AAZ
Votre santé sous contrôle

CE

TROD'DENGUE®

COMBO Rapid Test
NS1 antigen and IgG/IgM antibodies of dengue fever
(whole blood/serum/plasma)
Ref: TR-DEN-002
User manual

INDICATION FOR USE

TROD'DENGUE® is a rapid test for the qualitative detection of Dengue virus NS1 antigen and IgM/IgG antibodies to Dengue virus in whole blood, serum or plasma,
TROD'DENGUE® is a test for professional use to facilitate the diagnosis of Dengue viral infection.

INTRODUCTION

Dengue viruses, transmitted by the mosquitoes Aedes aegypti and Aedes albopictus, are widely distributed in tropical and subtropical areas of the world. There are four distinct known serotypes (Dengue virus 1, 2, 3 and 4). In children, the infection is often subclinical or causes self-limited febrile illness. However, if the patient is infected multiple times with a different serotype, a more severe disease, dengue, dengue hemorrhagic fever or dengue shock syndrome, is more likely to occur. Dengue fever is considered the most important viral disease transmitted by arthropods because of the human morbidity and mortality it causes. NS1 is a highly conserved glycoprotein that is present in high concentrations in the serum of patients infected with Dengue during the early clinical phase of the disease.
Dengue NS1 antigen is present from the first day and up to 9 days after the onset of fever in the sample of patients infected with primary or secondary dengue. The immune response includes IgM antibodies produced between the 3rd and 5th day of symptoms and persisting for 30 to 60 days. IgG antibodies appear around day 14 and persist for life. Secondary infections often result in high fever and, in many cases, bleeding events and circulatory failure. Secondary infections show that IgG levels increase within 1 to 2 days of symptom onset and induce an IgM response after 20 days of infection.

PRINCIPLE OF THE TEST

TROD'DENGUE® is a two-part qualitative membrane immunoassay assay for the simultaneous detection of NS1 antigens and IgG and IgM class antibodies to Dengue in whole blood, serum or plasma.

Dengue NS1 antigen rapid test:
The test uses anti-Dengue NS1 antibodies fixed at the test area (T) and an anti-mouse IgG antibody fixed at the control area (C).
When the sample and then buffer are added to the sample (S) well, the NS1 antigens interact with the NS1 dengue antibodies conjugated to the color particles to form a stained antibody-antigen complex.
This complex migrates by capillary action on the membrane to the test line (T) where it will be captured by the anti-Dengue NS1 antibodies attached to the membrane. The complex is then trapped and forms a colored band that confirms the reactivity of the test. The absence of a coloured band in the test region indicates a negative result.

Rapid test for differentiation of IgM and IgG antibodies against Dengue:
The test uses human antibodies to IgM (IgM test line), human antibodies to IgG (IgG test line) and mouse IgG (control line (C)) immobilized on the nitrocellulose strip. The Conjugate (recombinant dengue antigens labeled with colloidal gold) is also integrated into the strip.
When the sample and then buffer are added to the sample (S) well, IgM and/or IgG antibodies, if present, bind to dengue conjugates, forming antibody-antigen complexes. These complexes migrate through the nitrocellulose membrane by capillary action. When the complexes meet the line of the corresponding immobilized antibody (human IgM antibody and/or human IgG antibody), the complexes are trapped and form a burgundy band that confirms the reactivity of the test. The absence of a coloured band in the test region indicates a negative result.

EQUIPMENT PROVIDED

- 20 sealed pouches each containing a test cassette and a moisture absorber
- 1 sachet of 20 pipettes of 10µl
- 1 bag of 20 pipettes of 30µl
- 1 x 4.0 ml buffer bottle
- 20 lancets
- 20 alcohol swabs
- 1 user manual for professional use

MATERIALS REQUIRED BUT NOT PROVIDED

- Sample Collection Containers
- Laboratory pipettes (if plasma/serum is used)
- Centrifuge (for obtaining plasma)
- Timer

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). Do not freeze the kit. The kit is stable until the expiry date indicated on the kit. The test should remain in the sealed pouch until it is used. REMOVE THE KIT FROM THE SEALED POUCH ONLY JUST BEFORE PERFORMING THE TEST.

WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use. Do not use after the expiration date indicated on the package.
- Do not eat, drink, or smoke in the area where samples or kits are handled.
- Do not use if the sealed pouch is damaged or the buffer tube is broken.
- The test cassette is for single use. Do not reuse under any circumstances.
- Handle all specimens as if they contained infectious agents. Follow the safety instructions for microbiological hazards and follow the procedures in place for the proper disposal of samples.
- Wear protective clothing such as lab coats, disposable gloves, and goggles when samples are being tested.
- The equipment used must be disposed of in accordance with local regulations.

SAMPLE COLLECTION AND PREPARATION

TROD'DENGUE® can be performed from whole blood, serum or plasma.

- For whole blood samples: collect whole blood in a collection tube (containing anticoagulants such as heparin, EDTA, and sodium citrate) by venipuncture.
- For serum or plasma samples: collect blood by venipuncture and then separate the serum or plasma from the blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed samples.
- For fingerstick whole blood samples (using the pipette provided in the kit): collect the blood as described in the "Procedure" section below.
- Testing should be performed immediately after sample collection. Do not leave samples at room temperature for long periods of time. Serum and plasma samples can be stored at 2-8°C for up to 3 days. For long-term storage, samples should be stored at a temperature below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be performed within 2 days of collection. Do not freeze whole blood samples. Whole blood taken from the fingertip should be tested immediately.
- If samples are to be shipped, they must be packaged in accordance with local regulations for the transportation of etiologic agents.

PROCEDURE

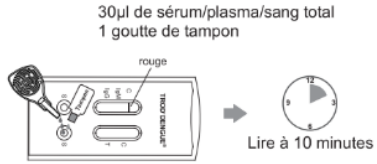
Ensure that the sample and test components are at room temperature (15-30°C) before proceeding with the test. Remove the cassette from the sealed pouch and just before performing the test.

1. Place the cassette on a clean, flat surface.
2. Perform the test as follows, depending on the type of sample:

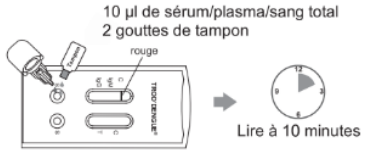
- For **fingerstick whole blood samples** (using the 10µ and 30µl pipettes provided in the kit):
  a) Disinfect the fingertip with the alcohol swab.
  b) Using the lancet, prick the side with the fingertip. Form a large drop of blood suspended.
  c) Take the 30µl pipette, hold it horizontally and bring the end of the pipette into contact with the blood. The pipette fills automatically. Fill it completely.
  d) Repeat the operation with the 10µl pipette.
  e) Take the 30µl pipette containing the sample, hold it vertically pointing down and gently touch the bottom of the sample well (S) on the right (NS1 area) of the cassette to empty it in its entirety. Immediately add 1 drop of tampon.
  f) Take the 10µL pipette and repeat the operation described above in the sample (S) well on the left (IgG/IgM zone). Immediately add 2 drops of tampon.
  g) Start the timer.
- For **venous whole blood/serum/plasma samples** :
  a) Collect 10µl of blood sample using a laboratory pipette and place it in the left sample (S) well (IgG/IgM zone) of the cassette. Immediately add 2 drops of tampon.
  b) Then take 30µl of blood sample using a laboratory pipette and place it in the right sample well (S) (NS1 zone) of the cassette. Immediately add 1 drop of tampon.
  c) Start the timer.

3. Wait for the colored line(s) to appear.
4. Read the results at 10 minutes. Do not interpret the result after 20 minutes.

Dengue NS1 Antigen Rapid Test



Rapid test for differentiation of IgM and IgG antibodies against Dengue



INTERPRETATION OF THE RESULTS

IgM/IgG Ag NS1	Result
Bleu, C, IgM, IgG — Red, C, T	**Positive NS1 Ag** Probable acute primary infection
Bleu, Rouge, C, IgM, IgG — Red, C, T	**Positive IgM and NS1 Ag** Probable primary infection
Bleu, Rouge, C, IgM, IgG — Red, C, T	**Positive IgM** Primary infection
Bleu, Rouge, Rouge, C, IgM, IgG — Red, C, T	**Positive IgM and IgG** Recovery phase
Bleu, Rouge, C, IgM, IgG — Red, C, T	**Positive IgG** Old infection
Bleu, Rouge, Rouge, C, IgM, IgG — Red, C, T	**Positive IgM, IgG and NS1 Ag** Probable secondary infection
Bleu, Rouge, C, IgM, IgG — Red, C, T	**Positive IgG and NS1 Ag** Probable acute secondary infection
Bleu, C, IgM, IgG — Red, C, T	Negative IgM, IgG and NS1 No infection or incubation phase

INVALID
IgM/IgG: The control line (C) is always (even partially) red and does not completely change from red to blue.
NS1 Ag: Control line (C) does not appear.

Insufficient buffer volume or incorrect execution are the most likely reasons for the control line failure. Review the procedure and repeat it with a new test. If the problem persists, stop using the kit and contact us.

EXPECTED VALUES

Dengue NS1 antigen should be detected as early as the first day after the onset of fever and may persist for up to 9 days in primary and secondary Dengue infections. But if anti-NS1 antibodies are produced, the detection of the NS1 antigen is inhibited.
Primary dengue fever is characterized by the presence of detectable IgM antibodies 3 to 5 days after the onset of infection.
Secondary dengue fever is characterized by elevation of specific IgG levels 1 to 2 days after the onset of infection, and in the majority of cases, this elevation is usually accompanied by elevated IgM levels.

QUALITY CONTROL

Internal quality control
Internal quality control is included in the test.
The appearance of a control red line (C) for the NS1 Ag test and the change of the control line (C) from red to blue for the IgM/IgG test confirms that the test procedure has been followed correctly.

BOUNDS

- TROD'DENGUE® is intended for in vitro diagnostic use. It should be used for the detection of Dengue virus NS1 antigen and IgM/IgG antibodies to Dengue virus in blood, serum, or plasma samples only. This qualitative test does not allow to determine the quantitative value or the rate of increase of the NS1 antigen of the Dengue virus.
- Failure to follow the test procedure may adversely affect the performance of the test and/or invalidate the test result.
- Cross-serological reactivity between flaviviruses (Dengue 1, 2, 3 and 4, St. Louis encephalitis, West Nile virus, Japanese encephalitis and yellow fever virus) is common.
- If the test result is negative and clinical symptoms persist, it is recommended that additional testing be done using other clinical methods. A negative result does not exclude the possibility of a Dengue virus infection at any time.

PERFORMANCE

Clinical Performance

Dengue NS1 Antigen Rapid Test
Samples obtained from a population of symptomatic and asymptomatic individuals. The results were confirmed by a reference ELISA test.

Method		Reference Test		Total
TROD'DENGUE®	Results	Positive	Negative	
	Positive	66	4	70
	Negative	3	226	229
Total Results		69	230	299

Relative sensitivity: 95.7%
Relative specificity: 98.3%
Accuracy: 97.7%

Rapid test for differentiation of IgM and IgG antibodies against Dengue
The samples are from a population of symptomatic and asymptomatic individuals. The results were confirmed by a reference ELISA test.

Infection par la Dengue	Result	Igm	IgG
Infection primary	Positive	14	0
	Negative	3	17
	Total	17	17
	Relative sensitivity	**82,4 %**	**0 %**
Secondary infection	Positive	39	55
	Negative	16	0
	Total	55	55
	Relative specificity	**70,9 %**	**>99.0%**
Non-Dengue Infection	Positive	0	0
	Negative	378	378
	Total	378	378
	Relative specificity	**>99.0%**	**>99.0%**

For primary and secondary infections, the overall sensitivity is 95.8%, the overall specificity is >99.0%, and the overall accuracy is 99.3%.

Limit of Detection (LOD)

The minimum detectable concentration of NS1 antigen is 0.1 ug/ml.

The positivity thresholds for the detection of IgG and IgM antibodies are:
- 1/32 for IgG antibodies
- 1/8 for IgM antibodies

Dilution IgG	CE marked rapid test	TROD'DENGUE®
	Positive /Total	Positive /Total
1/2	2/2	10/10
1/4	2/2	10/10
1/8	2/2	10/10
1/16	2/2	10/10
1/32	2/2	10/10
1/64	0/2	0/10
1/128	0/2	0/10

Dilution IgM	CE marked rapid test	TROD'DENGUE®
	Positive /Total	Positive /Total
1/2	2/2	10/10
1/4	2/2	10/10
1/8	2/2	10/10
1/16	0/2	0/10
1/32	0/2	0/10
1/64	0/2	0/10
1/128	0/2	0/10

The results show that the analytical sensitivity of the test is identical to the CE-marked rapid test used in comparison.

Interference
The substances listed in the table below have been tested:

Analyte	Concentration
4-Acetamidophenol	1mg/ml
Acetylsalicylic Acid (Aspirin)	0.2mg/ml
Bilirubin	0.3mg/ml
Gentisic acid	0.2mg/ml
Phenothiazine	0.2mg/ml
Ascorbic acid	0.2mg/ml
EDTA	0.2 mg/ml
Haemoglobin	10 mg/ml
Oxalic acid	0.2mg/ml
Creatine	1mg/ml
Ethanol	1%
Methanol	1%

The results showed no interference.

Precision
Within-lot: 9 samples (one IgG/IgM negative, one weak positive IgG, one strong positive IgG, one weak positive IgM, one strong positive IgM, one positive IgG/IgM on one side and one NS1 negative, one weak positive NS1, one strong positive NS1 on the other) were tested 10 times in 3 different batches. The results were all correct.

Inter-batch: 9 samples (one IgG/IgM negative, one weak positive IgG, one strong positive IgG, one weak positive IgM, one strong positive IgM, one positive IgG/IgM on one side and one NS1 negative, one weak positive NS1, one strong positive NS1 on the other) were tested 5 times on 3 different lots and for 4 days. The results were all correct.

Cross-reactions
Samples containing the substances listed below were tested.

Samples
Pregnant Serum
HBsAg positive
HIV positive
Positive RF
Syphilis positive
HCV positive
HAV positive
AFP positive
H. Pylori positive
PSA positive
Troponin I Positive

The results showed no cross-reaction.

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SYMBOL LEGEND

	Read the user manual		Expiry date
IVD	For in vitro *diagnostic use* only	LOT	Lot Number
2°C 30°C	Store between 2 and 30°C	Σ	Number of tests per kit
	Maker	REF	Reference
	Do not reuse	CE	CE marking

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