



ImmunoComb® II

Dengue IgM & IgG BiSpot



Code: 60483002

Format: 3 x 12 tests

For In vitro Diagnostic Use only

INTENDED USE

The **ImmunoComb® II Dengue IgM&IgG BiSpot Kit** is a test intended for the qualitative simultaneous detection of IgM and IgG antibodies to **Dengue virus** in human serum or plasma and for the differentiation between primary and secondary infection. Thirty-six tests may be performed with one kit.

INTRODUCTION

Dengue fever (DF), a mosquito-borne infection, is a major global epidemiological and public health problem affecting tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. Over half of the world population is under the risk of Dengue infection. Dengue is an arbovirus belongs to the genus *Flavivirus*. Four distinct serotypes of the virus, closely related antigenically, have been defined, Dengue-1, -2, -3, and -4. The virus is transmitted by mosquito vectors, primarily *Aedes aegypti* and *Aedes albopictus*.

The Dengue fever form of the illness, commonly observed in primary infection, is characterized by acute onset of high fever, frontal headache, retro-orbital pain and myalgias. This phase is frequently presenting with rash, bone or joint and muscular pain, nausea, vomiting and lymphadenopathy. The acute illness may last up to one week, with prolonged convalescence for several weeks. Secondary infection is often associated with the haemorrhagic form of the disease (DHF). DHF is commonly observed in children, but also occurs in adults. It is characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, often with hepatomegaly and in severe cases, circulatory failure signs.

In more severe cases these patients may develop hypovolaemic shock resulting from plasma leakage. This is known as Dengue shock syndrome (DSS), which could be rapidly fatal.

The immune response during the primary Dengue infection is characterized by a rise of IgM antibodies by the 5th day of the onset of symptoms and may persist as long as three months. IgG antibodies appear after two weeks of infection and persist for many years. Secondary infection immune response is characterized by rise of IgG within 1-2 day after onset of symptoms, whereas IgM antibodies are induced 20 days later.

Serological methods have been developed to assess laboratory confirmation of Dengue infection. The haemagglutination-inhibition (HAI) test has been traditionally used to differentiate between primary and secondary Dengue infection, where the HAI titer 1:2560 is defined as a

cut-off for secondary infection. However, the HAI test suffers from several limitations including difficulty to standardize, sample pretreatment, need for paired serum specimens and inapplicability for primary infection diagnosis.

The utilization of the ImmunoComb® system in simultaneous detection of the presence of Dengue IgM and IgG provides a presumptive diagnosis of Dengue infection, including differentiation between primary and secondary infection from a single serum or plasma specimen.

PRINCIPLE OF THE TEST

The **ImmunoComb® II Dengue IgM/IgG BiSpot** test is a solid-phase enzyme immunoassay (EIA), based on an *immunocapture* principle. The solid phase is a card with 12 projections ("teeth").

Each tooth is sensitized at three positions:

Upper spot – biotinylated bovine serum albumin (Internal Control)

Middle spot – rabbit antibodies to human IgG

Lower spot – rabbit antibodies to human IgM.

The Developing Plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation at each step.

At the outset of the test, serum or plasma specimens are added to the diluent in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Human IgM and IgG will be captured, respectively, by the anti-IgM and by the anti-IgG antibodies on the lower and middle spots on the teeth of the Card (Figure 1). In row B, anti-Dengue IgM and IgG captured on the lower and middle spots of the teeth will react with Dengue antigens. In row C, Dengue antigens captured on the teeth, will react with biotinylated anti-Dengue antibodies. In row D the biotinylated antigen/antibody complex on the lower and middle spots of the teeth, and biotinylated bovine serum albumin on the upper spots (Internal Control), will react with streptavidin labeled with alkaline phosphatase (AP). In row E unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card.

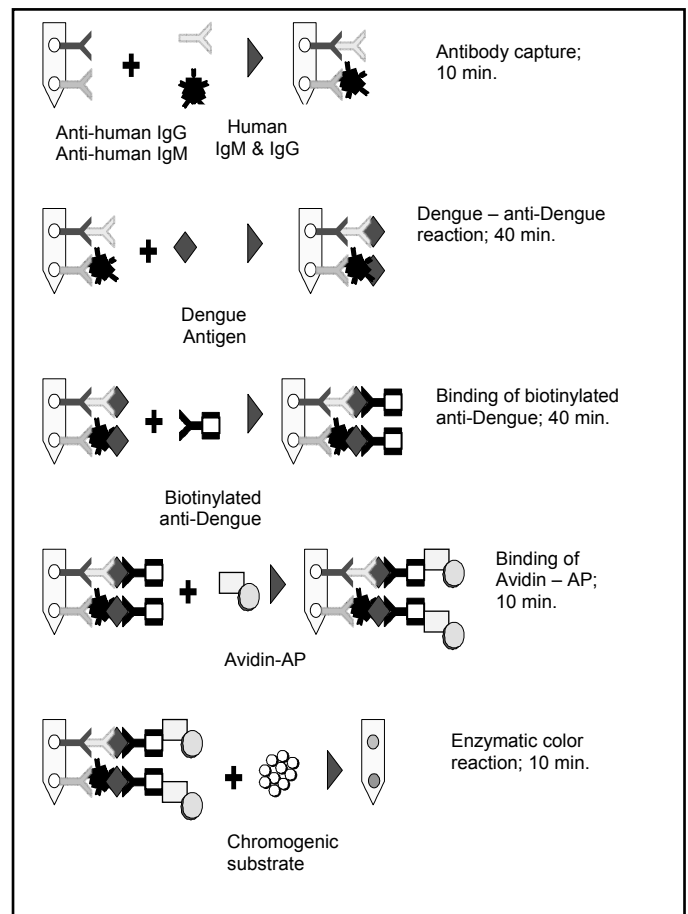


Figure 1. Principle of the Test

The kit includes a Positive Control (anti-Dengue IgM and IgG) and a Negative Control to be included in each assay run. Upon completion of the test, the tooth used with the Positive Control should show three gray-blue spots. The tooth used with the Negative Control should show the upper spot and either no lower or middle spots or faint spots. The

upper spot should also appear on all other teeth, to confirm that the kit functions properly and that the test was performed correctly.

KIT CONTENTS

Cards

The kit contains 3 plastic Cards. Each Card has 12 teeth, one tooth for each test (Figure 2). Each tooth is sensitized with two reactive areas:

Upper spot – biotinylated bovine serum albumin (Internal Control)

Middle spot – rabbit antibodies to human IgG

Lower spot – rabbit antibodies to human IgM.

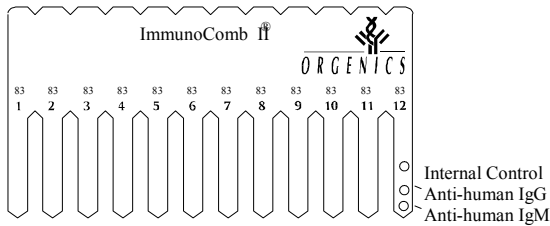


Figure 2. Card

The Cards are provided in aluminum pouches containing a desiccant bag.

Developing Plates

The kit contains 3 Developing Plates covered with aluminum foil. Each Developing Plate (Figure 3) contains all reagents needed for the test. The Developing Plate consists of 6 rows (A–F) of 12 wells each.

The contents of each row are as follows:

Row A	Specimen diluent
Row B	Dengue antigens
Row C	Biotinylated monoclonal antibody to Dengue proteins
Row D	Streptavidin conjugated to alkaline phosphatase
Row E	Washing solution
Row F	Chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT)

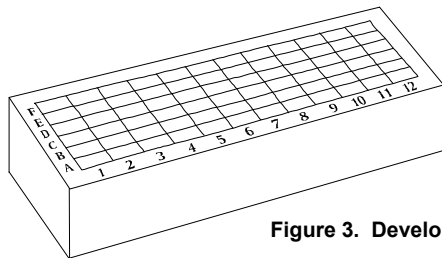


Figure 3. Developing Plate

Positive Control – 1 vial (red-colored cap) of 0.15 ml diluted human heat-inactivated plasma, containing anti-Dengue IgM and IgG antibodies.

Negative Control – 1 vial (green-colored cap) of 0.15 ml diluted heat-inactivated reconstituted human plasma, negative for antibodies to Dengue virus.

CombScale™ – for reading test results

Perforator – for perforation of the aluminum foil, covering the wells of the Developing Plate.

SAFETY AND PRECAUTIONS

- Human source materials used in the preparation of the kit were tested and found to be non-reactive for hepatitis B surface antigen, and for antibodies to HIV and to HCV. Since no test method can give complete assurance of the absence of viral contamination, all reference solutions and all human specimens should be handled as potentially infectious.
- Wear surgical gloves and laboratory clothing. Follow accepted laboratory procedures for working with human serum or plasma.
- Do not pipette by mouth.
- Dispose of all specimens, used Cards*, Developing Plates, and other materials used with the kit as biohazardous waste.
- Do not mix reagents from different lots.
- Do not use kit after the expiry date.

STORAGE AND STABILITY OF THE KIT

- The kit is shipped at 2–8°C. During transport the kit can be kept at ≤ 30°C for short time periods not exceeding a total of 48 hours. The

internal controls indicate that the kit has not been damaged during transport.

- Store the kit in its original box at 2–8°C.
- Do not freeze the kit.
- Following the first opening of the Kit the components have to be stored at 2–8°C.
- Performance of the Kit after the first opening is stable up to the expiry date of the Kit, when stored at 2–8°C.
- After first use, the card and plate cannot be used for more than three times.

Handling of Specimens

- You may test either serum or plasma.
- Specimens may be stored for 7 days at 2–8°C before testing. To store for more than 7 days, freeze specimens at –20°C or colder.
- After serum specimens have thawed, centrifuge them. Test the supernatant. Avoid repeated freezing and thawing.
- Anti coagulants such as heparin, EDTA and sodium citrate were found to have no effect on the test results.

TEST PROCEDURE

Equipment Needed

- Precision pipettes with disposable tips for dispensing 10 µl, 25 µl and 100 µl
- Scissors
- Laboratory timer or watch
- Micro tubes, or microtiter well strips

Preparing the Test

Bring all components, cards, reagents and specimens to room temperature and perform the test at room temperature.

Preparing the Developing Plate

- Incubate the Developing Plate in an incubator at 37°C for 30 minutes; or leave at room temperature (22–26°C) for 3 hours.
- Cover the worktable with absorbent tissue to be discarded as biohazardous waste at the end of the test.
- Mix reagents by inverting the Developing Plate a few times.

Note: Do not remove the foil cover of the Developing Plate. Break the foil cover by using the disposable tip of the pipette or the perforator only when instructed to do so by the Test Instructions.

Preparing the Card

Caution: To ensure proper functioning of the test, do not touch the teeth of the Card.

- Tear the aluminum pouch of the Card at the notched edge. Remove the Card.
- You may use the entire Card and Developing Plate or only a part. To use part of a Card:
 - Determine how many teeth you need for testing the specimens and controls. You need one tooth for each test. Each tooth displays the code number "83" of the kit, to enable identification of detached teeth.
 - Bend and break the Card vertically or cut with scissors (see Figure 4) to detach the required number of teeth (No. of tests including 2 controls).
 - Return the unused portion of the Card to the aluminum pouch (with desiccant bag). **Close pouch tightly**, e.g. with a paper clip, to maintain dryness. Store the Card in the original kit box at 2°–8°C for later use.

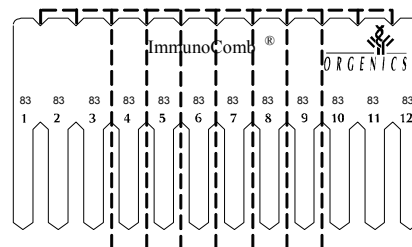


Figure 4. Breaking the Card

TEST INSTRUCTIONS

Antibody Capture (Row A of the Developing Plate)

- Pipette 10 µl of specimen. Perforate the foil cover of one well of row A of the Developing Plate with the pipette tip or perforator and dispense the specimen at the bottom of the well. **Mix** by repeatedly refilling and ejecting the solution. Discard pipette tip.

* Unless stored for documentation

2. Repeat step 1 for the other specimens and the two controls. Use a new well in row A and change pipette tip for each specimen or control.
3.
 - a. Insert the Card (**printed** side facing you) into the wells of row A containing specimens and controls.
Mix: Withdraw and insert the Card in the wells several times.
 - b. Leave the Card in row A and incubate for **10 minutes at room temperature**. Set the timer. Near the end of 10 minutes, perforate the foil of row B using the perforator. Do not open more wells than needed.
 - c. At the end of 10 minutes, take the Card out of row A.
Absorb adhering liquid from the **pointed tips** of the teeth on clean absorbent paper. Do not touch the front surface of the teeth.

Antigen-Antibody reaction (Row B)

4. Insert the Card into the wells of row B. **Mix** as in step 3a. Incubate Developing Plate with Card for 40 minutes (set timer) **at room temperature**. Perforate the foil of row C. After 40 minutes, withdraw the Card and **absorb adhering liquid** as in step 3c.

Binding of Biotinylated anti-Dengue (Row C)

5. Insert the Card into the wells of row C. **Mix** as in step 3a. Incubate Developing Plate with Card for 40 minutes (set timer) **at room temperature**. Perforate the foil of row D. After 40 minutes, withdraw the Card and **absorb adhering liquid**.

Binding of modified avidin/alkaline phosphatase (Row D)

6. Insert the Card into the wells of row D. **Mix**. Incubate Developing Plate with Card for 10 minutes (set timer) **at room temperature**. Perforate the foil of row E. After 10 minutes, withdraw the Card and **absorb adhering liquid**.

First Wash (Row E)

7. Insert the Card into the wells of row E. **Agitate:** Vigorously withdraw and insert the Card in the wells for at least 10 seconds to achieve proper washing. Repeat agitation several times during the course of 2 minutes; meanwhile perforate the foil of row F. After 2 minutes, withdraw the Card and **absorb adhering liquid**.

Color Reaction (Row F)

8. Insert the Card into the wells of row F. **Mix**. Incubate the Developing Plate with the Card for exactly 10 minutes (set timer) **at room temperature**. After 10 minutes, withdraw the Card.

Stop Reaction (Row E)

9. Insert the Card again into row E.
After 1 minute, withdraw the Card and allow it to dry in the air.

Waste Disposal

Dispose of used Developing Plates, pipette tips, micro tubes or microtiter well strips, absorbent paper, and gloves as biohazardous waste.

STORING UNUSED PART OF KIT

Developing Plate

If you have not used all the wells of the Developing Plate, you may store it for future use:

- Seal used wells with wide adhesive tape so that nothing can spill out of the wells, even if the Developing Plate is tipped over.

Other Kit Materials:

- Return remaining Developing Plate(s), Card(s), perforator, controls, specimen diluent, and instructions to the original kit box. Store at 2-8°C.

TEST RESULTS

Validation

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following three conditions must be fulfilled (see Figure 5):

1. The **Positive Control** must produce **three** spots on the Card tooth.
2. The **Negative Control** must produce an **upper** spot (Internal Control). The lower and the middle spot will either not appear or appear faintly, without affecting the interpretation of the results.
3. Each **specimen tested** must produce an **upper** spot (Internal Control).

If any of the three conditions are not fulfilled, the results are invalid, and the specimens and controls should be retested.

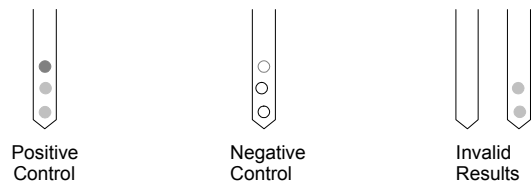


Figure 5. Test Validation

READING AND INTERPRETATION OF THE RESULTS

Visual Interpretation of Dengue IgM

Compare the intensity of the **lower** spot of each specimen tooth with that of the **lower** spot of the Positive Control tooth (Figure 6).

- A spot with intensity higher than or equal to that of the positive control indicates a **positive** result for the specimen.
- **No** spot or a spot with intensity **lower** than that of the Positive Control indicates the **absence** of anti-Dengue IgM antibodies in the specimen (Negative Result).
- Equivocal samples should be retested. If the interpretation remains equivocal, then a new sample must be collected.

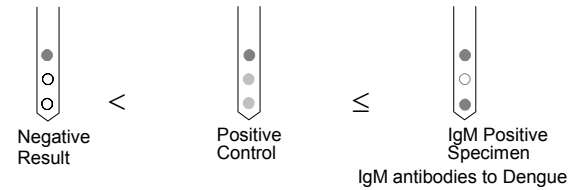


Figure 6. Results

CombScale™ reading and interpretation of Dengue IgG

The presence of specific anti-Dengue IgG in each specimen may be assessed by comparing the color intensity of the **middle** spot on each tooth, with the color scale on the CombScale™ provided with the kit. This is performed as follows (Figure 7):

1. Calibrate the CombScale™. Place the **middle** spot of the **Positive Control** tooth underneath the most similar color intensity of the color scale. Adjust the ruler so that "C+" appears in the window above the selected color intensity.
2. Read the results without changing the calibrated position of the ruler. Match the color intensity of each **middle** spot with the most similar intensity on the color scale.

Any **middle** spot with a color intensity that matches or higher than the intensity on the color scale underneath the "CO" in the window should be considered as positive to Dengue IgG.

In the absence of positive IgM, any **middle** spot with a color intensity that is higher than that of "CO", but lower than "C+" should be considered as **negative – no evidence for a present infection**.

A color intensity of the **middle** spot similar or higher than that of the "C+" can be used as an indication of **Dengue Secondary Infection**.

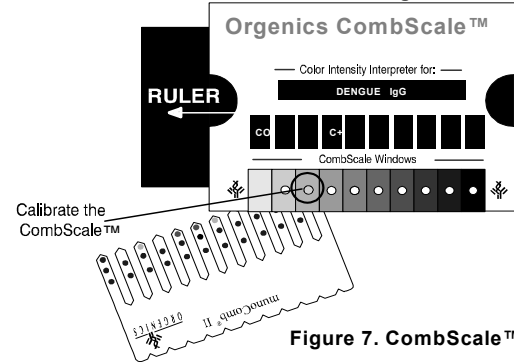


Figure 7. CombScale™

Summary of results interpretation

	Presence of:		Interpretation
	IgM	IgG	
Visual on CombScale™	< C+	< CO	No evidence for Dengue infection
Visual on CombScale™	< C+	CO ≤ S < C+	No evidence for Dengue infection.
Visual on CombScale™	≥ C+	< CO	May indicate primary Dengue infection
Visual on CombScale™	≥ C+	CO ≤ S < C+	May indicate primary Dengue infection
Visual on CombScale™	< C+	≥ C+	May indicate secondary Dengue infection
Visual on CombScale™	≥ C+	≥ C+	May indicate secondary Dengue infection

DOCUMENTATION OF RESULTS

As the color developed on the Card is stable, the Cards may be stored for later documentation.

LIMITATIONS

As with other tests intended for *in vitro* diagnostic use, the results of this test should be evaluated in relation to all symptoms, clinical history and other laboratory findings for the patient.

PERFORMANCE CHARACTERISTICS

A. Sensitivity

A study was carried out on 365 Dengue positive samples obtained from several health centers in endemic areas in Central America (Colombia, Guatemala and Venezuela) and in the Philippines. Results are detailed in Table 1.

Table 1. Sensitivity of the ImmunoComb® II Dengue IgM&IgG BiSpot kit

Source	Sample Number	Defined positive for:		Detected by ImmunoComb® II Dengue IgM&IgG BiSpot		
		IgG	IgM	IgG	IgM	IgG+IgM
Central America	324	304	213	302	207	320
Philippines	41	40	34	39	34	41
Total	365	344	247	341	241	361

Overall sensitivity:

For IgG: 99.1%

For IgM: 97.5%

For IgG + IgM: 98.9%

B. Specificity

A study was carried out on 669 Dengue negative samples including samples obtained from healthy blood donors from low and high prevalence for Dengue, pregnant women, non-infectious diseases (cancer and autoimmune diseases) and infectious diseases, such as HIV, HCV and HBV and from patients with antibodies to other flaviviruses (Japanese Encephalitis, Yellow Fever, West Nile Virus). Results are detailed in Table 2.

Table 2. Specificity of the ImmunoComb® II Dengue IgM&IgG BiSpot kit

Specimens	Number	Negative by ImmunoComb® II Dengue IgM&IgG BiSpot	Specificity (%)
Healthy Blood donors	312	310	99.3
Pregnant women	98	95	96.9
Non-infectious diseases	112	109	97.3
Infectious diseases	110	106	96.3
Flaviviruses	37	35	94.6
Total	669	655	97.9

The overall specificity of the kit: 97.9%

Positive predictive value (PPV):

For IgG: 91.4%.

For IgM: 91.4%.

Negative predictive value (NPV):

For IgG: 100%.

For IgM: 96.8%.

BIBLIOGRAPHY

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- World Health Organization.** 1997. Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control. 2nd edition. WHO publications, Geneva, Switzerland.

Manufacturer:



ORGENICS

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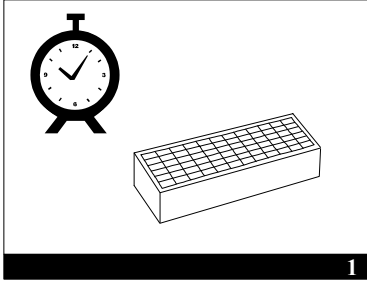
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Version: 60483002/E11/OR (02/2007)

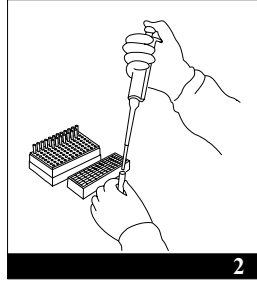
Symbols Legend

	ImmunoComb® Card
	Developing Plate
	Positive Control
	Negative Control
	Perforator
	CombScale™
	Consult Instructions for Use
	Caution, consult accompanying documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Contains sufficient for n tests
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by
	Serial number

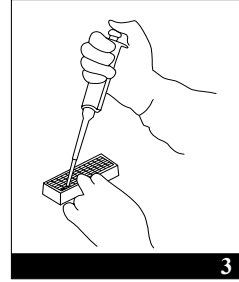
Summary of Main Test Procedures



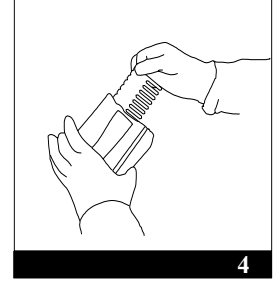
1
Bring the Developing Plate to room temperature: 30 minutes at 37°C.



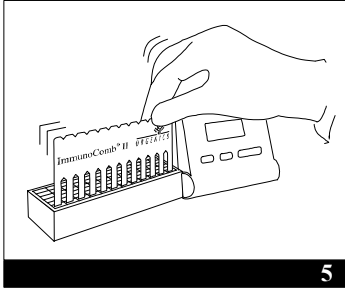
2
Drawing specimens and controls.



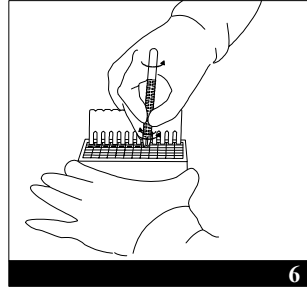
3
Adding specimens and controls to row A.



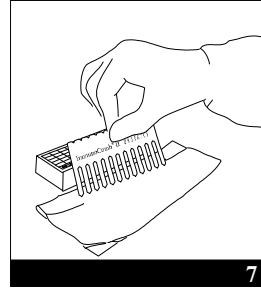
4
Removing Card from pouch.



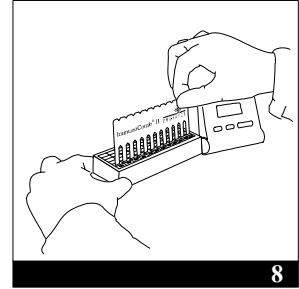
5
Insert Card in row A and mix. Incubate at RT.



6
Open row B.

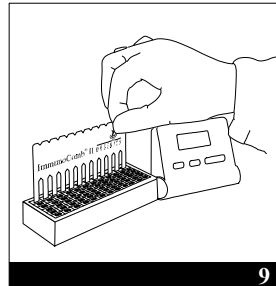


7
Absorb adhering liquid from teeth.

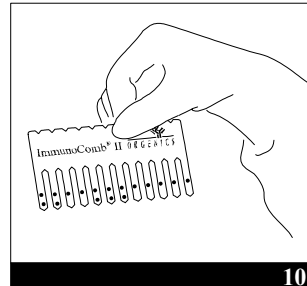


8
Insert Card and agitate in row B. Incubate.

After mixing/agitating & incubating in rows C, D and E ...



9
Color reaction in row F.



10
Results

SUMMARY OF THE TEST PROCEDURE

The abbreviated instructions below are for experienced users of the **ImmunoComb® II Dengue IgM&IgG BiSpot Kit**.

(For detailed instructions please refer to complete text inside)

1. Bring all reagents and specimens to room temperature and perform the test at room temperature.
2. Dispense 10 µl of each specimen and controls into the wells of row A of the Developing Plate.
3. Insert Card in row A, mix, and continue as described in Table 1.

Table 1. Summary of test procedure

Step	Row	Proceed as follows
Antibody capture	A	Mix; incubate 10 minutes; absorb.
Antigen-antibody reaction	B	Mix; incubate 40 minutes; absorb.
Binding of biotinylated anti-Dengue	C	Mix; incubate 40 minutes; absorb.
Binding of modified avidin/ alkaline phosphatase	D	Mix; incubate 10 minutes; absorb.
Wash	E	Agitate; incubate 2 minutes; absorb.
Chromogen	F	Mix; incubate 10 minutes.
Stop reaction	E	Incubate 1 minute; dry in air.

