Development of the highly accurate diagnostic kit for the dengue NS1 antigen detection

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1. Introduction

Dengue is the most important human diseases caused by mosquito-borne viruses and is the most rapidly spreading viral disease, with over one billion people at risk in the subtropics and tropics [1]. Dengue virus (DENV) belongs to the *Flavivirus* genus of the *Flaviviridae* family and exists as four known serotypes of the dengue virus (DENV1-4) [2]. Because the Korean Peninsula is turning into a subtropical zone as a result of global warming, South Korea is no longer a safe zone of tropical endemic diseases. There are no available vaccines or antivirals against DENV. In this reason, early diagnosis is very important in dengue virus related diseases.

The rapid diagnostic tests (RDT), also known as the lateral flow rapid test, are diagnostic assays designed for use at the point-of-care (POC). An RDT has several advantages, such as low-cost, simple to operate and read, sensitive, specific, stable at high temperatures, and works in a short period of time. As a consequence, the RDT has been widely used in clinics and elsewhere [3]. In this report, we describe the preparation of monoclonal antibodies specific for the nonstructural protein 1 (NS1) of DENV. We also detail the development of an RDT system using the antibodies and its clinical characteristics.

2. MATERIALS AND METHODS

2.1. Clinical samples

100 dengue-negative sera were provided by Chungbuk National University Hospital (Cheongju, Korea) and 42 dengue NS1 antigen-positive sera were from General Hospital Kuala Lumpur (15 cases; Kuala Lumpur, Malaysia), Rio de Janeiro Hospital (17 cases; Rio de Janeiro, Brazil), and Bombay Hospital (10 cases; Mumbai, India).

2.2. Rapid diagnostic kit

Composition of the RDT strip is shown in Figure 1. The test is positive for NS1 antigen when both control line © and test line (T) appear and the test is negative when only the control line appears in the test area. Commercial RDT purchased (SD BioLine Dengue NS1 Ag, Lot. No.: 127056) and compared with the RDT that we developed and introduced in this study. Both RDTs are designed to detect DENV NS1 using sandwich mmune-chromatographic method. These RDTs were evaluated in the laboratory using various sera which were obtained from several hospitals and countries.

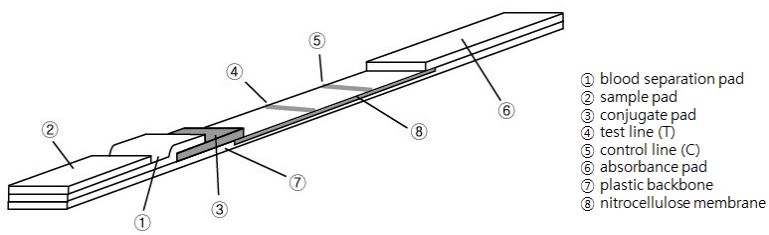

Figure 1. Diagram of the RDT strip for the diagnosis of dengue virus infection.

3. Clinical assessment of RDT

Sensitivity and specificity, expressed as percentages, were calculated as the number of positives or negatives from the RDT results divided by the number of positives or negatives from the RT-PCR or ELISA results that are generally using as a definitive diagnosis by hospital.

3.1. Specificity of the RDT kit developed in this study

Total 100 sera were used for the evaluation of the kit's specificity. The results showed that the specificity of RDT developed in this study was 100%. The background of the kit was also very clear and good for interpretation after 15 minutes. Till 30 minutes of reading time, we have checked that the kit did not have any traces in the test line and did not showed false positive results. The intensity of test line (T) was also clear to read the results.

3.2. Sensitivity of the RDT kit and its comparison with other commercial RDT kit

Total 42 sera were used for the evaluation of the kit's sensitivity. RDT developed in this study showed the 39 positive results among 42 positives, resulting to 92.9% of sensitivity. However, commercial one from the SD BioLine was just 35 positive results among them, resulting to 83.3% sensitivity. We assumed that this difference of NS1 antigens might be made the sensitivity difference of both RDTs.

[Table 1] Clinical performance of the RDT kit developed in this study

		RDT used in this study		
(n=142)		Negative	Positive	Total
ELISA/ RT-PCR	Negative	100	0	100
	Positive	3	39	42
Total		103	39	142
Sensitivity = 92.9%, Specificity = 100%				

4. Conclusion

Dengue is the most important human diseases caused by mosquito-borne viruses. Climate change as a result of global warming affects the survival and transmission of mosquito vectors as well as the development rates of mosquito-borne tropical diseases. Increased international travel is also an important factor in the spread of mosquito-borne diseases. Because the Korean Peninsula is turning into a subtropical zone as a result of global warming, South Korea is no longer a safe zone of tropical endemic diseases such as malaria and dengue fever. There are no available vaccines or antivirals against DENV. In this reason, early diagnosis is most important to control the dengue related diseases.

In this study, we developed RDT as a NS1 target for dengue virus infection. The RDT developed in this study are more sensitive and specific than those of other commercial RDT kit. Our RDT can serve as a point-of-care testing (POCT) tool because of its rapid assay time, ease of use, and no need for specialized machines or expert technicians. Therefore, the RDT kit developed in this study could be used as a promising POCT tool for diagnosing DENV infection in remote areas, and can contribute to the control of dengue related diseases.

5. References

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