MB *Millennium Biotechnology* Now with IgG cutoff for determination of secondary infection

Instructions for Use

# *BIAS -3* Dengue IgG/IgM

(Bi-directional Immuno Assay System™)

Two Step Assay for the Differential Detection of IgG and or IgM Antibodies to Dengue Virus in Serum or Whole Blood

*For In Vitro Diagnostic Use*

## Description

The Dengue virus belongs to the Flavavirus group of viruses. This virus is commonly found throughout the tropics and Australia. The symptoms of Dengue fever are sudden onset fever, headache, pain in the back and limbs, lymphaderopathy, maculopapular rash and retrobulbar pain.
Dengue fever causes approximately 20,000 deaths annually with nearly 3 million children hospitalized over the past 3 decades as a result of Dengue. The virus is transmitted by a day biting mosquito (Aeder). This species is common in urban settings. Tests such as Elisa and PCR are being used to aid in the diagnosis of Dengue fever.
New serological tests such as the *BIAS 3* Dengue IgG/IgM rapid test are among the simplest and fastest means of identifying Dengue antibodies.

## Principle of the Test

The *BIAS 3* Dengue IgG/IgM test kit is a rapid membrane based screening test to differentially detect the presence of antibodies to Dengue virus. This test is the newer generation lateral flow immunochromatographic type assay. These are among the simplest and easiest to use POC (point of care) assays.
The test can be used either with serum or whole blood. The test employs the use of two antibody binding proteins conjugated to colloidal gold particles and a unique combination of Dengue antigens immobilized on the membrane.
Once the sample is added to the sample pad along with the diluent, the mixture passes bi-directionally through two antibody binding/gold complexes, which then binds the immunoglobulins in the sample. As this complex passes over the immobilized antigens on the membrane, if any antibodies to Dengue (IgG or IgM) are present the antigens capture them in turn. This produces a pink/purple band in the Test zone of the device. The remaining complex continues to migrate to a control area on the test device and produces a pink/purple band each Control area. This control bands indicate that the test has been performed properly.

## Kit Components

Each test kit contains:

1. *BIAS 3* Dengue IgG/IgM test devices – 15 each
2. Diluent in dropper vial
3. Directions for Use

Needed but not provided:

1. Measuring pipet(s) capable of delivering 5 or 10ul's

## Stability and Storage Conditions

The *BIAS 3* Dengue IgG/IgM test kit is stable at any room temperature between 8-30'C when in the unopened pouches.
DO NOT FREEZE the kit or expose to temperature extremes.
Stability of the kit is 24 months from the date of manufacture – dating is indicating on the kit label.

## General Precautions

- The test is for *In Vitro Diagnostic* Use only.
- Appropriate infection control and handling procedures should be followed – do not smoke, eat or drink in the area where the test is to be performed. Use suitable clothing and gloves when handling samples and when performing the test.
- Do not pipet any samples or reagents by mouth.
- All materials should be considered as potentially infectious. To disinfect, either autoclave materials at 121.5'C for 1 hour or treat with Sodium hyprochlorite (1 percent solution).
- Do not use test beyond the expiration date indicated.

## Sample Collection

The *BIAS 3* Dengue IgG/IgM test can be run on serum or whole blood.
The test works best on fresh samples. If testing cannot be done immediately, they should be stored at 2-8'C after collection for up to 3 days. If testing cannot be done within 3 days, serum can be stored frozen at –20'C or colder. Whole blood samples cannot be frozen and it is recommended that finger prick blood be used not samples collected in EDTA. Shipment of samples should comply with local regulations for transport of etiologic agents.

## Test Procedure

1. Remove as many test devices from the pouches as needed. Lay on a clean flat surface.
2. **For WHOLE BLOOD** - add 10 uls of sample to the center sample well **(S)** of the test device using a measuring pipet.
   **For SERUM** – add 5 uls of sample to the center sample pad hole of the test device using a measuring pipet.
   ***NOTE:*** ***SAMPLE AND DILUENT IS ADDED ONLY TO THE CENTER HOLE INDICATED BY AN "S" ON THE DEVICE***

3. Follow sample addition with 6 drops of the diluent provided in the dropper bottle by holding the bottle vertically over the hole in the center sample well **(S).** Add the diluent SLOWLY to the Sample well (S) of the device **making sure to use the diluent that was supplied with the test lot**.

The test device and the diluent supplied are "matched reagents" and should not be interchanged with other lots.

4. Results are then read in as little as 5 minutes for strong positives or up to 30 minutes for weaker positives and to make sure negatives are confirmed.

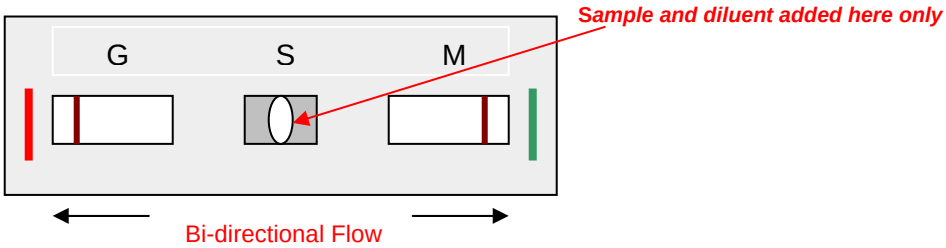
NOTE: If the dye has not cleared the membrane sufficiently or blood is still present after ~15 minutes, one more drop of diluent may be added to the center Sample pad.

## Reading the Test Results

### Negative

Only one pink/purple band appears in the Control areas of the *BIAS 3* test device

The RED and GREEN lines at the ends of the strip are there to indicate which end is IgG detection (RED) and which end is IgM detection (GREEN). They are NOT Test or Control lines



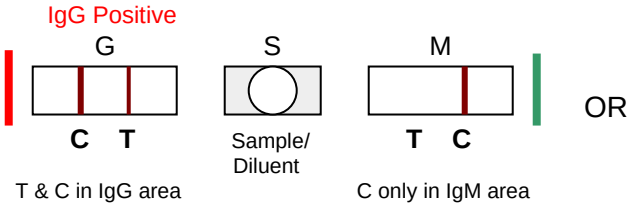

### Positive

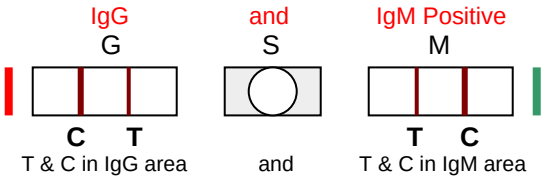
One or Two pink/purple bands appear in either or both of the Test area(s) of the *BIAS 3* test device and one line each in the Control area of the test device.

**The BIAS 3 Dengue IgG – IgM test is Interpreted as follows**:

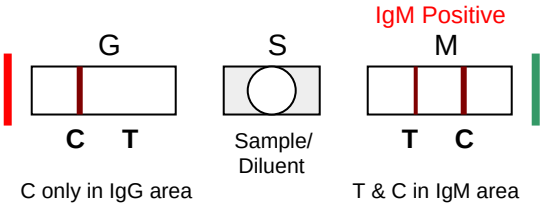
***Secondary Dengue Infection***

The IgG test line has a cutoff based on HIA titer of ~1:1280




***Primary Dengue Infection***




**KEY: T = Test line, C = Control line**

**PLEASE NOTE:** When reading this test, any visible ***pink-purple colored line*** in the Test area of the device within the prescribed time limit of the test indicates a POSITIVE result. ***IgM test lines are typically of lesser intensity than IgG test lines***

### Indeterminate

If only one band appears in the Test area, or no band appears at all in the Control area. It is then recommended that a fresh device be used and the test repeated carefully following the directions in this insert.

### Quality Control

It is recommended that a known positive and negative control and or patient samples be run to insure proper performance. Handle all controls/patient samples as infectious materials.

### Limitations of the Test

The instructions for use and reading of the test instructions must be followed carefully for the test to perform properly.

The *BIAS 3* Dengue IgG/IgM test is designed to detect antibodies against Dengue virus in serum or whole blood. Testing of any other body fluids has not been validated and may not yield appropriate results.

For samples that test positive by the *BIAS 3* Dengue IgG/IgM test, more specific confirmatory testing should be done. A clinical evaluation of the patient's situation and history should also be made before a final diagnosis is established. The use of a rapid test alone is not sufficient to diagnose Dengue fever even if antibodies are present. Also, a negative result does not preclude the possibility of infection with Dengue virus.

### Performance Characteristics

As there are no true standards established for determining the absence or presence of Dengue IgG or IgM antibodies in serum or whole blood samples it is recommended that the performance of the kit be compared to established serum panels or reference materials. The *BIAS 3* Dengue IgG/IgM kit is tested against ELISA characterized serum samples and has shown to be highly sensitive and specific for Dengue IgG and IgM antibodies.

***Sensitivity on IgM and IgG positive serum samples, versus another commercially available Dengue IgG-iGm rapid test, was shown to be*** 90 and 98 percent respectively. Specificity, using serum samples obtained from a regional blood bank in the US where Dengue infection is nil, was shown to be >99 percent.

Revised September 2005/Patent Pending