

### ABSTRACT

An estimated 100 million cases of dengue fever and 250,000 cases of the life-threatening dengue hemorrhagic fever occur annually on a worldwide basis. At this time, there is no specific treatment or vaccine available, however, early detection and proper medical care reduce fatality rates to below 1%. CTK Biotech meets this need with a complete line of dengue rapid test kits for screening of all phases of dengue infection. To ensure superior performance, an external evaluation recently performed in an ISO 9001: 2008 laboratory facility in Colombia, South America compared CTK's Dengue Duo Ag-IgG/IgM rapid test (R0062C) against the current market leader, SD Bioline Dengue Duo NS1 Ag + IgG/IgM. The true nature of any conflicting samples was determined by confirmatory ELISA kits. The external evaluation confirmed that CTK's Dengue Duo rapid test was in fact more accurate than SD Bioline.

### BACKGROUND

Dengue is an acute febrile disease that should be discriminated from other febrile diseases with similar clinical presentation (malaria, typhoid, etc) because acute infection can lead to dengue hemorrhagic fever. This can be life threatening and incorrect diagnosis and treatment can lead to misuse of antibiotics, and in extreme cases, death due to missing the critical detection period. In the 1990's, dengue infection was mainly diagnosed in laboratories by virus culture, however due to the high demand for optimal accuracy, affordability, Point of Care and simplified test procedure, the protocol has been changed to ELISA and rapid test methods. The recommended dengue diagnostic algorithm includes screening by rapid test followed by confirmation of the results with an ELISA kit (or the less popular method, PCR). The sensitivity and specificity of commercially available dengue rapid test kits may vary depending on the specimen panel tested and due to the antigen and antibody used to manufacture the product. Therefore, a comprehensive product evaluation must include verification of conflicting specimens by a confirmatory ELISA.

CTK Biotech is devoted to developing high quality dengue rapid tests. CTK started from developing the antigen and antibodies by culturing the virus, producing recombinant dengue E and NS1 protein in mammalian cells, and immunization of rabbits and mice to prepare antibodies to native protein. In 2014, CTK Biotech and the US Center for Disease Control (CDC) reached a license agreement that granted worldwide exclusive, diagnostic commercial rights to a novel and patented recombinant dengue virus antigen. Developed by the US CDC's dengue experts, this antigen identically preserves the envelope structure of the native virus including subunit number and conformation. This unique property makes it the most advanced recombinant dengue antigen technology available to date and is used in CTK's most recent revision of dengue IgG and IgM test kits.

### METHODS

Side by side, thirty suspicious specimens collected from outpatient visits were tested with Dengue Duo Ag-IgG/IgM Rapid Test (R0062C) and SD Bioline Dengue Duo NS1 Ag + IgG/IgM. Frozen specimens were pre-warmed to room temperature and mixed well prior to testing. The testing was performed according to the instructions provided by the manufacturers and the results were reported and analyzed by a trained technician. Conflicting specimens were further tested with market leading ELISA kits, Vircell Dengue IgG ELISA, Vircell Dengue IgM Capture, and Panbio Dengue Early ELISA, respectively.

### RESULTS

- **Tubular table results of testing by CTK's rapid test vs. SD Bioline**

	IgG	IgM	NS1
No. Specimens	30	30	30
No. Concordant Specimens	28	28	27
No. Discordant Specimens	2	2	3
Concordance Percentage of CTK test v.s. SD Bioline	93%	93%	90%

- Raw data of testing by CTK's rapid test vs. SD Bioline

SAMPLE	Dengue IgG Results			Dengue IgM Results			Dengue NS1 Results		
	CTK	SD Bioline	VIRCELL ELISA	CTK	SD Bioline	VIRCELL ELISA	CTK	SD Bioline	PANBIO Early ELISA
1	1310879	Positive	Negative	Positive	Positive	Positive	Positive	Positive	
2	8140725	Positive	Negative	Positive	Negative	Negative	Positive	Positive	
3	2063258	Positive	Positive	Positive	Negative	Positive	Negative	Negative	
4	2062641	Positive	Positive	Positive	Positive		Negative	Negative	
5	1204206	Positive	Positive	Positive	Positive		Negative	Positive	Negative
6	1310646	Positive	Positive	Positive	Positive		Negative	Negative	
7	1313215	Positive	Positive	Positive	Positive		Negative	Negative	
8	8014679	Negative	Negative	Positive	Positive		Positive	Positive	
9	1210448	Positive	Positive	Positive	Positive		Negative	Negative	
10	2063557	Positive	Positive	Negative	Negative		Negative	Negative	
11	2062953	Negative	Negative	Negative	Negative		Negative	Negative	
12	2060666	Positive	Positive	Negative	Negative		Negative	Negative	
13	2022845	Positive	Positive	Negative	Negative		Negative	Negative	
14	1310633	Positive	Positive	Negative	Negative		Negative	Negative	
15	1295296	Negative	Negative	Negative	Negative		Negative	Negative	
16	1303707	Negative	Negative	Negative	Negative		Negative	Negative	
17	1300204	Negative	Negative	Negative	Negative		Negative	Negative	
18	1300183	Positive	Positive	Positive	Positive		Negative	Negative	
19	1310324	Negative	Negative	Negative	Negative		Negative	Negative	
20	1310678	Negative	Negative	Negative	Negative		Negative	Negative	
21	1310787	Positive	Positive	Positive	Positive		Positive	Positive	
22	8130505	Positive	Positive	Positive	Positive		Negative	Positive	Negative
23	8142881	Negative	Negative	Negative	Negative		Positive	Negative	Negative
24	8111358	Negative	Negative	Negative	Negative		Positive	Positive	
25	8151948	Negative	Negative	Positive	Negative	Positive	Positive	Positive	
26	8133722	Negative	Negative	Positive	Positive		Negative	Negative	
27	8221445	Negative	Negative	Negative	Negative		Negative	Negative	
28	2060606	Negative	Negative	Negative	Negative		Negative	Negative	
29	2060612	Positive	Positive	Positive	Positive		Negative	Negative	
30	1310743	Positive	Positive	Positive	Positive		Negative	Negative	

- Results on conflicting specimens by confirmatory ELISA kits

SAMPLE	CTK	SD Bioline		Qualitative Interpretation	Quantitative Result
		IgG	IgG		
1	1310879	Positive	Negative	Positive	16.2
2	8140725	Positive	Negative	Positive	23.1
		IgM		VIRCELL Dengue IgM CAPTURE	
3	2063258	Positive	Negative	Positive	16.4
25	8151948	Positive	Negative	Positive	14.4
		NS1		PANBIO Dengue EARLY ELISA	
5	1204206	Negative	Positive	Negative	4.5
22	8130505	Negative	Positive	Negative	5
23	8142881	Positive	Negative	Negative	4.7

## CONCLUSION

In this well designed external evaluation, the overall agreement on detection of IgG, IgM, and NS1 by CTK Dengue Duo Ag-IgG/IgM Rapid Test against SD Bioline Dengue Duo NS1 Ag + IgG/IgM were 90% or above. Among the 7 conflicting specimens, confirmatory ELISA tests showed that 6 out of 7 specimens were correctly identified by CTK rapid test kits, while only 1 out of 7 was correctly identified by **the SD Bioline test with which the testing has more false positive and false negative results.** Therefore, CTK Dengue Duo Ag-IgG/IgM Rapid Test is a more accurate screening tool.