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Evaluation of a Rapid IgG4 Lateral Flow Dipstick Test to Detect *Strongyloides stercoralis* Infection in Northeast Thailand

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Abstract. *Strongyloides stercoralis* affects more than half a billion people worldwide, and hyperinfection in immunocompromised patients can be fatal. Elimination of this neglected tropical disease requires field-applicable diagnostic tools. We conducted a laboratory evaluation of a lateral flow rapid dipstick test (SsRapid™) using sera samples from a *Strongyloides*-endemic area in northeast Thailand. Group 1 was *S. stercoralis*-positive and larvae- and/or antibody-positive (according to the IgG ELISA) ($N = 100$). Group 2 had negative fecal examination and IgG ELISA results ($N = 25$). Group 3 had other parasitic infections and negative IgG ELISA results ($N = 25$). The results showed good diagnostic sensitivity (82%) and excellent specificity (96%). Suggested improvements in the SsRapid™ test include increased diagnostic sensitivity and conversion to the more robust cassette format. Field studies should be performed as well.

Strongyloides stercoralis is the main causative agent of strongyloidiasis, a neglected tropical disease affecting 613.9 million people worldwide.[1] A high prevalence of *S. stercoralis* infection (as high as 61%) has been reported in northeastern communities in Thailand.[2] The availability of a field-applicable rapid test is essential for epidemiological studies to assess its true prevalence and impact and to facilitate an elimination program. It is also beneficial for diagnosing patients in low-resource settings.

To date, there have been only three reports of the development of prototype lateral flow rapid tests for strongyloidiasis.[3–5] One of them, SsRapid™, is an IgG4 dipstick test lined with *S. stercoralis* recombinant proteins.[5] The IgG4 level is significantly increased in individuals with chronic strongyloidiasis, and this level is higher in treatment-resistant patients.[6,7] IgG4 is also known to be highly specific for the detection of strongyloidiasis and other helminth infections. Our previous study showed that the diagnostic specificity increased 13.3% when IgG4 replaced IgG as the secondary antibody used for an ELISA.[8]

The initial laboratory-based evaluation of SsRapid™ showed that its diagnostic sensitivity and specificity were 91.3% and 100%, respectively.[5] Further studies are needed to evaluate the diagnostic value of the rapid test and to suggest further improvements. Therefore, the present study aimed to evaluate SsRapid™ using serum samples collected from an endemic area in northeast Thailand.

The sample subjects recruited during this study were residents of a suburban area of Khon Kaen province, northeast Thailand. The map of the study area is available elsewhere.[9]

After the project protocols were explained, written informed consents were requested from the sample subjects. Blood and fecal samples were collected from the volunteers. Finally, 150 matched samples were used for the analysis during this study. Blood samples were collected by venipuncture. Fecal samples were collected in wide-mouth containers, kept at ambient temperature, and transported to the laboratory. The study protocol for collecting and examining clinical samples was approved by the Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (HE621073).

The participants were assigned to three groups based on fecal examination and serum based-IgG ELISA results (Table 1). Group 1 was *S. stercoralis*-positive and larvae- and/or antibody-positive (IgG ELISA) ($N = 100$). Group 1 was divided into group 1.1 (positive for larvae and antibody) and group 1.2 (positive for antibody). Group 2 was negative according to both testing methods ($N = 25$). Group 3 had other parasitic infections and negative IgG ELISA results ($N = 25$).

Fecal samples were processed to detect *S. stercoralis* larvae using the agar plate culture technique (APCT) and formalin-ethyl acetate concentration technique (FECT).[10–12] When using the FECT, the results of triplicate examinations of each fecal sample were combined. The results of the sample were defined as positive if either the APCT or the FECT found at least one larva. The serum IgG ELISA using the *S. ratti* antigen was performed according to previously reported prototcols.[13,14]

The dipsticks were prepared at the Institute for Research in Molecular Medicine, Universiti Sains Malaysia, as previously described.[5] Mouse anti-human (Merck Millipore, Darmstadt, Germany) was conjugated to colloidal gold particles (optical density 10 at 530 nm).[15] A volume of 25 μL of the conjugate in drying buffer (0.05 M Na_2HPO_4, 1% w/v bovine serum albumin, 0.1% w/v NaN_3, 5% v/v trehalose) was added to the middle well of a triplet-well unit of a flat-bottomed ELISA microplate and dried at 35°C. In a dry room (≈20% humidity), aluminum test pouches were prepared. Each pouch contained a dipstick, a triplet-well unit (wells A, B, C), and a desiccant. All pouches were sealed. One-hundred fifty rapid tests and two microplate well holders were couriered from Universiti Sains Malaysia to Khon Kaen University at room temperature. On arrival at Khon Kaen University, they were kept for approximately 2 months at 4°C before use.

The test was performed as reported previously.[5] Briefly, two drops of buffer A were placed into well C, and 25 μL of buffer B was pipetted in well B to reconstitute the dried IgG4–gold. In well A, serum (10 μL) was mixed with an equal volume of buffer

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TABLE 1
Summary of the evaluation results of the SsRapid™ dipstick test using serum samples from a *Strongyloides* endemic area in northeast Thailand

Group	Assessed, n	SsRapid™ reactivity		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Group 1 Positive *S. stercoralis*	100	82	18		
1.1 Positive *S. stercoralis* larvae and antibody	84	69	15	69/84 (82)	
1.2 Positive *S. stercoralis* antibody	16	13	3		
Group 2 Negative in endemic areas	25	6	19		
Group 3 Other parasites	25	1	24		24/25 (96)
Echinostoma sp.	1	0	1		
Hookworm	9	1	8		
Minute intestinal fluke	1	0	1		
Opisthorchis viverrini	7	0	7		
Taenia sp.	1	0	1		
Minute intestinal fluke + hookworm	1	0	1		
O. viverrini + hookworm	1	0	1		
O. viverrini + minute intestinal fluke	1	0	1		
O. viverrini + *Taenia* sp.	2	0	2		
O. viverrini + minute intestinal fluke + *Echinostoma* sp.	1	0	1		

Group 1: Positive for *S. larvae* and/or antibody (*S. ratti* ELISA). Cutoff: 132 units.
Group 2: Negative for *S. stercoralis* antibody and fecal examination. This group resides in endemic areas.
Group 3: Positive for other parasites according to the fecal examination (formalin-ethyl acetate concentration technique) results.
The positive and negative predictive values of the SsRapid are 98.6% and 61.5%, respectively, and the accuracy is 85.3%. The receiver-operating characteristic curve shows an area under the curve of 0.8303 to 0.9631 ($P < 0.0001$) (Supplemental Figure S5).

B. After the mixture migrated up the dipstick, it was transferred to well B. When the IgG4–gold conjugate was fully absorbed, the dipstick was transferred to well C for a washing step. After approximately 10 minutes, the background cleared and the dipstick result was read: two red lines (control and test lines) on the dipstick indicate a positive result, and one red control line denotes a negative result. Several documents, including a quick procedure guide (flow chart), an instruction sheet, a video of how to perform the test, a dipstick line intensity chart, and images of some dipstick results, were sent via e-mail to Professor Sithithaworn at Khon Kaen University (Supplemental Figures S1, S2, S3, and S4).

Table 1 shows the evaluation results. The positive and negative results were converted into binary data for Receiver Operating Characteristic (ROC) analysis using GraphPad Prism version 8.0.2. The area under the curve (AUC) is 0.8303 to 0.9631 ($P < 0.0001$) (Supplemental Figure S5). The diagnostic sensitivity of SsRapid™ based on the serum samples (larvae-positive, IgG-positive) of group 1.1 was 82% (69/84), and the diagnostic specificity based on the serum samples (other parasitic infections, IgG-negative) of group 3 was 96% (24/25). The positive and negative predictive values were 98.6% and 61.5%, respectively, and the accuracy was 85.3%.

SsRapid™ was positive for 81.3% (13/16) of group 1.2 serum samples (larvae-negative, IgG-positive). The IgG4 rapid test was positive for 24% (6/25) of group 2 sera from apparently healthy people from a *Strongyloides*-endemic area. The group 2 samples were excluded from the diagnostic specificity determination because some group members may have been infected but asymptomatic and IgG-negative. An analysis of the IgG antibody levels and positive rates found by the IgG4 dipstick revealed a statistically significant positive association (chi-square test, $P < 0.05$) (Supplemental Table S6). Figure 1 shows the representative images of the dipstick results.

Table 2 shows a comparison of the results and parameters of the present study and the other reported studies using lateral flow rapid tests for strongyloidiasis. The diagnostic specificity of the cassette tests using larvae extract or *S. stercoralis*

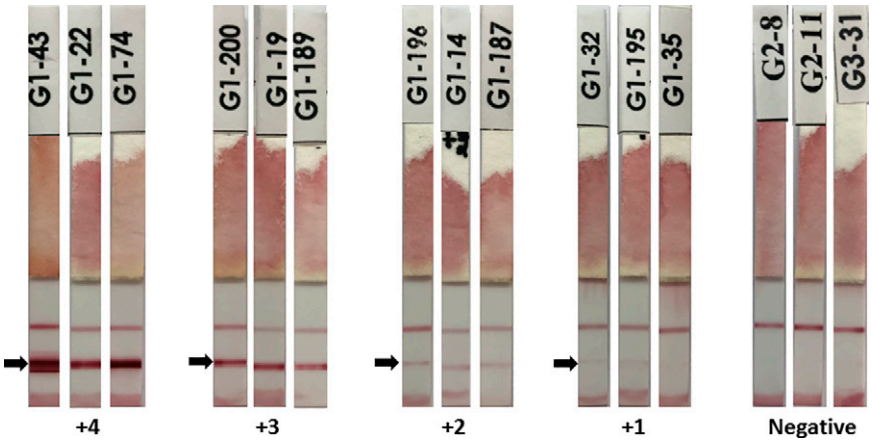

FIGURE 1. Representative images of the SsRapid™ dipstick results. The arrow shows the test line. The control line is above the test line. The reddish color at the bottom end of the dipstick, which may appear like a line, is the stain of the IgG4–gold conjugate; therefore, it is ignored when reading the results. This figure appears in color at www.ajtmh.org.

TABLE 2
Studies of lateral flow rapid tests for the detection of *Strongyloides stercoralis* infection

No.	Format	Antigen	Detecting antibody (gold-conjugated)	Sample dilution	Diagnostic sensitivity	Diagnostic specificity	Positive and negative predictive values	Intensity reading chart	Location	Reference
1a	Cassette	Recombinant protein (SsIR)	IgG	1:100	91.7% (55/60)	83.8% (88/105)	76.4%, 94.6%	Nine line intensity levels, 8–0.5; cutoff intensity level ≥ 1	Thailand	Boonroumkaew et al. (2020)
1b	Cassette	Recombinant protein (SsIR)	IgG4	1:100	78.3% (47/60)	84.8% (89/105)	76.4%, 87.3%	Nine line intensity levels, 8–0.5; cutoff intensity level ≥ 0.5	Thailand	Boonroumkaew et al. (2020)
2	Cassette	Larvae extract of *Strongyloides stercoralis*	IgG	1:50	93.3% (56/60)	83.7% (87/104)	76.7%, 95.6%	Nine line intensity levels, 8–0.5; cutoff intensity level ≥ 0.5	Thailand	Sadaow et al. (2020)
3	Dipstick	Recombinant proteins (rNIE and rSs1a)	IgG4	1:2	91.3% (21/23)	100% (82/82)	100%, 7.6%	Intensity of the test line was quantified using image analysis software	Malaysia	Yunus et al. (2019)
4	Dipstick	Recombinant proteins (rNIE and rSs1a)	IgG4	1:2	82% (69/84)	96% (24/25)	98.6%, 61.5%	Four intensity scores for positive results: +1 to +4; negative result: no test line	Thailand	Present study

SsIR = *S. stercoralis* immunoreactive recombinant antigen. All evaluation studies except study 4 were performed at the location where the tests were developed.

immunoreactive (SsIR) recombinant antigen protein was approximately 84% to 85%. The diagnostic sensitivity of the rapid IgG cassette tests using larvae extract and *S. stercoralis* immunoreactive recombinant antigen protein were similar (91.7% and 93.3%, respectively). The sensitivity rates were higher than that of the IgG4 cassette rapid test (78.3%) using the same recombinant protein.[3,4] *Strongyloides* IgG4 antibody levels are increased during chronic infection, whereas IgG is detected with both acute and chronic strongyloidiasis.[7] Therefore, it is not surprising that, generally, the infection prevalence detected by IgG is higher than that detected by IgG4.

SsRapid™ is an IgG4 test. The first evaluation study was performed in-house (at Universiti Sains Malaysia) using positive samples from Malaysia, and it showed high diagnostic sensitivity (91.3%) and specificity (100%). During the present study, SsRapid™ was evaluated using serum samples from a northeast Thailand endemic area. The results showed reduced diagnostic sensitivity (82%), but the specificity remained high (96%). Whether the reduced sensitivity can be attributed to the fact that the *Strongyloides* serum samples were from a different country or to technical issues must be investigated. The dipstick format requires two pipetting steps and moving the dipstick from well to well. Therefore, it may be more prone to technical inconsistency than a cassette test format with a much simpler procedure. Nevertheless, the diagnostic sensitivity of SsRapid™ during the present study (82%) was slightly higher than that of the IgG4 cassette test (78.3%) as reported by Boonroumkaew et al.[2] There was no cross-reactivity with *Opisthorchis viverrini* and other parasites (*Taenia*, minute intestinal flukes, and *Echinostome*) in northeast Thailand. A cross-reaction with hookworms (1 of 9 cases) was observed. In northeast Thailand, *Strongyloides* and *O. viverrini* are the two most common parasites, followed by a very low prevalence of the other helminth infections (Table 1). To date, there has been no report of *Schistosoma* in the area; therefore, they were not included in the specificity evaluation. During our previous study performed at the Universiti Sains Malaysia, 11 schistosomiasis sera were negative when tested with the rapid dipstick test.[5] Other notable results from this study were the significant area under the curve of the receiver-operating characteristic curve, significant correlation between the IgG ELISA units and SsRapid™ positive rates, and good test accuracy.

A simpler and more robust cassette format of SsRapid™ needs to be developed. The diagnostic sensitivity should be at least 90% when tested with samples from larvae-positive individuals, and the diagnostic specificity should remain higher than 95%. Some of the samples with false-negative results during the present evaluation will be used to optimize the SsRapid™ cassette test to help improve its diagnostic sensitivity. Afterwards, it should be evaluated at Khon Kaen University and other places, and during field studies. The test should also work well with whole blood and eluted blood samples to make it field-applicable.

Near the end of a disease elimination program, the infection prevalence is low; therefore, a diagnostic test with high specificity is crucial to support decisions to stop mass drug administration.[16] The high specificity of SsRapid™ may indicate its suitability for such use in the future.

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