MULTISURE® DENGUE Ab/Ag RAPID TEST

CE

Instructions For Use

REVISION DATE: 2016-09
MDZ0011-ENG-2

Note: Changes Highlighted.

REF 43590-020 (20 tests)

TRADE NAME AND INTENDED USE

The MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test is a qualitative *in vitro* immunochromatographic test to detect and differentiate IgA/ IgM/ IgG antibodies against dengue virus and NS1 dengue antigen in human plasma, serum, finger pricked whole blood or whole blood with anti-coagulant. It is intended for professional use as a clinical diagnostic test for diagnosing dengue infected patients.

INTRODUCTION

Dengue fever, a breakbone fever, is a mosquito-borne tropical disease caused by the dengue virus, transmitted by several species of mosquito within the genus Aedes, mainly A.argypti. There are four different serotypes which all cause clinical disease. Dengue virus is now circulating in Asia, Africa and Americas, a dramatically different scenario from that which prevailed 20 or 30 years ago [1, 3]. It is estimated that more than 2.5 billion people live in dengue endemic countries are at risk of infection [1, 2].

In general, diagnostic of dengue is dependent on the phase of the infection, current dengue diagnostics are based on either detection of viral agent (antigen/genome) or antibodies (IgA/ IgM/IgG) produced against it [3, 9]. Dengue nonstructural protein 1 (NS1), produced in both membrane-associated and secreted forms, has been utilized as an early marker due to its presence in the serum of patients during the viraemic phase of infection. NS1 circulates in the serum from 1 to 9 days after the onset of clinical signs, with a peak from 3 to 5 days [4, 5]. The host immune response to dengue infection consists of the production of antibodies that are primarily directed against the dengue virus envelope proteins [3]. Understanding the features of host humoral immune response is important for the interpretation of dengue infection [6]. A primary antibody response is observed in individuals who are not immune to dengue and a secondary immune response is observed in patients who have had a previous dengue infection. In primary infection, IgM antibodies develop by 3-10 days after the onset of infection and reach its peak level ~2 weeks later. IgM generally decline to undetectable levels over the next 2-3 months [7, 8]. After the end of first week of infection, IgG is detectable at low titre and slowly increases, and persists for life. By contrast, during a secondary infection, high level of IgG antibodies is detectable even in the acute phase and rapidly rises over the following 2 weeks [9]. IgM antibodies may be detectable after 20 days of infection and are usually lower than in primary infections. IgA typically appears a day after IgM at a low level and decreases to undetectable level within 45 days following detection [10, 11, 12].

MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test is a reverse-flow immunochromatographic test and uses immobilized goat anti-human IgA, mouse anti-human IgM and IgG antibodies; and anti-dengue NS1 monoclonal antibodies for capturing IgA, IgM, IgG antibodies and NS1 dengue antigen in the human serum, plasma, finger pricked whole blood or whole blood with anti-coagulants. The presence of IgA, IgM, IgG antibodies against dengue virus and NS1 dengue antigen are detected by the colloidal gold-labeled dengue recombinant proteins and anti-dengue NS1 monoclonal antibody.

CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The **MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test** kit is a direct solid-phase immunochromatographic assay, based on MP Biomedicals' proprietary Reverse Flow technology (US Patent No.: 6,316,205) for simultaneous and differential detection of dengue antibodies and antigen in human serum, plasma, finger pricked whole blood or whole blood with anti-coagulants.

Goat anti-human IgA, mouse anti-human IgM and IgG; and anti-dengue NS1 monoclonal antibodies are striped on the membrane as 4 separate test lines.

The dengue antibodies and/ or antigen in the test sample (serum, plasma, finger pricked whole blood or whole blood with anti-coagulants) form antibody-antigen complexes with anti-human antibodies and dengue NS1 monoclonal antibodies immobilized on nitrocellulose membrane in the test viewing window as the test sample migrates upward from the sample well.

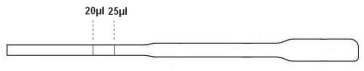
The bound antibody-antigen complexes are subsequently detected by dengue antigens and dengue NS1 monoclonal antibody conjugates carried by chase buffer that flows downward giving a pink-purplish color. In addition, immobilized Biotinylated-BSA which can be recognized by colloidal gold-labeled goat anti-biotin is used as a control for proper function of the reagents.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on MP Diagnostics' products and packaging. These symbols are the most common ones appearing on medical devices and their packaging.

	Use by	IVD	*In vitro* diagnostic medical device
LOT	Batch Code *Synonyms for this are:* *Lot Number* *Batch Number*	REF	Catalogue Number *Synonyms for this are:* *Reference Number* *Reorder Number*
			Caution
	Temperature Limitation		Consult Instructions for Use
	Manufacturer		Do not reuse
Σ	Contains sufficient for <n> tests	EC REP	Authorized representative in the European Community

KIT COMPONENTS

DEVICE	MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test Device Incorporated with anti-human and dengue NS1 monoclonal antibodies. Packed in individually sealed pouch with a desiccant. Store at 2°C - 28°C	20 devices
APPLICATOR	SAMPLE APPLICATOR With marking of 20µl and 25µl.	20 pieces
		
BUFFER	CHASE BUFFER Contains sodium azide as preservative. Store at 2°C - 28°C	1 bottle (5 ml)
	INSTRUCTIONS FOR USE	1 copy

HEALTH AND SAFETY INFORMATION

1. In case of an accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
3. Wipe any spills of sera, plasma or blood promptly with 1% sodium hypochlorite solution.
4. Autoclave all used and contaminated materials at 121°C, 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.

Pursuant to EC regulation 1272/2008 (CLP), hazardous components as classified and labelled as follows:

Component:	**Chase Buffer**
Signal Word:	**Warning**
Pictogram:	
Hazard Statements:	H319 Causes serious eye irritation.
Precautionary Statements:	P264 Wash hands thoroughly after handling. P280 Wear protective gloves/protective clothing/ eye protection/ face protection.
Supplemental Statements:	EUH 210 Safety Data Sheet is available on request
Contains:	1% Triton X-100

ANALYTICAL PRECAUTIONS:

1. The sample applicator is for single use only. DO NOT re-use the sample applicator.
2. Each sealed test device is for single use only. DO NOT re-use the test device.
3. For *in vitro* diagnostic use only.
4. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when samples are assayed.
5. Handle all samples as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
6. Optimal assay performance requires STRICT ADHERENCE to the assay procedure described in this Instruction For Use. Deviations from the procedure may lead to aberrant results.
7. Do not interchange reagents between kit lots.
8. Do not use kit components beyond the expiry date printed on the kit box.
9. The chase buffer reagent contains sodium azide as a preservative. Handle reagent carefully to avoid spills on work surface. Clean up spills with absorbent paper and water.
10. Reagents supplied should not present a hazard to health if used in accordance to the instructions stated.
11. For best results, allow all reagents and samples to reach room temperature (25°C ± 3°C) before use.
12. Humidity and temperature can adversely affect results.
13. DO NOT use the test device if the seal of the pouch is broken.
14. DO NOT use the test device if the pull tab is not fully inserted, i.e. pre-pulled.

STORAGE

1. Store the kit and its components at 2°C - 28°C.
2. Do not freeze the kit and its components.
3. The test device must remain in the sealed pouch until use.
4. Do not use after the expiry date printed on the pouch.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Timer
2. Lancet
3. Alcohol swabs

SPECIMEN COLLECTION

Whole blood can be collected in anti-coagulant containing tubes and used as outlined in the assay procedure immediately or stored at 2°C to 8°C for not more than 72 hours before use.

The presence of 6 anti-coagulants, Acid Citrate Dextrose (ACD), Citrate-phosphate-dextrose (CPD), Ethylenediaminetetraacetic acid (EDTA), Potassium Oxalate (K-Oxalate), Lithium Heparin (Li Hep) and Sodium Citrate (Na Citrate) were found to have no effects on the performance of MULTISURE® Dengue Ab/ Ag Rapid Test.

Serum / plasma samples should be stored at 2°C to 8°C if the test is to be run within 7 days of collection or frozen at -20°C or colder if the test is to be delayed for more than 7 days. Clear, non-hemolyzed samples are preferred. Lipemic, icteric or contaminated (particulate or bacterial) samples should be filtered (0.45µm) or centrifuged before testing.

Sample can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follow:
1. Loosen cap of sample container.
2. Heat-inactivate sample at 56°C for 30 minutes in a water bath.
3. Allow sample to cool down before retightening cap.
4. Sample can be stored frozen until analysis.

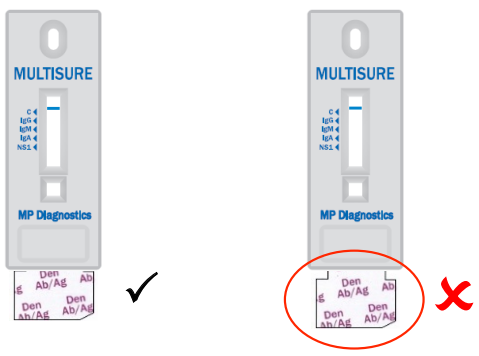
Repeated freeze-thawing of the sample is not recommended. Do not use specimens (serum & plasma) with more than five (5) freeze-thaw cycles.

ASSAY PROCEDURE

IMPORTANT: Strict adherence to the assay procedure will ensure optimal performance. Deviations from the procedure may lead to aberrant results.

Note :
- Allow the kit to warm to room temperature (25°C ± 3°C) before running the assay.
- For best results, conduct the assay at room temperature (25°C ± 3°C).

1. Open the pouch by tearing at the tear notch on the right side of the pouch to avoid accidental pulling of pull tab.
2. Discard the device if pull tab is not fully inserted into test device. Refer to diagrams below.



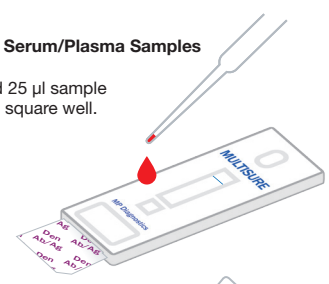
3. Conduct the test immediately after removing the test device from the pouch.
4. Label the test device with the sample name.
5. Proceed with the assay procedures as shown in the diagrams below.

Assay Procedures for MULTISURE® Dengue Ab/Ag Rapid Test

1

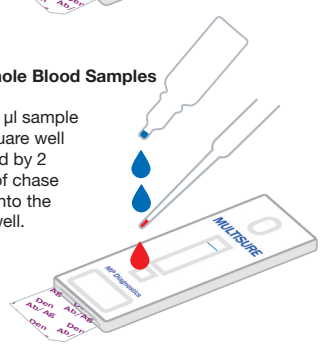
For Serum/Plasma Samples

Add 25 µl sample into square well.



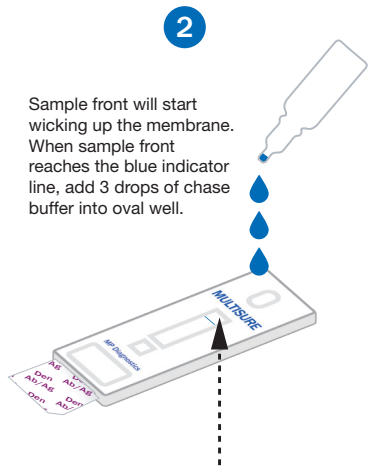
For Whole Blood Samples

Add 20 µl sample into square well followed by 2 drops of chase buffer into the same well.



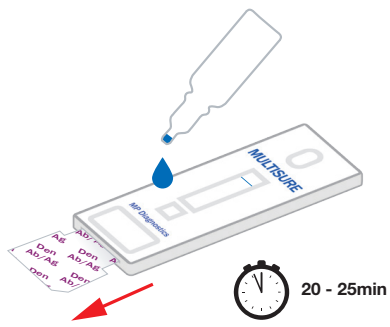
2

Sample front will start wicking up the membrane. When sample front reaches the blue indicator line, add 3 drops of chase buffer into oval well.



(Caution: Do not allow sample front to flow out of test viewing window.)

3



- Pull out the "Den Ab/Ag" pull tab until resistance is felt.
- Add 1 drop of chase buffer into square well.
- Start timer. Read results between 20 and 25 minutes. (Note: Background of test line area should be clear at time of reading.)
- Do not read results after 25 min.

Example of test device with all visible test line

QUALITY CONTROL

The control line is an internal procedure control. Absence of control line indicates incorrect assay technique used or faulty device. The assay procedure should be reviewed before repeating with a new device.

Positive and negative controls are not provided with this kit. However, it is recommended that controls are used as good laboratory practice to verify proper assay technique and performance.

INTERPRETATION OF RESULTS

1. **Positive for Dengue**

   A test is dengue positive if Control Line (C) appears with any visible test lines (IgG, IgM, IgA, NS1).

   - Presence of IgG indicates secondary or past infection.
   - Presence of IgM and/or IgA indicates current infection.
   - Presence of NS1 indicates acute dengue infection.

2. **Negative for Dengue**

   A test is negative if Control line (C) appears with no visible test line(s). Retest in 3-5 days if dengue infection is suspected.

3. **Invalid**

   A test is invalid if Control Line (C) is absent. The assay should be repeated using a new device.

LIMITATIONS OF THE PROCEDURE

The **MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test** is a qualitative *in vitro* immunochromatographic test to detect and differentiate IgA/ IgM/ IgG antibodies against dengue virus and NS1 dengue antigen in serum, plasma, finger pricked whole blood or whole blood with anti-coagulants. This test is not for determining the quantitative value in dengue antibodies/ antigen and in the diagnosis or prognosis of disease.

Optimal assay performance requires the strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results. A **POSITIVE** result may indicate infection with dengue antibodies and/ or dengue antigen. The positive results should be further confirmed by more specific supplemental tests. A **NEGATIVE** result does not exclude the possibility of infection with dengue.

PERFORMANCE CHARACTERISTICS

Total Diagnostic Performance (In-house study)

Diagnostic Parameter	Performance of MULTISURE® Dengue Ab/Ag Rapid Test	95% Confidence Interval
Sensitivity (n=291)	94.16%	90.81% to 96.56%
Specificity (n=400)	94.75%	92.09% to 96.72%
Positive Predictive Value (PPV)	92.88%	89.32% to 95.54%
Negative Predictive Value (NPV)	95.71%	93.22% to 97.48%

PPV and NPV values may vary according to prevalence rate in different regions and time.

Diagnostic Sensitivity (In-house study)

Sensitivity performance of individual markers

Markers	Reference Commercial ELISAs and/ or Rapid Tests (n=291)	MULTISURE® Dengue Ab/Ag Rapid Test (n=291)	
	Total^	Positive	Sensitivity
IgG	156	136	87.18%
IgM	224	206	91.96%
IgA	206	114	55.34%
NS1	101	84	83.17%
Overall	**291**	**274**	**94.16%**

^ Total number of samples positive with respective marker defined by reference methods.

Diagnostic Specificity (In-house study)

Specificity performance of individual markers

Method used	Reference assay: commercial ELISAs (n=400)#		
	Markers	Negative	Specificity
MULTISURE® Dengue Ab/ Ag Rapid Test	IgG	393	98.25%
	IgM	386	96.50%
	IgA	398	99.50%
	NS1	400	100.00%
	All markers	379	94.75%

Dengue negative samples are defined by Commercial dengue IgG, IgM and IgA ELISAs.

Specificity performance in various populations

Sample Category	Total Sample Size	Diagnostic Specificity of MULTISURE® Dengue Ab/Ag Rapid Test
Blood Donors / Healthy Donors	153	96.73% (148/153)
Hospitalized / Clinical	91	94.51% (86/91)
Cross-reactive	62	96.77% (60/62)
Interference	50	98.00% (49/50)
Pregnancy	44	81.82% (36/44)
Total	**400**	**94.75% (379/400)**

Specificity performance in potential cross-reactive specimens

Sample Profile	Total Sample Size	Performance of MULTISURE® Dengue Ab/Ag Rapid Test
Rheumatoid Factor (RF)	10	100.00% (10/10)
Hepatitis A	10	100.00% (10/10)
HBsAg	3	100.00% (03/03)
Hepatitis C	6	100.00% (06/06)
Measles IgG	9	100.00% (09/09)
Human T-lymphotropic Virus Type I (HTLV-I)	10	90.00% (09/10)
HIV 1/2	9	88.89% (08/09)
Hepatitis E	5	100.00% (05/05)
Total	**62**	**96.77% (60/62)**

Specificity performance in potential interference specimens

Sample Profile	Total Sample Size	Performance of MULTISURE® Dengue Ab/Ag Rapid Test
Icteric	8	100.00% (08/08)
Hemolyzed	8	100.00% (08/08)
Triglyceride	8	100.00% (08/08)
Lipemic	6	100.00% (06/06)
Total Protein	4	100.00% (04/04)
Total bilirubin	7	100.00% (07/07)
Antinuclear Antibody (ANA)	9	88.89% (08/09)
Total	**50**	**98.00% (49/50)**

External evaluation study

Sensitivity performance on different types of dengue infection

Dengue Type	MULTISURE® Dengue Ab/Ag Rapid Test (n=80)			
	Positive	Negative	Total	Sensitivity
DENV-1	17	3	20	85.0%
DENV-2	16	4	20	80.0%
DENV-3	20	0	20	100.0%
DENV-4	19	1	20	95.0%
Total	**72**	**8**	**80**	**90.0%**

All specimens were confirmed by PCR test.

Specificity performance on cross-reactivity test with other mosquito-borne diseases

Sample Profile	Total Sample size	Specificity
Chikungunya	5	100.0% (5/5)
Japanese Encephalitis	9	88.9% (8/9)
Total	**14**	**92.9% (13/14)**

Cross-reactivity with other flavivirus mediated and mosquito-borne diseases are known to be common but have not been tested.

Analytical Sensitivity

Analytical sensitivity for MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test was found to be comparable to a leading commercial dengue rapid test for antibodies and NS1 antigen detection.

Precision

The inter-assay (between-run) and intra-assay (within-run, within-day and day-to-day) reproducibility of MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test have been evaluated using a set of control panel members. All results obtained were consistently fall within the acceptance criteria, indicating the MP Diagnostics MULTISURE® Dengue Ab/Ag Rapid Test is robust, reproducible and consistent across three lots studied.

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LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as an *in vitro* diagnostic assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be a technical problem / complaint, please do the following:

1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

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