

Not for Sale or Distribution in the United States of America

Dengue Duo Cassette

Catalogue No. R-DEN03D

INTENDED USE

The Panbio Dengue Duo Cassette is for the qualitative presumptive detection of IgM and IgG antibodies to dengue virus in human serum, plasma and whole blood. The assay can be used for the presumptive differentiation between primary and secondary infection. This test should only be used for patients with signs and symptoms that are consistent with dengue virus infection. Positive results are presumptive and must be confirmed by virus isolation, paired serum analysis, antigen detection by immunohistochemistry or viral nucleic acid detection for confirmation of dengue virus infection.

INTRODUCTION

Dengue, a flavivirus, is found in large areas of the tropics and subtropics. Transmission is by mosquito, principally *Aedes aegypti* and *Aedes albopictus*. Dengue virus infection causes a spectrum of clinical manifestations ranging from asymptomatic to fatal haemorrhagic disease. Classic dengue is characterised by the sudden onset of fever, intense headache, myalgia, arthralgia and rash. A biphasic febrile course is common, as is insomnia and anorexia with bitter or loss of taste. Dengue haemorrhagic fever and dengue shock syndrome are severe complications often associated with secondary dengue infection.

In endemic regions, patients diagnosed with dengue fever generally have secondary infection¹. Consequently, detection of antibodies to dengue is a valuable procedure, particularly in second and subsequent infections where the occurrence of complications is high. Traditionally, haemagglutination-inhibition (HAI) titers have been used to classify infections as primary or secondary. The current definition depends on an assay of paired serum specimens separated in time by at least 7 days, although any acute specimen with an HAI titer $\geq 1:1280$ is defined as coming from a patient with secondary flavivirus infection².

In the Panbio Dengue Duo Cassette, IgM and IgG are determined simultaneously using a single addition of serum, plasma or whole blood. Therefore, differentiation between primary and secondary infection can be made through a single application of serum, plasma or whole blood rather than a series of dilutions as needed in the HAI assay. In primary infections, serum IgM antibodies can be detected from dengue patients as early as 3-5 days after the onset of fever, generally persisting for 30-90 days, although detectable levels may be present for 8 months post-infection³.

Secondary infection is characterised by high IgG levels that may or may not be accompanied by elevated IgM levels. The sensitivity of this assay has been set so that in patients with primary dengue, IgM is positive while IgG is negative. In contrast, patients with secondary infections will have a positive IgG result with or without a positive IgM result. Serological cross-reactivity across the flavivirus group is common (i.e. between dengue 1, 2, 3 & 4, St. Louis encephalitis, West Nile, Japanese encephalitis and yellow fever viruses)^{4,5}.

PRINCIPLE

When present in the patient sample, dengue-specific IgM or IgG antibodies bind to anti-human IgM or IgG antibodies immobilized in two lines across the cassette membrane. Colloidal gold complexes containing recombinant dengue 1-4 antigens are captured by the bound patient's IgM or IgG to give visible pink line(s). A procedural control is included to indicate that the assay has been performed correctly.

PRECAUTIONS

1. All human blood products should be handled as potentially infectious material. The Centers for Disease Control and the National Institutes of Health recommend that potentially infectious agents be handled at Biosafety Level 2⁶.
2. Never pipette by mouth or allow reagents or patient sample to come into contact with skin.
3. Optimal results will be obtained by strict adherence to this protocol. Reagents must be added carefully to maintain precision and accuracy.
4. Performing the assay outside the prescribed time and temperature ranges may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
5. The components in this kit have been quality control tested as a master lot unit. Do not mix components from different lot numbers. Do not mix with components from other manufacturers.
6. Care should be exercised to protect the reagents in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.
7. Do not heat-inactivate samples.
8. Keep storage boxes dry.
9. Do not reuse test cassettes.
10. Do not use test cassettes if foil pouch is punctured or damaged.
11. Testing materials should be disposed of in accordance with local, state and/or federal regulations.

STORAGE AND SHELF LIFE OF REAGENTS

1. Store kit between 2 and 30°C. Constant storage temperature must be maintained for the reagents to be stable until the expiry date of the kit. Refer to package label for expiry date.
2. Do not freeze kit components.
3. The test kit may be used until the expiry date marked on the package label.
4. Do not use reagents beyond the expiry date.

SPECIMEN COLLECTION AND PREPARATION

1. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) and then centrifuged according to the National Committee for Clinical Laboratory Standards (NCCLS), (Approved Standard - Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, H3-A4, 1998). The serum should be separated as soon as possible and refrigerated at 2-8°C or stored frozen at -20°C or colder if not tested within two days. The use of icteric sera or sera exhibiting haemolysis, lipaemia or microbial growth is not recommended.
2. Self-defrosting freezers are not recommended for storage as they may cause the specimens to go through freeze-thaw cycles and degrade the antibodies, thus yielding spurious results.
3. Fingertip blood should be tested immediately after the sample is taken. The NCCLS recommends that whole blood containing EDTA or heparin as anticoagulant may be used immediately without centrifugation or may be stored at 2-8°C for up to 72 hours (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A2, 1999).
4. Test accuracy is dependent on the time of sample collection following the onset of fever. Optimal results are obtained on samples collected between 6 and 14 days following the onset of fever.

KIT COMPONENTS

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

- 25 x Pouches. *Each pouch contains one test cassette and one MicroSafe[®] 10 μ L pipette.*
- 1 x Instructions for Use.
- 1 x 3 mL Bottle of Buffer (contains 0.1% Proclin)

MATERIALS REQUIRED BUT NOT SUPPLIED

- Timer

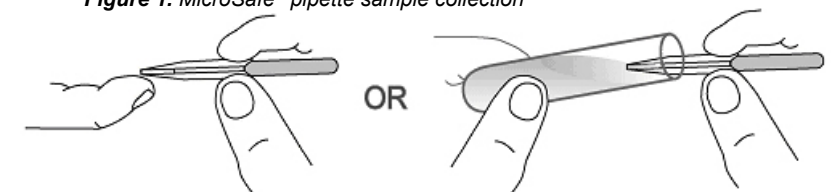
GENERAL PROCEDURE

MicroSafe[®] Pipette Operation:

*The MicroSafe[®] pipette can be used with whole blood, plasma or serum. Please **strictly** adhere to the following instructions (Figure 1) when pipetting:*

1. Hold the pipette **horizontally**.
2. To collect, touch the test sample with the tip of the pipette (see Figure 1, below).
3. To expel the sample, gently squeeze the bulb.

Figure 1. MicroSafe[®] pipette sample collection



CAUTION! Please do NOT squeeze the bulb when collecting; filling is automatic.

ASSAY PROCEDURE:

NOTE: Ensure all reagents are equilibrated to room temperature (20-25°C) before commencing the assay.

Remove the cassette and MicroSafe® pipette from the pouch just prior to use.

1



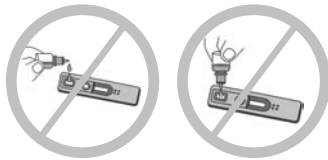
- Add 10 µL of whole blood, serum or plasma to the circular well using a micropipette or the MicroSafe® pipette provided.
- Allow the sample to absorb entirely into the specimen pad within the circular well.

2



- Hold the buffer bottle vertically and 1 cm above the square well.
- Add 2 drops of buffer to the square well at the base of the cassette.

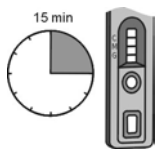
Incorrect Use



Do not hold bottle horizontally when dispensing.

Do not allow bottle to touch sample well when dispensing.

3



- Read the result exactly 15 minutes after adding the buffer to the cassette.
- Any trace of a pink line in the test area indicates a positive result.
- Any results read outside 15 minutes should be considered **invalid** and must be repeated.

QUALITY CONTROL

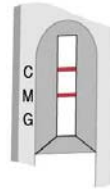
1. The test is invalid and should be repeated if the control line does not appear. If the test is invalid, patient results cannot be reported.
2. Whole blood samples may cause a red background to appear in the viewing window. If this is not masking the test line, the result remains valid.
3. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard QC procedures. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1202(c) for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

Primary dengue is characterised by the presence of detectable IgM antibodies 3-5 days after the onset of infection. Secondary dengue is characterised by elevation of specific IgG antibodies 1-2 days after the onset of infection, often accompanied by an elevation of IgM.

Interpretation should be based on the combined results of the IgG and IgM test lines. Individual line analysis may be inaccurate.

C: Control line M: IgM test line G: IgG test line



Primary Infection

Pink bands appear in the IgM and Control regions.

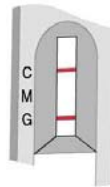
The test is positive for IgM antibodies and is suggestive of primary dengue infection.



Secondary Infection

Pink bands appear in the IgM, IgG and Control regions.

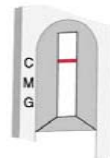
The test is positive for IgM and IgG antibodies and is suggestive of a secondary dengue infection.



Secondary Infection

Pink bands appear in the IgG and Control regions.

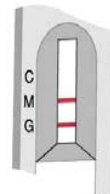
The test is positive for IgG antibodies and is suggestive of secondary dengue infection.



Negative

A pink band appears in the Control region only.

No detectable IgG and IgM antibodies to dengue. The result does not exclude dengue infection. Retest in 3-4 days if dengue infection is suspected.



Invalid

No pink band appears in the Control region.

The test is invalid and should be repeated.

TEST LIMITATIONS

1. The analysis of a single test sample should not be used as the sole criterion for diagnosis.
2. In early infections and some secondary infections, detectable levels of IgM antibodies may be low. Some patients may not produce detectable levels of antibody within the first seven to ten days after infection. Where symptoms persist, patients should be re-tested 3-4 days after the first specimen.
3. Serological cross-reactivity across the flavivirus group is common (i.e. between dengue 1, 2, 3 & 4, St. Louis encephalitis, West Nile, Japanese encephalitis and yellow fever viruses)^{4,5}.
4. The final diagnosis should be based on test results in conjunction with other clinical and laboratory findings.
5. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of the virus being present. Testing should only be performed on patients with clinical symptoms or when exposure is suspected.
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.
8. Optimal results are obtained on samples collected 6-14 days following the onset of fever.

PERFORMANCE EVALUATION DATA

SENSITIVITY AND SPECIFICITY

Studies were conducted in-house and at independent trial sites in Malaysia and Sri Lanka to assess the sensitivity and specificity of the Panbio Dengue Duo Cassette using serum, plasma and whole blood.

Serum

Samples from patients with clinically suspected cases of dengue infection were selected retrospectively from a bank of frozen sera collected after hospital admission from a trial site in Malaysia. A panel consisting of 30 paired sera from patients with primary dengue, 30 paired sera from patients with secondary dengue and 80 sera from patients negative for dengue on laboratory testing were assessed. Dengue diagnosis was based on the in-house IgM ELISA and the haemagglutination-inhibition (HAI) assay using a titre $\geq 1:2560$ to define a secondary infection. Results are summarised in Table 1.

Plasma

A clinical study was conducted at Panbio Ltd to assess the performance of the Panbio Dengue Duo Cassette using 208 plasma samples. The panel consisted of 208 plasma samples comprised of 93 seronegative, 32 primary and 83 secondary samples characterised by the Panbio Dengue Duo IgM Capture and IgG Capture ELISA (E-DEN01D). Results are summarised in Table 2.

Whole Blood

A research institute in Sri Lanka tested 331 whole blood and serum samples collected from patients who had presented to hospital with signs and symptoms that are consistent with dengue virus infection. The panel was comprised of 74 seronegative, 77 primary and 180 secondary samples. The Panbio Dengue Duo Cassette was tested on finger-prick blood collected with the MicroSafe[®] pipette provided in the kit. Serum was obtained by venous collection and characterised by the Panbio Dengue Duo IgM Capture and IgG Capture ELISA (E-DEN01D). Results were compared to dengue serological status to determine the sensitivity, specificity and agreement of the assay for whole blood samples. Results are summarised in Table 3.

**Table 1: Panbio Dengue Duo Cassette
Summary of Sensitivity & Specificity (Serum)
Study Site - Malaysia**

Specimen Characterisation (HAI & in-house IgM ELISA)	Panbio Dengue Duo Cassette				
	Seronegative	Primary Infection	Secondary Infection	Total	
Seronegative	76	4	0	80	
Primary	S1 ^a	0	18	0	18 ^a
	S2	0	16	14	30
Secondary	S1	3	3	24	30
	S2	1	1	28	30
Total	80	42	66	188	

S1= 1st bleed S2= 2nd bleed

^a Adjusted sample numbers following the removal of 12 acute sera with HAI titres for Den-2 and Den-3 ≤ 10 .

Relative Serological Sensitivity ^a	=	104/108	96.3%	95% CI*	90.8 - 99.0%
Relative Serological Specificity	=	76/80	95.0%		87.7 - 98.6%
Relative Serological Agreement	=	162/188	86.2%		81.2 - 91.1%

*CI = Confidence Interval

**Table 2: Panbio Dengue Duo Cassette
Summary of Sensitivity & Specificity (Plasma)
Study Site - Australia**

Specimen Characterisation (Panbio Dengue IgM & IgG Capture ELISAs)	Panbio Dengue Duo Cassette			
	Seronegative	Primary Infection	Secondary Infection	Total
Seronegative	77	3	13	93
Primary infection	2	21	9	32
Secondary infection	3	0	80	83
Total	82	24	102	208

Relative Serological Sensitivity	=	110/115	95.7%	95% CI*	92.9 - 98.4%
Relative Serological Specificity	=	77/93	82.8%		77.7 - 87.9%
Relative Serological Agreement	=	178/208	85.6%		80.8 - 90.4%

*CI = Confidence Interval

**Table 3: Panbio Dengue Duo Cassette
Summary of Sensitivity & Specificity (Whole Blood)
Study Site - Sri Lanka**

Specimen Characterisation (Panbio Dengue IgM & IgG Capture ELISAs)	Panbio Dengue Duo Cassette			
	Seronegative	Primary infection	Secondary infection	Total
Seronegative	66	6	2	74
Primary infection	15	52	10	77
Secondary infection	11	13	156	180
Total	92	71	168	331

Relative Serological Sensitivity	=	231/257	89.9%	95% CI*	86.2 - 93.6%
Relative Serological Specificity	=	66/74	89.2%		82.1 - 96.3%
Relative Serological Agreement	=	274/331	82.8%		78.7 - 86.8%

*CI = Confidence Interval

CROSS-REACTIVITY

A panel of 208 sera from patients confirmed with diseases other than dengue fever was tested to establish the analytical specificity of the Panbio Dengue Duo Cassette. A panel of serum from subjects with no evidence of infection was also tested. Refer to Table 4 for a summary of the results.

**Table 4: Cross-reactivity Study -
Panbio Dengue Duo Cassette**

Disease State	No. IgM Negative / Total	No. IgG Negative / Total	No. IgM or IgG Negative / Total
No evidence of infection	48/50	50/50	48/50
West Nile Virus	8/9	9/9	8/9
Japanese Encephalitis	28/30	29/30	27/30
Ross River Virus	10/10	10/10	10/10
Malaria	10/10	10/10	10/10
Leptospirosis	8/10	10/10	8/10
Epstein-Barr virus	18/20	20/20	18/20
Cytomegalovirus	8/10	10/10	8/10
Hepatitis A	10/12	11/12	10/12
Hepatitis B	10/11	11/11	10/11
Hepatitis C	11/11	11/11	11/11
Rheumatoid factor	12/15	15/15	12/15
Anti Nuclear Antibody	10/10	10/10	10/10
Total	191/208 (91.8%)	206/208 (99.0%)	190/208 (91.4%)

REPRODUCIBILITY

The reproducibility of the Panbio Dengue Duo Cassette was determined by testing nine sera on three batches on three different days. The nine sera were representative of primary dengue, secondary dengue and no dengue infection, and the test results were compared to the reference results. The intra- and inter-batch test results were read at 15 minutes and are summarised in Table 5.

**Table 5: Intra- and Inter-batch Reproducibility -
Panbio Dengue Duo Cassette**

P = Primary S = Secondary N = Negative

Serum Panel	Reference Diagnosis	R-DEN03D-04097			R-DEN03D-04098			R-DEN03D-04099		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
CDEN 08	P	P	P	P	P	P	P	P	P	P
CDEN 11	P	P	P	P	P	P	P	P	P	P
CDEN 12	P	P	P	P	P	P	P	P	P	P
CDEN 01	S	S	S	S	S	S	S	S	S	S
CDEN 05	S	S	S	S	S	S	S	S	S	S
CDEN 07	S	S	S	S	S	S	S	S	S	S
CDEN 18	N	N	N	N	N	N	N	N	N	N
CDEN 19	N	N	N	N	N	N	N	N	N	N
CDEN 20	N	N	N	N	N	N	N	N	N	N

Primary diagnosis is based on an IgM positive and IgG negative reading. Secondary diagnosis is based on an IgG positive and IgM positive or negative reading. All control lines were positive.

TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	SOLUTION
Sample does not flow along the viewing window	Insufficient buffer	<ul style="list-style-type: none"> ➤ Ensure bottle is vertical when adding drops and not in contact with square well. ➤ When dispensing, hold bottle 1 cm above square well.
Blood obscuring test window	Blood not absorbed into specimen pad in circular well	<ul style="list-style-type: none"> ➤ Ensure sample is absorbed entirely into material prior to buffer addition. This can take up to 30 seconds for blood samples.
No control line	Insufficient buffer	<ul style="list-style-type: none"> ➤ Repeat test.
MicroSafe® pipette not functioning	Squeezing bulb when filling Incorrect angle of pipette when filling	<ul style="list-style-type: none"> ➤ Do not squeeze bulb when filling. Only squeeze the bulb to expel the sample. ➤ Hold pipette horizontally when filling.

BIBLIOGRAPHY

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2. Lam SK, Lum LCS. (1998). Rapid dengue diagnosis: a prospective study using a commercial rapid test. *In Dengue Bulletin* Vol. 22. World Health Organization Regional Office for South-East Asia and Western Pacific Regions.
3. Ruechusatsawat K, Morita K, Tanaka M, Vongcheree S, Rojanasuphot P, Warachit P, Kanai P, Thongtradol P, Nimnakorn P, Kanungkid S, Igarashi A. (1994). Daily observation of antibody levels among dengue patients detected by enzyme-linked immunosorbent assay (ELISA). *Japanese J. Trop. Med. Hygiene* **22**:9-12.
4. Innis BL, Nisalak A, Nammanitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH. (1989). An enzyme-linked immunosorbent assay to characterise dengue infections where dengue and Japanese encephalitis co-circulate. *Am. J. Trop. Med. Hygiene* **40**: 418-427.
5. Makino Y, Tadano M, Saito M, Maneeakam N, Sittisombut N, Sirisanthana V, Poneprasert B, and Fukunaga T. (1994). Studies on serological cross-reaction in sequential flavivirus infections. *Microbiol. Immunol.* **38**: 951-955.
6. Centers for Disease Control and Prevention and National Institutes of Health. (1999). *Guidelines: Biosafety in Microbiological and Biomedical Laboratories 4th Edition*. U.S. G.P.O. Washington.

GLOSSARY OF SYMBOLS:



Manufactured by



Authorised Representative



Catalogue Number / Reference Number



For *In Vitro* Diagnostic Use Only



Batch Code / Lot Number



Store between 2°C and 30°C



Use By / Expiry Date



Sufficient for X Tests



CE marking according to IVD Medical Devices Directive 98/79/EC



Attention!



See Instructions for Use



Cassette and Collection Pipette



Buffer



Authorised Representative:

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