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Short Communication

Development and Clinical Evaluation of Rapid Diagnostic Kit for Hemorrhagic Fever with Renal Syndrome

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Hemorrhagic fever with renal syndrome (HFRS) is one of the acute febrile illnesses. This study has analysed 'Immune Med Hanta Rapid Kit', a medical device for *in vitro* diagnosis of HFRS using immunochromatographic assay. This kit was evaluated by the test of the IgM and IgG to the Hantavirus. In a clinical evaluation with 194 specimens (91 HFRS, 48 other infectious diseases, and 55 healthy controls), the kit was shown 96.7 % sensitivity and 97.1 % specificity. These results demonstrate that this kit is the potentially useful diagnostic device which can perform a rapid, accurate, and simple diagnosis of HRFS clinically.

Keywords: Rapid Diagnostic Kit, Immunochromatographic assay, HFRS, Nucleocapsid protein, Baculovirus expression

TEXT

Hemorrhagic fever with renal syndrome (HFRS) is distributed around the world, containing not only in Northeast Asia including South Korea, China, and Russia but also Europe including Scandinavia, and North and South America. WHO estimates that about 150,000 patients occur annually. Approximately 50 % ~ 90 % of HFRS patients in the world occur in China (Jonsson et al., 2010; Zou et al., 2016), and several hundred patients arise in Russia each year (Bi et al., 2008).

HFRS, commonly known as epidemic hemorrhagic fever, is an acute febrile illness (AFI) in humans (Kim et al., 2009) infected through the urine, feces, and saliva of rodents which were infected with Hantavirus (Pettersson et al., 2008). The infected rodent as reservoir has been increasing with climate change caused by global warming. As a result, the number of patients has recently increased due to the increased contacts between human beings and virus-infected rodents (Wu et al., 2016).

HFRS has begun to be known as hundreds of people

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had died among thousands of UN soldiers suffering from an unidentified acute hemorrhagic fever during the Korean War. According to the statistics from Korea Centers for Disease Control and Prevention, approximately 100 patients were reported annually from the late 1970s to the late 1990s. The number steadily increased from 1998, and about 400 patients were reported annually after 2003 to now. In Korea, the illness is mainly caused by the two species, Hantaan virus and Seoul virus. Of these, Hantaan virus accounts for most of the cases and shows a more severe clinical symptoms than Seoul virus (Kim, 2011; Kim, 2009; Noh et al., 2013).

Because there is no effective therapy for HFRS (Escadafal et al., 2012), for complete cure, the early diagnosis in the stage of disease and the early symptomatic treatments such as control of fluid, electrolyte, blood pressure and hemorrhage are very important (McCaughey and Hart, 2000). If the diagnosis was delayed or missed, the mortality rate is increased to 5 to 10 % (Jiang et al., 2016; Wang et al., 2007).

Diagnostic methods include indirect fluorescent assay (IFA) as standard diagnostic test, real-time polymerase chain reaction (RT-PCR), and enzyme-linked immunosorbent assay (ELISA) etc (Jiang et al., 2016). IFA is a serological diagnostic method, which assesses any increase of antibody titers against the virus in blood collected twice with a 1-week interval. If both serum specimens in early and convalescent stage of the disease cannot be collected, the diagnosis may not correct. But in most cases, the serum at the early stage alone is used to measure the IgM antibody against Hantavirus, thus delivering a putative diagnosis (Wang et al., 2007). However, IFA cannot rule out errors in the assessment, because it involves a complex process, such as to be done by a skilled expert in a space where there is a fluorescence microscope, and results in subjective judgment. A currently available RT-PCR and ELISA also have limitations in early diagnosis of HFRS in that there is a long processing time (Wichmann et al., 2001). Therefore, the conventional diagnostic methods are insufficient to provide for an early, simple, and accurate diagnosis (McCaughey and Hart, 2000). So, it requires a rapid and accurate serological diagnostic method to perform early diagnosis and proper treatment of a patient (Lagerqvist et al., 2016).

To solve these problems, a development of accurate diagnostic antigens is required to use simple and rapid immunochromatographic assay (ICA). Therefore, Immune Med has used nucleocapsid protein among viral proteins, as a diagnostic antigen, which is known as an immunodominant antigen of non-pathogenic Soochong virus which shows 96 % amino acid homology with Hantaan virus and 82 % with Seoul virus. This is consistent with other reports that the antigenicity of Hantavirus nucleocapsid protein is conserved rather than the antigenicity of envelope glycoproteins and therefore used in serological or seroepidemiological studies (Yoshimatsu and Arikawa, 2014).

The viral RNA was isolated from the Vero cells which were infected with the Soochong virus and cDNA was synthesized. In order to amplify the gene encoding the complete nucleocapsid protein (CNP) of Soochong virus, the primers were designed using GenBank AY675350 (data not shown) and PCR was performed. As a result, the CNP gene was identified as a band of 1,300 bp as shown in Figure 1(a). The gene was cloned into baculovirus expression vector. After the confirmation of inclusion body formation in insect cells (Sf9) infected with recombinant baculovirus, the cells were lysed with lysis buffer containing 6 M Urea. The lysate was purified by His-bind affinity chromatography and then followed to SDS-PAGE. It was confirmed that the recombinant CNP proteins were expressed as 50 kDa of molecular weight of protein (Figure 1(b)). In addition, it was confirmed that the recombinant CNP protein was purely purified using affinity chromatography (lane 7 to 9 of SDS-PAGE). To confirm the antigenicity of the expressed recombinant CNP, dot-blot was performed with the positive serum to HFRS and the negative serum. No negative serum does react with CNP. As the five positive sera to HFRS were reacted with CNP, the antigenicity of the expressed recombinant CNP was confirmed (data not shown).

After confirming its antigenicity of the recombinant protein as a diagnostic antigen, ImmuneMed has developed a rapid diagnostic kit (RDK) that adopts lateral flow immunochromatographic assay, a kind of ICA. In this study, clinical performance test was done for 'ImmuneMed Hanta Rapid Kit' by using 194 domestic specimens for validity assessment. The test procedure was briefly as follows: 300 µl diluent buffer including 6 µl of whole blood or 3 µl of serum or plasma was applied to the sample port of the kit. A complex of serum antibody-gold conjugated anti-IgM or IgG ran on each IgM or IgG test strip. The result was read at 15 minutes. As a result, control line (C) of the IgM and IgG strips appear as red bands (Figure 2(a)). And if the test line (T) appears as a red band on one or both of the IgM and IgG, it was judged as positive (Figure 2(b), 2(c) and 2(d)). The chromogenic reaction at the test line indicates an HFRS patient who contains antibody against Hantaan virus or Seoul virus in the specimen. The control line indicates the performance of the kit. It should always be red (Figure 2(a)). If it does not show a chromogenic reaction, the result is invalid (Figure 2(e)).

The evaluation of clinical performance was performed using totally 139 patients specimens suspected to HFRS. IFA results have showed 91 specimens were positive in HFRS and 48 were negative. And 55 healthy control specimens used in this study were all negative in HFRS. The 91 HFRS-positive specimens were used for the sensitivity evaluation. Among these 91 specimens, 74 were positive to IgM and 88 were positive to IgG at IFA. Among 120 specimens that were negative at either IgM or IgG at IFA, each IgM negative 120 or IgG negative 106

Table 1. Sensitivity and specificity of the ImmuneMed Hanta Rapid Kit compared with the results of IFA for each IgM and IgG using domestic specimens which are from 91 HFRS, 48 other infectious diseases, and 55 healthy controls

Reaction at IFA	IgM		IgG		IgM/IgG	
	+*	-†	+*	-†	HFRS‡	Others§
RDK positive	69	3	82	2	88	3
RDK negative	5	117	6	104	3	100
Total	74	120	88	106	91	103
Sensitivity, %	93.2		93.2		96.7	
Specificity, %	97.5		98.1		97.1	
Sensitivity, % (95 % CI)	84.52-98.39		87.95-98.45		93.03-100.37	
Specificity, % (95 % CI)	94.72-100.28		95.49-100.71		93.87-100.33	

* : positive at IFA IgM or IgG among HFRS sera
† : negative at IFA IgM or IgG among HFRS, other infectious diseases and healthy controls
‡ : diagnosed HFRS by IFA
§ : other diseases or healthy controls with negative at IFA IgM and IgG to HFRS

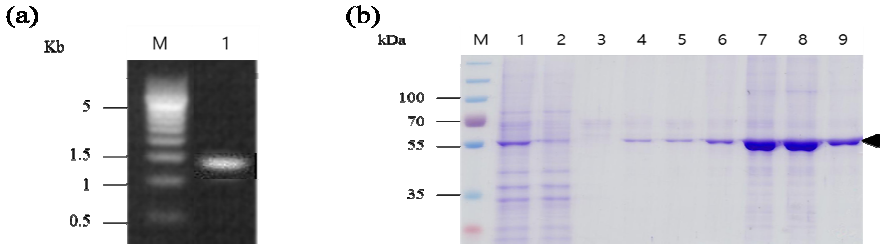

Figure 1. Amplification and purification of complete nucleocapsid protein (CNP). Amplification of CNP gene from Soochong virus (a) (lane 1: 1.3Kb). The DNA base pair size marker (lane M). SDS-PAGE of recombinant CNP (about 50kDa) (b). Loading sample (lane 1), loading waste (lane 2), binding waste (lane 3), washing waste (lane 4), elution 1 (lane 5), elution 2 (lane 6), elution 3 (lane 7), elution 4 (lane 8), elution 5 (lane 9)

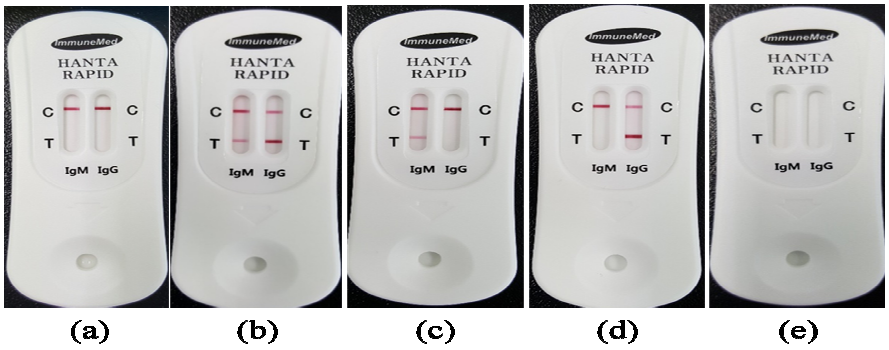

Figure 2. Representative pictures for rapid diagnostic kit (RDK) indicating negative and positive results. Red colorization of the test line (T) indicates the presence of human antibody against Hantavirus and the red colorization of control line (C) represents the validation of test. The RDT result shows negative (a), both IgM and IgG positive (b), IgM positive (c), IgG positive (d), and invalid (e).

was used to assess each specificity of RDK to IgM or IgG. When the result of IFA is standard, variables indicate the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Accuracy (ACC) was calculated by (TP+TN)/(TP+FP+FN+TN). Sensitivity and specificity were calculated by TP/(TP+FN) and TN/(TN+FP), respectively.

In a clinical performance evaluation with 194 specimens collected in Korea, it showed 96.7 % sensitivity and 97.1 % specificity. Specifically, the IgM evaluation (to IFA with positive 74 and negative 120 specimens) showed 93.2 % sensitivity and 97.5 % specificity, while IgG evaluation (to IFA with positive 88 and negative 106 specimens) showed 93.2 % sensitivity

and 98.1 % specificity (Table 1).

HFRS is one of the AFI. As such AFI diseases share similar clinical symptoms and epidemiology, clinicians have difficulty in carrying out differential diagnosis merely based on a patient's clinical symptoms and history taking. Therefore, HFRS can be easily diagnosed using the diagnostic kit developed in this study. Also this diagnostic kit can detected IgM and IgG separately and simultaneously, HFRS can be diagnosed at early stage through detection of IgM, and the progression of the disease can be understood by detection of IgM and IgG.

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