ABSTRACT
Yellow fever (YF) is an acute viral haemorrhagic disease transmitted by infected genus *Aedes* mosquitoes. The yellow fever virus (YFV) is mostly found in tropical and subtropical areas of Africa and South America. Outbreaks recently reached considerable proportions in terms of spatial distribution and total numbers of cases, with multiple exports, including to China. There is no medicine to treat or cure infection. Rapid diagnostic tests (RDTs) for the detection of YFV antigens are based on the immunochromatographic test principle. A total of 124 prospective serum samples submitted for yellow fever virus (YFV) IgM and IgG testing. All 124 serum samples among 124 in Brazil showed IgG positive results with YFV. Thus sensitivity of the GenBody Company RDT was 100.0% for IgG. 11 samples among 12 were negative. Specificity of GenBody kits was 91.6% (11/12) for YFV. It is suggested that the rapid diagnosis kit of GenBody company can be used as a useful test for Yellow fever virus management.

KEYWORDS: Yellow fever virus (YFV), IgG, IgM, rapid diagnostic tests (RDTs), RT-PCR of YFV.

INTRODUCTION
Yellow fever is a viral disease of typically short duration. The disease is caused by the yellow fever virus and is spread by the bite of an infected female mosquito.[1] It infects only humans, other primates, and several species of mosquitoes. The yellow fever virus (YFV) is found in tropical and subtropical areas of Africa and South America. Although it is now commonly accepted that YFV originated in Africa[2] between 1500 and 3000 years ago[3] the first outbreaks of the “Black Death”, “Yellow Jack” or “Blood Vomit” (Xekik in Mayan) were reported in Barbados and in St. Christophe (now St. Kitts) in 1647.[4] According to the World Health Organization, an estimated 200,000 people become infected each year.[5] Most cases occur in 32 countries in Africa, including Rwanda and Sierra Leone, and in 13 countries in Latin America, including Bolivia, Brazil, Colombia, Ecuador, and Peru.[5] Outbreaks recently reported in Central Africa (2015-2016) and Brazil (since late 2016), reached considerable proportions in terms of spatial distribution and total numbers of cases, with multiple exports, including to China.[6]

Yellow fever is a serious, potentially deadly flu-like disease spread by mosquitoes. It’s characterized by a high fever and jaundice. Jaundice is yellowing of the skin and eyes, which is why this disease is called yellow fever. There is no medicine to treat or cure infection. Large epidemics of yellow fever occur when infected people introduce the virus into heavily populated areas with high mosquito density and where most people have little or no immunity, due to lack of vaccination. In these conditions, infected mosquitoes of the *Aedes aegypti* specie transmit the virus from person to person.

Rapid diagnostic tests (RDTs) for dengue, West Nile fever, YFV have become increasing available over the last 5 years because of the need for point-of-care diagnosis in resource limited settings.[7] Laboratory diagnosis generally is accomplished by testing serum to detect virus-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies by serologic assays.[8] Early in the illness (during the first 3-4 days), yellow fever virus or yellow fever virus RNA can be detected in the serum by virus isolation or nucleic acid amplification testing such as reverse transcription-polymerase chain reaction (RT-PCR). However, by the time overt symptoms are recognized, the virus or viral RNA usually is undetectable. Therefore, negative virus isolation and RT-PCR results cannot rule-out the diagnosis of yellow fever. Immuno-histochemical staining of formalin-fixed material can detect yellow fever virus antigen in histopathologic specimens.[8]
There are not many commercial serologic diagnostic tests for detection of anti-YF virus (anti-YFV) enzyme-linked immunoglobulin M (IgM) or IgG. Tests commonly used are in-house assays such as enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), and plaque neutralization tests (PRNT).[9]

Immunoglobulin M is the first antibody isotype to be synthesized, and it is the first antibody produced in response to a foreign antigen.[10] It is mostly found in the bloodstream, although mucosal IgM has been noted in physiologic amounts. It has been hypothesized that it provides a level of protection in those patients with selective IgA deficiency who are asymptomatic.

Several rapid diagnostic tests (RDTs) have been developed to simplify point-of-care testing for YFV. In the present study, we assessed the utility of the commercially available blood tests approved by the rapid diagnostic tests (RDTs) of GenBody Company (Korea) for the detection of Yellow fever virus. Our aim was to test the commercial RDT anti-YFV IgM and IgG assays to the reference RT-PCR-ELISA and PRNT currently in use.

We report the development and evaluation of the RDT, known as the genus Aedes mosquitoes which can identify infections YFV.

MATERIALS AND METHODS

Clinical specimens and clinical evaluation of RDT

Aedes albopictus (C6/36) cells were grown in Liebovitz L-15 media with 10% FBS, 10% tryptose phosphate broth (Sigma-Aldrich, St. Louis, MO), pen-strep, and 1% L-glutamine at 28°C.[11] A. albopictus (C710) cells were grown in Dulbecco's minimum essential medium (Gibco, Carlsbad, CA) with 10% FBS, pen-strep, L-glutamine, and maintained at 30°C. Total RNA was extracted from serum or blood using the QIAamp Viral RNA Mini kit (Qiagen, Germany), according to the manufacturer’s instructions. For reverse transcription-polymerase chain reaction (RT-PCR), 5 μL RNA was added to a 50 μL master mixture containing reaction buffer, enzyme mixture, and primers. PCR products were purified by using a QIAquick PCR purification kit (Qiagen). Nucleotide sequences of the full-length coding regions of all RNA segments from each virus were determined by direct cycle sequencing system. The sequences were compared with accession no. AY640589 in GenBank.

RDTs were based on the immunochromatographic test principle. Hybridoma was made by fusing non-protein NS1 of yellow fever virus cells with mouse bone marrow cancer cells. Among the nine monoclonal antibodies purified in this hybridoma, two monoclonal antibodies cross-reacting with yellow fever virus were selected and the remaining seven monoclonal antibodies were screened for the two most sensitive hybridomas (C08M, C09M). It was used as T Line and Gold Line to make kits. RDT kits for the detection of YFV antigens were shipped to only one of the reference laboratories where they were subsequently shipped to the evaluation laboratories under conditions specified by the manufacturer.

When 5 μL of whole blood was dropped by the immunochromatography principle, the anti-human IgM antibody or the anti-human IgG antibody binds to the test line when the anti-yellow fever antibody was present along the nitrocellulose. When the reaction solution was dropped to 3 to 4 drop, the reaction solution will dissolve the yellow NS1 recombinant protein + anti-yellow NS1 monoclonal antibody-gold condensate in the condensation pad. It binds with anti-yellow fever antibodies in the sample and reacts with anti-mouse IgG on the control. Network laboratories tested a limit of detection panel to ensure comparable sensitivity of their RT-PCR assay as a reference standard. The methods of RNA extraction and RT-PCR used in the network laboratories included: nested RT-PCR (Polymerase Chain Reaction). The samples were compared with 113 positive and 11 negative samples by RT-PCR method provided by University Hospital of Rio de Janeiro, Brazil based on IRB regulations. To use RDT Kits, the fingertip was pierced using a lancet, and 10 μL of whole blood or 5 μL of serum or plasma was collected using capillary blood vessels. The collected specimen was dropped in the specimen injecting section. Then, the buffer solution is applied to the buffer injecting part of 3 ~ 4 drop, and the reaction is carried out for 15 ~ 20 minutes and then the result is read. RDTs were tested in duplicates, with two readers evaluating each result independently.

Statistical analysis

The sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for the assays were calculated based on true positive Yellow fever samples (virus isolation/PCR positives, sero-negative acute sera, acute primary, acute secondary).

Statistical analysis was performed with Statistical version 18 (StatSoft, Inc., Tulsa, OK). Significance was assigned at p<0.05 for all parameters and were two-sided unless otherwise indicated. Uncertainty was expressed by 95% confidence intervals. Categorical variables between groups were compared by Fisher's exact test. The t-test was used for continuous variables.

RESULTS

A total of 124 prospective serum samples submitted for yellow fever virus (YFV) IgM and IgG testing. Focus Diagnostics YFV IgM and IgG EIAs were also tested by the IgM and IgG YFV assays (Figure 1). Figure 2 was shown the typical evaluation methods of the rapid
diagnostic test for YFV. The appearance of the control line alone indicated a negative result. The results were compared and the data summarized in Table 1.

Table 1 showed results of analysis for IgG, IgM, PRNTs, and ELISA of specimens obtained during the study populations (n = 124 cases). Specimens were divided into primary and secondary infections on the basis of antibody testing results of acute-phase specimens. All 124 serum samples among 124 in Brazil showed IgG positive results with YFV. Thus sensitivity of the GenBody Company RDT was 100.0% for IgG 11 samples among 12 were negative. Specificity of GenBody Company Kits was 91.6% (11/12) for YFV.

DISCUSSION

Yellow fever is difficult to diagnose, especially during the early stages. Once contracted, the yellow fever virus incubates in the body for 3 to 6 days. Many people do not experience symptoms, but when these do occur, the most common are fever, muscle pain with prominent backache, headache, loss of appetite, and nausea or vomiting. In most cases, symptoms disappear after 3 to 4 days.[14]

Several methods for detection of antibodies against YFV have been developed, such as the hemagglutination inhibition test, PRNT, IFA, and ELISA.[15-16]

The first commercial indirect immunofluorescence assay (IFA) using Euroimmun Biochip technology was evaluated for the serodiagnosis of immunoglobulin G (IgG) and IgM antibodies against YFV and was compared with the plaque reduction neutralization test (PRNT), which is currently the gold standard test for.[9] They reported that the sensitivity and specificity, calculated using the 150 sera from vaccines and 150 sera from healthy blood donors, were 95% and 95%, respectively, for the IgG IFA and 94% and 97% for the IgM IFA.

The ELISA (enzyme-linked immunosorbent assay) is a well-established antibody-based tool for detecting and quantifying antigens of interest. Ready-to-use ELISA Kits are available from a variety of manufacturers. For example, MBS9344675 by MyBiosource (Vancouver, British Columbia, Southern California, San Diego, USA) is a ready-to-use microwell, strip plate ELISA (enzyme-linked immunosorbent assay) Kit for analyzing the presence of the Yellow Fever Virus Antibody IgG (YFV-IgG) ELISA Kit target analytes in biological samples. The concentration gradients of the kit standards or positive controls render a theoretical kit detection range in biological research samples containing YFV-IgG. The ELISA analytical biochemical technique of the MBS9344675 kit is based on YFV-IgG antibody-YFV-IgG antigen interactions (immunosorbency) and an HRP colorimetric detection system to detect YFV-IgG antigen targets in samples. The ELISA Kit is designed to detect native, not recombinant, YFV-IgG. Appropriate sample types may include undiluted body fluids and/or tissue homogenates, secretions. Human Anti-Yellow Fever Virus Envelop protein (YFV-Env) IgG ELISA kit was also developed by Life Technologies (India). Genekam Biotechnology AG, Germany had developed a DNA kit for yellow fever virus. This kit is with CE-IVD and can

![Figure 1: The schematic representation of yellow fever virus parental and chimeric genes used in this study.](image1)

![Figure 2: The methods of the yellow fever IgG/IgM rapid diagnostic test (RDT).](image2)

**Table 1: Laboratory diagnosis of yellow fever virus using RT-PCR and RDTs.**

<table>
<thead>
<tr>
<th>IgG (N=124)</th>
<th>RDTs</th>
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<tr>
<td></td>
<td>RT-PCR</td>
<td>Negative</td>
<td>Positive</td>
<td>Total</td>
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<tr>
<td>IgG</td>
<td></td>
<td>11</td>
<td>1</td>
<td>12</td>
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<tr>
<td>Positive</td>
<td></td>
<td>0</td>
<td>112</td>
<td>112</td>
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<td>Total</td>
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<td>11</td>
<td>113</td>
<td>124</td>
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be used in diagnostic in Europe and other CE accepting countries.

Conclusionally this study assayed the commercial IgM and IgG kits to the reference ELISA in 124 clinical serum specimens. The developed GenBody Company's quick diagnostic kits are available at low cost without any special equipment such as microscopes or PCR equipments, and can be quickly inspected by shortening the inspection time, and are suitable for reading results and field inspection. It is suggested that the rapid diagnosis kit of GenBody Company can be used as a useful test for Yellow fever virus management.

REFERENCES
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