MONTHLY DENGUE UPDATE

A publication of the National Dengue Control Unit
Ministry of Health, Sri Lanka

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Contents

Laboratory tests for the diagnosis and treatment of dengue

There are several tests that can be performed to diagnose dengue and their results may vary depending on the time of infection and the different stages of the disease.

➢ COMPLETE BLOOD COUNT

Complete Blood Count test values vary according to the stage of the disease.

- Progressive leukopenia (≤5000 white blood cells/mm^3) is the earliest laboratory abnormality in dengue virus infection.
- It is usually followed by a decline in the platelet count to about 100,000 cells/mm3 or below before the emergence of signs of plasma leakage.
- Plasma leakage is heralded by a rising haematocrit above the baseline value, and the extent of haemoconcentration reflects the severity of fluid leakage.
- The haematocrit level normalizes or may be lower than the baseline due to the dilutional effect caused by fluid overload or bleeding.

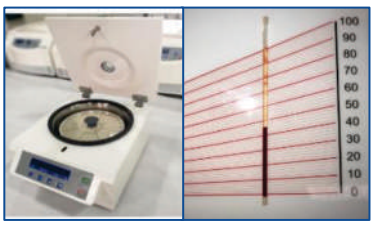

Figure 1 – Microhaematocrit reader

- The white cell count usually starts to rise soon after defervescence, while the recovery of the platelet count is typically delayed.

➢ BIOMARKERS OF DENGUE

The timing of the appearance and duration of these biomarkers in both primary and secondary dengue infection is graphically presented in Figure 2.

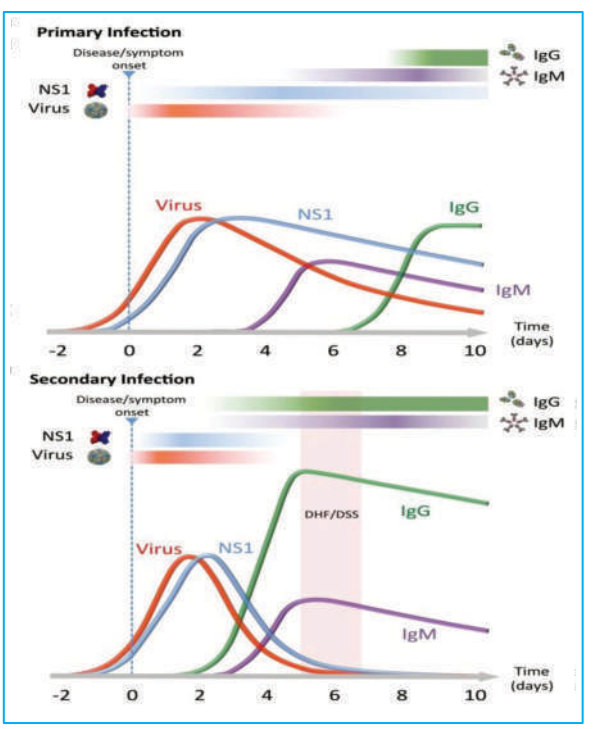

Figure 2 - Timeline of dengue biomarker appearance in patients experiencing primary and secondary infection. (Source: *J Infect Dis*, Volume 215, Issue suppl_2, 1 March 2017, Pages S89–S95)

➢ DENGUE VIRUS DETECTION

• VIRUS ISOLATION

Virus isolation has been the most common diagnostic method for detecting DENV infection. However, it has gradually been replaced by Reverse-Transcription Polymerase Chain Reaction (RT-PCR). More recently, NS1 antigen-capture Enzyme-Linked Immunosorbent Assays (ELISAs) has emerged for more rapid diagnosis. For virus isolation, clinical samples taken from patients are cultured in a variety of cell lines of either mosquito or mammalian origin or in live mosquitoes. Blood samples taken from infected patients experiencing febrile phase of the illness, up to 5 days after the onset of disease give the most successful results. Although detection of DENV by virus isolation is definitive, it is not particularly practical and useful in clinical settings, as isolation can take days to weeks to give results.

• DENGUE VIRUS ANTIGEN DETECTION

Dengue NS1 antigen test

NS1 tests detect the non-structural protein of dengue virus, which is secreted into the blood during dengue infection. The test should be performed during the acute phase of dengue virus infections; 12 hours after the onset of symptoms and within the first five days. After five days, the sensitivity of the test declines.

A positive NS1 test result is indicative of a dengue infection but it does not distinguish between different serotypes of dengue virus. It has been found that the sensitivity of the test is higher in primary infections (first-time infections) than in secondary infections.

A negative NS1 test result does not rule out infection. Patients with negative NS1 results should be tested for the presence of dengue IgM antibodies to determine possible recent dengue exposure.

Though studies show that NS1 can be found in whole blood or plasma, most NS1 tests have been developed and evaluated in serum samples. Dengue NS1 tests are available as commercial diagnostic kits.

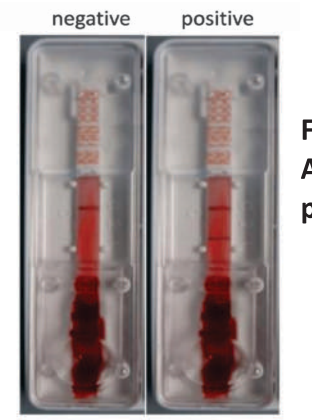

Figure 3 - Dengue NS1 Antigen negative & positive test

• NUCLEIC ACID AMPLIFICATION TEST (NAAT)

A NAAT is a generic term referring to molecular tests used to detect viral genomic material. For symptomatic patients during the first 1-7 days of illness, any serum sample should be tested with a NAAT and for IgM antibody since both tests can be performed in serum. Performing both these tests can detect more cases than performing just one test. After day 7 of illness, few cases can be detected by NAAT. Serum specimens have been the most extensively validated. The relative sensitivity of serum, plasma, and whole blood is not as well documented. Dengue virus has occasionally been detected in cerebrospinal fluid.

A positive NAAT result confirms dengue virus infection. **A negative NAAT** result does not rule out infection. People with NAAT negative results should be tested for the presence of IgM antibodies against dengue virus to determine possible recent dengue exposure. If both the NAAT and IgM antibody results from the acute phase of illness are negative, a convalescent serum should be obtained for IgM antibody testing.

- **REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION (RT–PCR)**

It is a primary test used to detect dengue virus in the early stage of the infection. A positive result not only confirms the infection but also helps to identify the different serotypes of the dengue virus. The test is around 90% sensitive and almost 100% specific. Molecular methods such as RT-PCR and nucleic acid hybridization have been used to great effect in successfully diagnosing DENV infection. PCR-based methods provide same or next day diagnosis of DENV during the acute phase of disease. Now RT-PCR method was then modified to a single-step multiplex real-time RT-PCR (rRT-PCR) assay, which was adopted worldwide. A major advantage of PCR-based techniques is that viral RNA can be detected from the onset of illness and is sensitive, specific, fast, less complicated and cheaper than virus isolation methods. Although PCR-based methods are fast and accurate, they require a laboratory with specialized equipment and trained staff to run the test.

- **SEROLOGIC TESTS FOR DENGUE VIRUS**

Immunoglobulin M (IgM)

As the immune system fights the infection, IgM antibodies against dengue virus are detectable starting 3-5 days after onset of symptoms and are reliably detectable for approximately 12 weeks. These antibodies are detectable in 80% of patients by day 5 and 99% of patients by day 10. This test detects IgM (antibodies) in the blood and shows it in the early stages of the disease. This test can distinguish between primary and secondary infection. If you have had dengue symptoms for more than a week, a dengue antibody IgM test can detect the presence of dengue. This test is not recommended if you have a secondary dengue infection because IgM levels are significantly lower (or undetectable) in secondary infections.

Positive IgM

Patients with a positive IgM test result are classified as probable, recent dengue virus infections.

Negative IgM

Patients with negative IgM results before day 8 of illness and absent or negative NS1 results are considered unconfirmed cases. For these cases, a second sample should be obtained after day 7 of symptoms for additional serologic testing. Patients with negative IgM results after 7 days of symptoms, and absent or negative NS1 (dengue virus antigen detection) are classified as negative for recent infection. Patients with a change from negative to positive IgM results in paired samples (first sample collected during the first 7 days of illness, and second sample collected after symptoms subside) are classified as current dengue infections.

Immunoglobulin G (IgG)

The test is used to detect subsequent infections in dengue because the IgG level in the blood slowly rises after infection. Generally, the level of infection rises in 6 to 10 days and antibodies can remain in the blood for about 90 days or throughout your life.

IgG / IgM ratio testing is widely used to distinguish between primary and secondary dengue infections. Even a few months after infection, these antibodies can still be detected in the blood, so you can get tested 15 days later.

Dengue IgM detection kit is commercially available in the market and IgM serologic tests also are available as laboratory-developed tests in clinical laboratories or as diagnostic kits.

➢ TISSUE TESTS FOR DENGUE VIRUS DETECTION

Nucleic Acid Amplification Test (NAAT) for Fixed Tissue Specimens.

Tissue tests for dengue virus detection may be performed on biopsy or autopsy specimens of liver, kidney, spleen, and lung tissue. A NAAT detects dengue virus RNA. Fixed tissue samples can be tested using NAAT and the sensitivity of the test is less after day 7 of illness. A positive result confirms the presence of dengue virus in tissue. However, other causes of death should be considered. A negative result does not preclude infection. Diagnosis should be interpreted within the context of histopathologic findings, clinical and epidemiologic history, and other laboratory studies.

Special considerations:

Cross reactivity: Cross reactivity is a limitation of dengue serologic tests. Serologic tests to detect antibodies against other flaviviruses such as Japanese encephalitis, St. Louis encephalitis, West Nile yellow fever, and Zika viruses may cross react with Dengue viruses. This limitation must be considered for patients who live in or have traveled to areas where other flaviviruses co-circulate. Therefore, a patient with other recent or past flavivirus infection(s) may be positive when tested to detect IgM antibodies against dengue virus. To more precisely determine the cause of infection in IgM positive patients, the IgM-positive specimens can be tested for specific neutralizing antibodies by plaque reduction neutralization test (PRNT) (against the four dengue virus serotypes and other flaviviruses; however, PRNT does not always conclusively distinguish specific flaviviruses.

Diagnostic Tests for Dengue and Specimens

Diagnostic method	Specimen	Time of collection after onset of symptoms	Time to results	Diagnosis of acute infection
Virus isolation and serotype identification	Whole blood, Serum, Tissue	1 - 5 days	1 - 2 weeks	Confirmed
Nucleic Acid detection	Whole blood, Serum, Plasma, Tissues	1 - 5 days	1 or 2 days	Confirmed
NS1 Antigen detection/ NAAT	Serum	1 - 5 days	1 day	Not yet determined
	Tissue samples	> 1 day	NA	Confirmed
IgM ELISA	Serum, Plasma, Whole Blood	After 5 days	1 – 2 days	Probable
IgM rapid Test			30 minutes	

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Compiled by Dr. K.A.S.D.Kumarapperuma, Medical Officer, National Dengue Control Unit

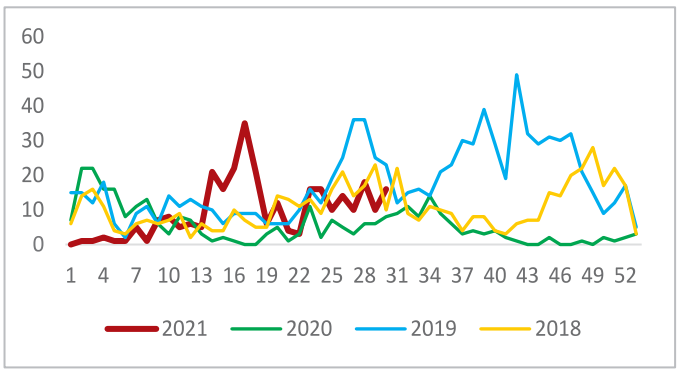
2. SUMMARY OF ENTOMOLOGICAL AND EPIDEMIOLOGICAL SURVEILLANCE DATA – July 2021

Province	District	Entomological surveillance data (Source - returns of entomology surveys received by NDCU)				Epidemiological surveillance (Source-DenSys)	
		No. of Premises			Main type of containers positive for larvae and Percentage positivity	Month	
		Inspected	Positive Found	Positive %		July	Cumulative
WP	Colombo	1403	127	9.05	Temporary removed items 18.3%, Discarded items 21.4%, Tyres 18.33%	1286	5155
	Colombo MC				Data not received to NDCU		
	Gampaha	1013	159	15.7	Temporary removed items 25%, Discarded items 18.2 %, Tyres 11%	642	2219
	Kalutara	1556	128	8.2	Discarded items 64.7 %, Temporary Removed items 10.3%, Tyres 4.8%, Covering items 4.8%	304	1042
	NIHS	508	51	10	Temporary removed items 45.5%, Discarded items 16.3%, Covering items 12.7%		
CP	Kandy	1258	91	7.2	Temporary removed items 24.5%, Water storage barrels 16.3%, Discarded items 14.5%	159	629
	Matale	600	39	6.5	Discarded items 40.9%, Natural items 25.8%, Tyres and Water storage barrels 6%	51	127
	NuwaraEliya				Data not received to NDCU	5	43
SP	Galle	3201	416	13	Discarded items 24.3%, Water storage other16.5%, Ornamental 10.9%	56	292
	Hambantota	1258	99	7.9	Temporary removed items 19%, Water storage barrels 17.4%, Water storage other 15.2%	51	266
	Matara	2200	273	12.4	Discarded items 37.8%, Water storage other 10.3%, Covering items 8.8%	103	421
NP	Jaffna	1477	67	4.5	Ornamental items 24%, Water storage other items 18.3%, Discarded items 16.9%	3	37
	Kilinochchi	400	9	2.3	Pet feeding cups 33.3%, Temporary removed items and Water storage other items 22%	1	13
	Mannar	550	12	2.2	Discarded items 46.7%, Water storage barrel 26.7%, Water storage other items 20%	1	23
	Vavuniya	2257	59	2.6	Water storage cement tanks 22%, Discarded items 17%, Pet feeding cups 16.6%	4	32
	Mullativu				Data not received to NDCU	0	0
EP	Batticaloa	936	31	3.1	Temporary removed items 32.1%, Wells 21.4%, Discarded items 14.3%	21	3373
	Ampara				Data not received to NDCU	11	62
	Trincomalee				Data not received to NDCU	2	129
	Kalmunai	900	72	8	Discarded items 68.6%, Ponds 8.5%, Wells 8.5%	1	232
NWP	Kurunegala	1538	212	13.8	Discarded items and Tyres17.2%, Pet feeding cups13%	222	870
	Puttalama	800	34	4.3	Discarded items 24.3%, Water storage other items 21.6%, Water storage barrel 13.5%	41	301
NCP	Anuradhapura	251	21	8.4	Temporary removed items 33.3%, Water storage containers 23.8%, Tyres 19%	46	242
	Polonnaruwa	500	13	2.6	Ornamental items 45.5%, Discarded items 27.3%, Pet feeding cups 9.1%	21	50
UP	Badulla	107	13	12.1	Discarded items 50%, Natural items and other items 18.2%	52	206
	Monaragala	2143	190	8.9	Discarded items 50.6%, Water storage barrel 14.5%, Covering items 11%	43	115
SGP	Rathnapura	1561	170	10.9%	Discarded items 34.4%, Covering items 11.9%, Water storage other items 11%	93	475
	Kegalle	1458	146	10	Ornamental items 30.6%, Discarded items 21.8%, Water storage barrels 15%	73	406
Sri Lanka					**Discarded items 28.8%, Temporary removed items 10.7%, Water storage other item 9.6%**	**3292**	**16760**

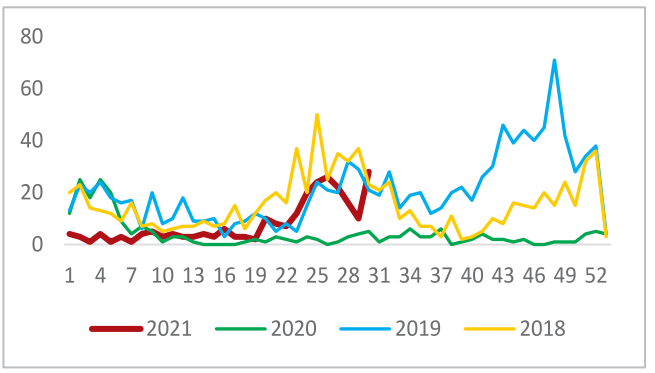
Summaries of Adult Surveys				
District	**MOH**	**GN area**	**Findings**	
Kalmunai	Akkaraippattu	PHI area: Kathiriya (sentinel site) Locaity: Beach Road	8.30am - 11.45pm No.of premises Examined = 10	No adult mosquitoes positive
			8.30am - 12.00 pm No.of premises Examined =m10	*Aedes aegypti* (1male, 1female)
Hambantota	Tangalle	Kudawella Central	8.00am - 4.00pm No.of premises Examined = 45	*Aedes albopictus* (1 male, 2 females;2 premises)

Current high risk MOOH - Epidemiological trends (Source: DenSys data)

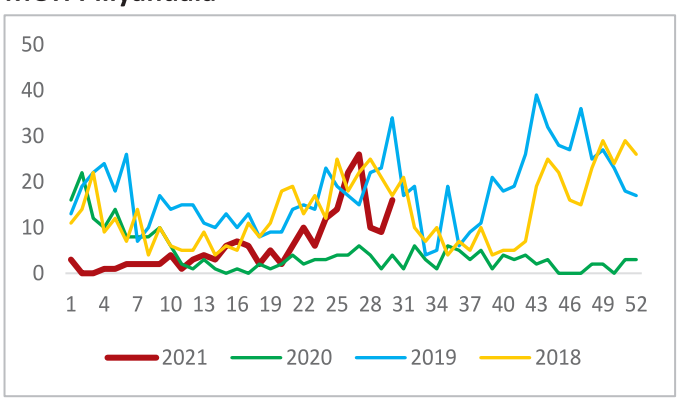
MC Homagama



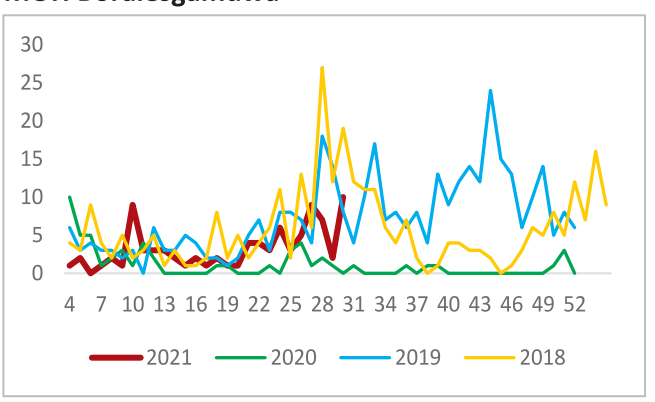
MOH Dehiwala



MOH Piliyandala



MOH Boralesgamuwa

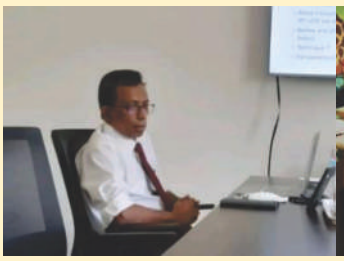


Entomological forecast of high risk areas		
RDHS	**MOH**	**GN Division**
Gampaha	Kelaniya	Dalugamgoda (locality: CEAT tyre Company)
	Attanagalla	Walgammulla
	Ja-ela	Indiwitiya
	Katana	Kadirana South
	Mahara	Enderamulla (locality:Sampaya Mw)
	Wattala	PHI area: Mabola, Locality: Duwawatta
	Ragama	PHI area:Tewatta, Locality: Vihara Mw
Colombo	Ratmalana	Wedikanda (Locality: St.Reeta Rd, Dammarama Rd, Mahindarama Rd)
	Kohuwala	Wilawala(Locality:Sri Maha Vihara Rd,Sujatha Mw)
	Kolonnawa	Gajabapura (locality: Ceylon Petrolium Corporation)

Kurunegala	Kurunegala	Malkaduwawa (Locality: Bandaranayakapura)
Jaffna	Nallur	J/110 (Locality:Parmeswara College Lane, Campus Lane)
Matara	Matara MC	Locality: Sunanda Mw
		Hittatiya Central (Rajapaksha Hena, Raturalagewatta)
		Tibbotuwawa
	Dondra	Kapugama - North (Locality:Pilawewa,Arewatta)
	Welipitiya	Maduragoda(Locality:Denipitiya)
	Weligama	Bandaramulla
Ratnapura	Eheliyagoda	locality: Hospital Junction to Thalagala Junction
Anuradhapura	Nachchaduwa	Locality: Town area
Kalmunai	Pottuvil	Pottuvil-2,3,4(Locality:Sea Thayar Rd,Main Street,Nawady Mw,Drazoor Central Rd,Dharul Falah School Rd)

Special activities and events conducted by National Dengue Control Unit

Live webinar on management of Dengue in children and adolescents on 14.07.2021 by Dr. Lak Kumar Fernando, Consultant Pediatrician/ DGH Negombo




Inter-sectoral meeting chaired by DGHS on 15.07.2021


Meeting chaired by Hon. Minister of Health to review the current Dengue situation – 19.08.2021

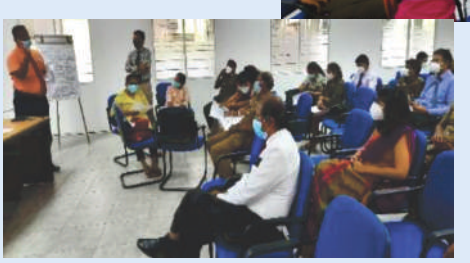
Entomology review of Kalutara district – 27.08.2021

Knowledge Sharing Session – 28.07.2021

Resource persons:

- Dr. Thilina Wijetunga
- Dr. N. Ariff
- Mrs. Rasika Dalpadado

Training programme for the MOOH of Colombo district – 29.07.2021

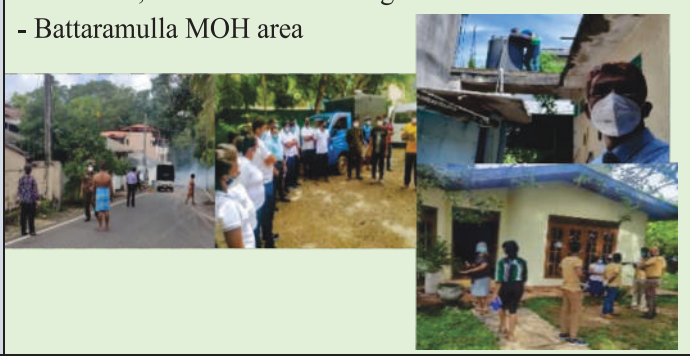
Training on Management of Dengue for Medical Officers and Nursing Officers of Armed Forces and Police – 05.08.2021

Media briefing to update on the current Dengue situation - 06.08.2021

Knowledge Sharing Session for the technical staff of NDCU - 11.08.2021

Resource persons:
-Dr. Indira Kahawita
-Dr. Thilina Wijetunga
-Ms. Ayesha Sarathchandra

Field Supervisions done by NDCU team
- Dehiwala MOH area
- Ragama, Negombo and Mahara MOOH areas
- Panadura, Horana and Bandaragama MOOH areas
- Battaramulla MOH area



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Comments and contributions for publication in the MDU Sri Lanka are welcome

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