ASTAM DIAGNOSTICS

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PRATHAM®

Dengue NS1 Antigen and Antibodies (IgG & IgM) Test Device

INTENDED USE

PRATHAM® Dengue NS1 Antigen and Antibodies (IgG & IgM) Test Device is an in vitro diagnostic rapid test based on the principle of immunochromatography on a membrane, for simultaneous and differential detection of Dengue specific antigen (NS-1) and antibody (IgM/IgG) in human serum / plasma for diagnosis of Dengue infection. For professional use only.

INTRODUCTION

Dengue is caused by any of the four distinct but antigenically related serotypes of flavivirus (DEN-V1, DEN-V2, DEN-V3 & DEN-V4) found largely in tropic and subtropic areas. The viruses are transmitted to human by mosquito Aedes aegypti and Aedes albopictus causing dengue fever with severe flu like symptoms. WHO estimates that 55-60 million cases of dengue fever occur worldwide each year, including a more severe form called the dengue hemmorraghic fever (DHF) and dengue shock syndrome (DSS). During primary infection the classical symptoms are sudden onset of fever, intense headache, myalgia, arthralgia and rash. Secondary infection is most common in many parts of South East Asia and South America.

During Primary infection Non Structural Protein (NS-1) level is raised and can be detected in early clinical phase of the disease from day 1-9. NS-1 Ag appears in serum / plasma before the onset of IgM & IgG antibodies and its detection in suspected dengue patient can be used as an early marker for the disease.

IgM antibodies increase to a detectable level in Primary Dengue infection (4-10days) after the onset of fever and stays upto 30 -90 days. IgG appears after 14 days and is there for life time in primary infection. In secondary infection, high levels of IgG antibodies appears within 1-2 days after the onset of the infection prior to or simultaneous with IgM antibodies. Therefore the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections. The secondary form of disease is severe and can lead to DHF and DSS. It is, therefore, important to detect Dengue infection at an early stage in order to avoid complications.

TEST PRINCIPAL

Pratham® Dengue NS1 Antigen and Antibodies (IgG & IgM) Test Device is a two site sandwich immunoassay based on the principle of immunochromatography on a membrane. It has two strips, one for detection of NS-1antigen and the other for differential detection of IgM/IgG antibodies in human serum /plasma specimen.

Dengue NS-1 nitrocellulose strip is predispensed with anti-dengue NS-1 antibodies at the test line region "T" and Goat anti rabbit IgG at control line region "C". When the sample is dispensed into the sample well (S) of the test device it flows through the membrane assembly of the device, comes in contact with the conjugate pad containing anti dengue NS-1antibody-colloidal gold conjugate and rabbit IgG colidal gold conjugate. Dengue NS-1 antigen if present, binds to the anti dengue NS-1 antibody gold conjugate making antigen antibody complex. This complex moves further on the membrane to the test region where it comes in contact with immobilized anti dengue NS-1 antibodies at the test line region "T" leading to the formation of a colored band confirming positive result for dengue NS-1 antigen.

Dengue Ab (IgM/IgG) nitrocellulose strip is a three line test predispensed with anti human IgM at the test line region "M", anti human IgG/ protein-A at the test line region "G", and a protein ligand at control line region "C ". When sample is added in sample well followed by sample running buffer, Ig G /IgM antibodies if present in the sample reacts with gold conjugated with recombinant antigens of dengue. This complex moves further up and reacts with the anti human IgG /IgM antibodies coated on the membrane leading to the formation of colored band at the IgM/IgG region. The appearance of colored band in a specific regions is considered as positive for that particular antibody. If the specimen does not contain dengue antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region in both the strips confirming that the test has performed properly.

KIT COMPONENTS

1. Pouch contents: Test Cassette, Sample dropper, Desiccant
2. Assay Buffer
3. Instruction for use

MATERIAL REQUIRED BUT NOT PROVIDED

Timer, Gloves, Micropipette, tips & centrifuge etc.

STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 2-30ºC till the duration of shelf life as indicated on the pouch. Do not freeze. Once the pouch is opened, test card must be used immediately.

SPECIMEN COLLECTION AND STORAGE

Human Serum: Collect the whole blood into the clean collection tube (Not containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture. Leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.

Plasma: Collect the whole blood into the clean collection tube (Containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture. And centrifuge blood to get Plasma specimen.

Storage: Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8ºC or if storing more than 3 days, then freeze the specimen at -20°C or below.

WARNING AND PRECAUTION

1. For professional use only, not to be used by the general public.
2. The test must be carried out by or under the direction of a registered medical practitioner or by a technician at the request of registered medical practitioner.
3. Bring all reagents and specimen to room temperature before use.
4. Do not pipette any material by mouth.
5. Do not eat, drink or smoke in the area where testing is done.
6. Use protective clothing and wear gloves when handling samples.
7. Use absorbent sheet to cover the working area.
8. Immediately clean up any spills with sodium hypochlorite.
9. Dispose off all the reagents and material used as if they contain infectious agent.
10. Neutralize acid containing waste before adding hypochlorite.
11. Do not use kit after the expiry date.
12. Do not mix components of one kit with another
13. Sample running buffer contains sodium azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing.

TEST PROCEDURE

1. Bring the sealed pouch to room temperature, if the pouch of the test card is damaged discard the card and take a new one for the test. Open the pouch and remove the card. Label the card appropriately with patient identity. Once opened, the card must be used immediately. Refrigerated specimen must be bought to room temperature prior to use. Take out the test cards from the pouch by tearing from notch provided and place on flat surface.

A. Test Procedure for dengue antigen (NS1)

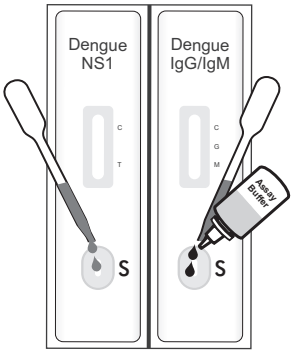
1. Add 2-3 drops (about 50-70 µl) of serum / plasma into the sample well (S) using dropper provided for NS-1 test.
2. Read results at the end of 15-20 minutes. Do not interpret after 20 minutes.

B. Test procedure for dengue antibody (IgM/IgG)

1. Using a micro pipette/dropper provided for antibody test, dispense 5 µl of serum / plasma in the well (S+B).
   Alternately use provided dropper if micro pipette is not available.
2. Add 2 drops of sample running buffer (approximately 60 µl) into well (S+B).
3. Read results at the end of 20 minutes.
4. Discard used card in a biomedical waste container after interpreting the results.

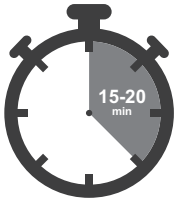
INTERPRETATION OF RESULTS

Add 2-3 drops (about 50-70 µl) of serum/plasma

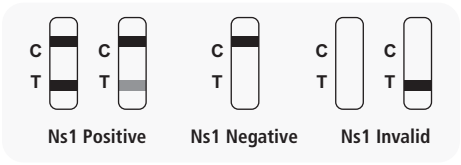


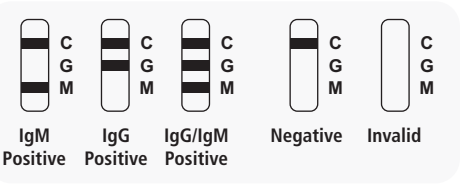
Add 5 µl of serum / plasma

Add 2 drops of sample buffer



Read results with 15-20 minutes.





NEGATIVE: Appearance of only one colored band at control line region 'C'. The result should be considered negative for Dengue antibody and NS-1 antigen.

POSITIVE:

Dengue Antigen (Ns1): Appearance of bands at 'T' and 'C' regions indicate that the specimen is positive for dengue NS-1 antigen.

Dengue Antibody (IgG/IgM): Appearance of colored bands at 'M' & 'C' regions indicates that specimen is positive for IgM Dengue antibody. Appearance of colored bands at 'G' & 'C' regions indicates that specimen is positive for IgG Dengue antibody. Appearance of colored bands at M, G & C regions indicates that specimen is positive for IgG & IgM Dengue antibody.

Appearance of colored band at T & either M or G or both region indicate specimen is positive for Dengue Antigen Ns1 & antibody IgG/ IgM or both.

NOTE: The intensity of the color in the test line regions (T,M & G) will vary depending on the concentration of Dengue antigen/ antibodies in the specimen.

INVALID: Appearance of no coloured band in the control line region C, the assay should be considered invalid regardless of any colored band in the region M/G in Dengue antibody test or T in NS-1 test . Repeat the test with a new card.

INTERNAL QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control region (C) is considered and internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique. External controls are not supplied with this kit. It is recommended that positive and negative controls should be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance. Handle the negative and positive controls in the same manner as patient specimens.

PERFORMANCE CHARACTERISTICS

Clinical Performance with positive and negative specimen: Samples from susceptible subjects were tested with DENGUE Ag (NS-1) AND Ab (IgM/IgG) TEST DEVICE and compared with commercially available test.

1. **Sensitivity:** Dengue antigen (NS1) : 98% | Dengue antibody (IgM/IgG) : 97%
2. **Specificity:** Dengue antigen (NS1) : 99% | Dengue antibody (IgM/IgG) : 98%
3. **Accuracy:** The results obtained by DENGUE Ag (NS-1) AND Ab (IgM/IgG) TEST DEVICE correlated very well when run in parallel with other commercially available tests, using known positive and negative specimens. For the primary and secondary infection, the overall accuracy is 99.3%
4. **Precision:** Repeatability and reproducibility (inter-assay and inter-lot) were evaluated on a number of negative and positive samples. No variations were found in the outcome of the different tests.

LIMITATION OF THE TEST

1. Pratham® Dengue NS1 Antigen and Antibodies (IgG & IgM) Test Device is a in vitro diagnostic test. The test should be used for qualitative detection of dengue antigen NS-1/ IgM, IgG antibodies in serum or plasma specimens only. Neither the quantitative value nor the rate of increase in dengue antigen/ antibody concentration can be determined by this qualitative test.
2. The test detects the presence of dengue NS1 antigen and IgM / IgG antibodies to dengue virus in the specimen and should not be used as the sole criteria for the diagnosis of Dengue virus infection.
3. A negative result can occur if the quantity of Dengue virus NS-1 antigen or IgM/ IgG antibody present in the specimen is below the detection limit of the assay, or the antigen/antibody that are detected are not present during the stage of disease in which a sample is collected. A negative test result cannot exclude a recent infection.
4. If the result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative

result does not at any time preclude the possibility of dengue infection.

5. Serological cross-reactivity across the flavivirus group (Dengue 1, 2, 3 & 4. St. Louis encephalitis West Nile virus, Japanese encephalitis and yellow fever viruses) is common. Positive results should be confirmed by other tests.
6. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
7. Results from immunosuppressed patients should be interpreted with caution.

DISPOSAL

Consider all test devices run with human specimen as potentially infectious and discard using standard biosafety practices.

DISCLAIMER:

Every precaution has been taken to ensure diagnostic ability and accuracy of this product. This product is used outside the control of manufacturer and distributors. Various factors including storage temperature, environment conditions, and procedural errors may affect the result. A person who is subject of the diagnosis should consult a doctor for further confirmation.

WARNING

The Manufacturer and Distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative in the use of this product.

BIBLOGRAPHY OF SUGGESTED READING

1. Ampaiwan C, Wathanee C, Viroj P, Kanchana T, Sarapee L, Sutee Y. (2008). The use of Nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. J Paed Infect Dis ;27:43-8
2. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol 2010; 28:107-10
3. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanium G. (2007). Evaluation of a dengue NS1 capture ELISA assay for the rapid detection of dengue. J Infect Developing Countries; 1:182-8
4. Shu PY, Huang JH. (2004) Current advances in dengue diagnosis. Clinical and Diagnostic Laboratory Immunology; 11:642-50.
5. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue, Rev. Infect. Dis. 1984; 6:251-264
6. Ruechusatsawat K, et al. Daily observation of antibody levels among dengue patients detected by enzyme -linked immunosorbent assay (ELISA). Japanese J. Trop. Med. Hygiene 1984; 22: 9-12

SYMBOLS

Read instructions for use	Name of Manufacturer	For single use only
No. of test	Expiry Date of Kit.	Date of manufacturing of IVD Kit
IVD In-vitro diagnostic use	Keep away from Sunlight	REF Reference Catalogue Number
Storage Condition		

Manufactured By:
ASTAM DIAGNOSTICS PVT. LTD.
Plot No. H-125, RIICO Indl. Area, Kaharani, Bhiwadi Extn.
Alwar, Rajasthan-301019 (INDIA)
For feedback/queries contact customer care : 011-27358101

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