	It is recommended to adhere to the manufacturers' storage conditions for different types of test kits.
Quality of products and registration	The manufacturer should provide evidence that the dengue kits are manufactured under a recognized quality management system, such as Good Manufacturing Practices or International Organization for Standardization certification. Notably, ISO 13485:2016 is a specific standard for medical devices.
Cost-effectiveness	The total cost of dengue diagnostics extends beyond the price of the test. Additional costs to consider are transportation expenses, import tariffs, storage and shelf-life, end-user training and post-purchase quality control testing needed to ensure the proper implementation of tests. In addition, laboratory-based tests (such as NAATs) require higher upfront cost whereas RDTs are lower-cost but may require further confirmatory testing. Therefore, testing strategies should be developed considering the laboratory system capabilities and longer-term sustainability.

Vaccine and immunization

Dengue vaccines have been developed to protect individuals against dengue virus disease. At the moment Qdenga® (TAK-003) is the only available licensed vaccine. It is a live-attenuated tetravalent vaccine given to specific age groups and in specific circumstances according to WHO recommendations *(108, 109)*.

Process and methods for developing this guidance

This interim guidance was developed as part of the response to the global dengue emergency declared by WHO in December 2023. This document was prepared by WHO in consultation with subject matter experts with knowledge and experience in laboratory diagnosis, surveillance and clinical management of dengue.

Step 1: Defining priorities and key diagnostic questions
The WHO incident management support team convened for the global dengue emergency identified key gaps in existing WHO guidance for laboratory testing of dengue. Key updates to be addressed in the interim guidance were defined including: the optimal methodologies for accurately detecting dengue (virus, NS1 antigen, and serological components) in different contexts; the timing for application of different tests relative to days post symptom onset; the utility of RDT in resource-limited contexts; the differentiation of dengue from co-circulating pathogens; and the definition and feasibility of a testing algorithm for a suspected dengue case.

Step 2: Evidence review
The WHO secretariat conducted a targeted literature review using PubMed and Google Scholar (January 2009- June 2024) using key search terms covering the identified gaps Due to the urgent need for the interim guidance and the broad scope of the guidance, it was not feasible to undertake a formal GRADE process (PICO questions; systematic reviews; formal documentation of values and preferences and incorporation of considerations of costs, resources, and feasibility). Therefore, a two-level literature screening (title and abstract, followed by full-text review) was conducted with key studies selected, peer-reviewed studies, evaluations, and guidelines, prioritizing methodological rigor and relevance to the identified gaps. English language publications only were included.

Step 3: Draft of interim guidance
The initial draft of the guidance was developed by WHO secretariat based on existing guidance and updated published literature and evidence.

Step 4: Expert consultation
WHO convened a multidisciplinary panel of external experts (laboratory specialists, clinicians, epidemiologists, public health officials) from all WHO regions with experience in dengue diagnostics, surveillance, outbreak response and/or clinical management. Two 90 minutes online consultations were held with internal and external experts in September and December 2024.

During the consultations experts had the opportunity to comment on, and contribute to, the draft guidance, to discuss in detail the different approaches used for dengue diagnosis in various settings, and to make key recommendations for dengue laboratory testing. In-depth discussions were held on the proposed diagnostic algorithm for dengue suspected cases with consensus reached during the second consultation. Any other areas of disagreement were documented and resolved through in-depth moderated discussions during the two expert consultations.

Step 5: Iterative review
The guideline underwent two rounds of written review by the experts. Prior to each of the online consultations the draft guidance was shared with experts for review. WHO integrated all written comments and all feedback received during the consultations and reshared revised drafts for review. The iterative process ensured the recommendations were evidence-based practical and globally applicable.

Step 6: Publication and dissemination
The final document was submitted and approved for WHO executive clearance and was disseminated via WHO official channels.

Plans for updating

This interim guidance was developed as part of the response to the global dengue emergency declared by WHO in December 2023. This version of the guidance incorporates the latest understanding and considerations for diagnosis of dengue. WHO closely monitors developments related to dengue and will revise and publish updated recommendations as necessary. Otherwise, this interim guidance will expire one year after the date of publication.

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Declaration of interests

Non-WHO experts completed a confidentiality agreement and declaration of interest. Declarations of interest were reviewed, and no conflicts regarding the support of this guidance document were identified.

[1] Contributions were made in 2024.

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