

Development and Evaluation of an Immunochromatographic Kit for the Detection of Antibody to *PLASMODIUM VIVAX* Infection in South Korea

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Malaria is a major parasitic disease in tropical areas. Three to five hundred million people suffer from the disease and it kill a million people per year. Blood smear observation was developed for the diagnosis of malaria, but the examination needs skilled experts and exact diagnosis is time consuming. A kit based on immunochromatography can be a reliable and rapid method for clinical diagnosis, even in the hands of inexperienced personnel. However, all such currently developed kits can only diagnose *P. falciparum* malaria. In our previous report, the C-terminal region of *P. vivax* merozoite surface protein 1 (PvcMSP) was cloned and expressed in *E. coli*. In the present study, we developed an immunochromatographic kit using this PvcMSP for the diagnosis of specific antibody to *P. vivax* malaria in serum samples. The kit was used to examine sera from vivax malaria patients and non-malaria-infected person and the test showed 100% sensitivity (78/78) and 98.3% specificity (58/59). This result demonstrated that the immunochromatographic kit for *P. vivax* antibody detection is applicable for the rapid and precise diagnosis of *P. vivax* malaria.

Key Words: *Plasmodium vivax*, immunochromatographic kit, merozoite surface protein

Malaria is a major parasitic disease causing hygienic problems in tropical areas. Since the 1970s, Korea has been classified as a malaria-free region. However, a case report in 1993 was a relapse sign of *vivax* malaria in Korea, and since then the number of malaria patients has been gradually increasing. Though more than three thousand patients have been reported, many researchers accept that Korea is reclassified as a non-endemic area.^{1,2} Currently, the diagnosis for malaria is performed with various methods using the antigen-antibody reaction. However, it remains to be elucidated whether the antibodies that were induced by past-infection of malaria can affect the current malaria diagnosis. Thus, the search for a specific antigen at a molecular level has been required to rule out this problem. Otherwise, the detection technique of *vivax* malaria antigen using specific antibodies is necessary.

Notable antigens for the diagnosis of malaria are histidine-rich protein II (PfHRPII) and lactate dehydrogenase (PfLDH) of *P. falciparum*, which are expressed in merozoite-staged malaria, secreted to erythrocyte cytoplasm, or passively diffuse to human plasma.^{3,4} ICTTM Malaria P.f/P.v., OptiMAL[®], and ParaSightTM F apply these antigens for the diagnosis of *falciparum* malaria.⁵⁻⁷ Though these kits can also diagnose *vivax* malaria, their sensitivity and specificity are lower than those for *falciparum* malaria.⁸ Thus, for accurate

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diagnosis of *vivax* malaria, a specific antigen derived from *P. vivax* is strongly required.

Merozoite surface protein (MSP) has been proposed as one of most probable candidate antigens for diagnosis and vaccination in many studies.^{9,10} The C-terminal fragment of merozoite surface antigen-1 of *P. vivax* (PvcMSP) was cloned in our previous study.¹¹ The recombinant PvcMSP showed high sensitivity and specificity for the diagnosis of *vivax* malaria. In the present study, an immunochromatographic kit composed of the recombinant PvcMSP was developed for the diagnosis of *vivax* malaria. The performance of the kit was examined using the sera obtained from patients of *vivax* malaria who showed signs with high fever and were identified by blood examination in Won Kwang Medical College Hospital (Iksan, Korea). For control groups, an anti-nucleic acid antibody and other sera obtained from healthy persons and patients with hepatitis B, hepatitis C, and rheumatoid arthritis (provided by the Department of Clinical Pathology, Won Kwang Medical College Hospital, Iksan, Korea) were used. The *vivax* malaria infections were reconfirmed by blood smear examination under a light microscope. The patients with rheumatoid arthritis and anti-nucleic acid were classified as autoimmunity patients. The sera from healthy individuals were collected from persons free of malaria, HBV, HCV, rheumatoid arthritis, and autoimmune disease with anti-nuclear antibody.

For the conjugation of PvcMSP with colloidal gold, 100 μ l of 10 μ g/ml PvcMSP was added to 10 ml of 40 nm colloidal gold (British Biocell International, Cardiff, England) and stirred at room temperature for one hr. One hundred microliters of 10% BSA was added and stirred at room temperature for one hr. The mixture was centrifuged at 12,000 rpm at 15°C for one hr, the supernatant was removed, and the precipitate was resuspended with 1 ml of 5% trehalose. The concentration was determined by absorbance at 540 nm.

The immunochromatographic kit (so-called HB kit) was produced according to the following description, as indicated diagrammatically in Fig. 1. Sample pad (S&S, Eninbeck, Germany) was treated with 100 mM Tris pH 8.2 containing 1% Tween 20. Conjugate pad (Millipore, Massachusetts, USA) was treated with PvcMSP colloidal gold conjugate

at the absorbance of 1.0 in 5% trehalose. A mixture of 1.0 mg/ml of anti-mouse IgG and PvcMSP was dispensed at the speed of 1 μ l/cm on nitrocellulose membrane (Millipore) for control and test lines. All pads and membranes were completely dried and laminated on plastic card and cut to 0.4 cm width. The kit was positioned on a flat table and 100 μ l of serum was applied to the specimen window (Fig. 1A). The result was assessed with distilled water at 20 minutes after color development. Purple-red band on the test line indicates the presence of antibodies to *vivax* malaria. Purple-red color on the control line indicates that the kit was properly working (Fig. 1B).

A total of 147 serum specimens were tested to determine the sensitivity and specificity of the immunochromatographic kit. The sensitivity was 100% (78/78) (Table 1) and samples of positive and negative results are shown in Fig. 1B. In detail, 75 of 78 malaria serum specimens showed strong positive reactions, while the other 3 showed weak positive reactions (data not shown). Reactions immediately observed within two to three minutes were identified as strong positive, while reactions requiring up to 20 minutes to show sufficient developments were termed weak positive. No color change indicated a negative reaction.

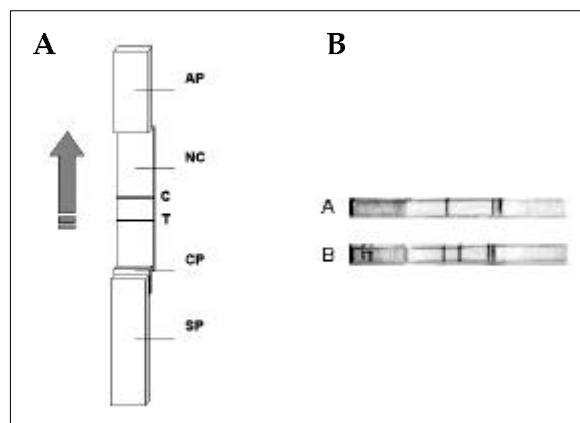


Fig. 1. Illustration of the immunochromatographic kit (1A). Sample pad (SP), conjugate pad (CP), nitrocellulose membrane (NC), and absorbent pad (AP) were laminated on sticky plastic cards. The positions of control (C) and test (T) lines were assigned on nitrocellulose membrane. The complete strip was assembled in plastic housing. The arrow indicates the flow direction of serum specimen; HB kit (1B) showing detection of *P. vivax* (B) and a negative serum sample (A). A, Serum of healthy person; B, Serum of *P. vivax* patients.

Table 1. Diagnosis of *Vivax* Malaria and Other Infections by the HB Kit

Sera from patients of	(n)	positive	negative	%
Healthy persons	(29)	1	28	96.6
<i>Vivax</i> malaria	(78)	78	0	100
Other diseases				
Hepatitis B	(8)	0	8	100
Hepatitis C	(7)	0	7	100
Rheumatoid arthritis	(7)	0	7	100
Other*	(8)	0	8	100

*Anti-nuclear antibody; positive.

Table 2. Sensitivity and Specificity of the HB Kit for *Vivax* Malaria Compared with the ICTTM Malaria P.f/P.v Kit

Test kits	Sera of malaria patients			Sera of non-malaria persons		
	Positive	Negative	Sensitivity(%)	Negative	Positive	Specificity(%)
HB kit	143	5	96.6*	202	19	91.4 [†]
ICT TM Malaria P.f/P.v	78	22	78.0	90	10	90.0

* t-test, $p < 0.05$.

[†] not significant.

Our previous work revealed that the sensitivity of recombinant PvcMSP by ELISA was 99.8%,¹¹ and confirmed that this sensitivity was both sufficient for practical use and was higher than that of other kits developed for the diagnosis of malaria. The HB kit, which was developed with the PvcMSP antigen in this study, showed a similar level of sensitivity to the ELISA test. Most of the kit reactions were readable within five minutes, suggesting the applicability of the kit for rapid diagnosis. As described in our previous report, the intensity on the test line is not proportional to the merozoite density in the blood because the individual immune reactions of the patients to malaria infection differ depending on the genetic characteristics of each patient.

The specificity of the immunochromatographic kit was examined using healthy persons or other sera obtained from patients with hepatitis B or C, rheumatoid arthritis or autoimmune disease with anti-nucleic acid antibody (Table 1). Sera from the HBV (n=8) and HCV (n=7) infections did not show positive reactions. Sera from the patients with rheumatoid arthritis (n=7) and autoimmune disease with positive anti-nuclear antibody (n=8)

also did not show positive reaction. However, one of the 29 sera from healthy persons showed false positive reaction. The specificity, including the results of sera from healthy persons, was 96.6% (28/29). It is not known what caused the non-specific result in the sera from one healthy person. In an endemic area, it was reported that the antibodies to malaria, however, are often present in cured patients.⁶

For the comparison of sensitivity with other developed kits, the sera from *P. vivax* malaria patients were examined with the HB (n=148) and ICTTM Malaria P.f/P.v (AMRAD) (n=100), and the sensitivities were determined as 96.6% (143/148) and 78.0% (78/100), respectively (Table 2). The sensitivity of the HB kit was significantly higher than that of the ICTTM Malaria P.f/P.v (t-test, $p < 0.05$). For a comparison of specificity (Table 2), the two test kits were examined with the sera from non-malaria-infected persons and showed 91.4% (202/221) and 90.0% (90/100) specificity, respectively. The ICTTM Malaria P.f/P.v kit is used mainly for the diagnosis of falciparum malaria. Diagnosis of *vivax* malaria depends on pan-malarial antigen, for which the sensitivity to *vivax*

malaria has been fully evaluated to date. Our data suggest that the use of PvcMSP antigen can improve the sensitivity of a diagnostic kit for *vivax* malaria.

In this report, we found that an immunochromatographic kit using PvcMSP as a *vivax* malaria-specific antigen is a specific and rapid method for the diagnosis of malaria in the field examination setting. The higher sensitivity recorded confirms that this kit is more accurate than the ICTTM Malaria P.f/P.v. kit for the diagnosis of *vivax* malaria.

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