Accuracy of Urine and Serum Assays for the Diagnosis of Strongyloidiasis by Three Enzyme-Linked Immunosorbent Assay Protocols

Sirowan Ruantip,¹ Chatanun Eamudomkarn,² Anchalee Techasen,^{3,4} Chompunoot Wangboon,^{1,2} Jiraporn Sithithaworn,⁵ Jeffrey M. Bethony,⁶ Makoto Itoh,⁷ and Paiboon Sithithaworn^{2,3*}

¹Biomedical Science Program, Graduate School, Khon Kaen University, Khon Kaen, Thailand; ²Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; ³Cholangiocarcinoma Research Institute (CARI), Khon Kaen University, Khon Kaen, Thailand; ⁴Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand; ⁵Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand; ⁶Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, District of Columbia; ⁷Department of Infection and Immunology, Aichi Medical University School of Medicine, Nagakute, Japan

Abstract. To evaluate the accuracy and reliability of urine assay for the diagnosis of strongyloidiasis, three different immunoassays were used to assess the diagnostic accuracy of anti-*Strongyloides* immunoglobulin G (IgG) in urine and compared with those in serum samples. Analyses by InBios enzyme-linked immunosorbent assay (ELISA) kit (recombinant NIE antigen), SciMedx ELISA kit (*Strongyloides stercoralis* antigen), and our in-house ELISA (*Strongyloides ratti* antigen) yielded comparable diagnostic performances between urine and serum assays. Levels of *Strongyloides*-specific IgG in urine significantly correlated with those in serum. Tests for diagnostic agreement between urine and serum IgG assays showed substantial to fair agreement ($\kappa = 0.207\text{--}0.615$). The observed quantitative and qualitative concordance between urine and serum assays in strongyloidiasis suggests that urine has similar diagnostic value to that for serum. Because of the ease and noninvasiveness of clinical sample collection, urine assay has a high potential for the initial diagnosis and mass screening of strongyloidiasis.

Strongyloidiasis is a neglected tropical disease caused by *Strongyloides stercoralis* infection. It has a worldwide distribution, but is particularly prevalent in tropical and subtropical regions.^{1,2} The diagnosis of strongyloidiasis has traditionally been based on fecal examination; however, this showed suboptimal sensitivity.² Several alternative methods have been developed to raise the diagnostic performance of strongyloidiasis. The most widely used serological assay was the enzyme-linked immunosorbent assay (ELISA) for detecting *Strongyloides*-specific antibody in serum by using several types of parasite-specific antigens.^{3,4} Detection of parasite-specific DNA by conventional and real-time polymerase chain reaction (PCR) has been established in feces and in urine for diagnosis of strongyloidiasis.^{5–8} As an alternative to blood, urine assay gave a similar diagnostic performance to serum IgG for immunodiagnosis of strongyloidiasis.⁹ The recently established urine assay approach has not been validated by other immunoassay protocols. We hypothesized that urine antibodies are detectable by different ELISA protocols and provide diagnostic value similar to serum antibody assay.

The aim of this study was to explore the reliability and performance of the urine assay by evaluating its diagnostic accuracy for strongyloidiasis in comparison with conventional serum assays using three different types of antigen in the ELISA. In addition, we also assessed the quantitative relationship between levels of parasite-specific IgG in urine and in serum by the same ELISA protocols.

This project was a prospective cross-sectional study, and the clinical samples for the reference tests and the other index tests were blinded during the specimen analyses. The laboratory staff involving in the study had no access to the serum, urine, and fecal code of participants. The sample population participated in this study were residents in a known endemic

area of strongyloidiasis in a suburban area in Amphoe Meuang district, Khon Kaen Province, northeast Thailand.

The participants (≥ 15 years old) present in the study area during the study period from September 2014 to January 2015 were recruited for the project. Written informed consents were obtained from each participant before the project initiation. Then, arrangements for sample collections were carried out as previously described.⁹ The experimental protocol in this study was approved by the Khon Kaen University Institutional review board (HE601434). The parasite-positive participants were treated with suitable anthelmintic.¹⁰

The status of parasitic infection was assessed by the agar plate culture technique (APCT) using 4 g of feces¹¹ and the formalin-ethyl acetate concentration technique (FECT).¹² The combined results of these fecal examination methods were used for either mono or mixed infection with *S. stercoralis*.

Analyses of serum and urine sample for *Strongyloides*-specific IgG antibody were carried out by our in-house ELISA and by two commercial kits. For the in-house ELISA,⁹ an undiluted sample was used in the urine assay and a sample dilution of 1:8,000 was used in the serum assay. The optical density (OD) was measured at a wavelength of 492 nm. For the commercial kits, the InBios Strongy Detect IgG ELISA (InBios International, Inc., Seattle, WA) and the SciMedx *Strongyloides* serology microwell ELISA (SciMedx Corporation, Denville, NJ) were used. The protocols supplied by the manufacturer were followed for the serum analyses. Based on our preliminary study, the ELISA protocol for urine analysis of the index tests was modified by extending the incubation time for the tested sample, conjugate, and substrate to 60 minutes. The OD was measured at a wavelength of 450/620 nm.

Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic parameters of the urine and serum ELISA compared with the primary reference standard by APCT and FECT. The cutoff values for the in-house ELISA of serum and urine were determined using arbitrary units and the values higher than or equal to 89.44 and 107.44 were interpreted as positive for the urine and serum ELISA, respectively.

* Address correspondence to Paiboon Sithithaworn, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. E-mail: paibosit@gmail.com

TABLE 1

Positive infection rates for strongyloidiasis by different immunoassay methods and tests for diagnostic agreement between urine and serum assays in the sample population ($n = 54$)

ELISA	Serum		Urine	
	No positive (%)	No positive (%)	P-value*	Kappa
In-house	41 (75.9)	39 (72.2)	> 0.05	0.615
InBios strongy detect	37 (68.5)	36 (66.7)	> 0.05	0.207
SciMedx <i>Strongyloides</i>	43 (79.6)	33 (61.1)	0.031	0.232

ELISA = enzyme-linked immunosorbent assay. Diagnosis by combined fecal examination methods (agar plate culture technique and formalin-ethyl acetate concentration technique) detected *Strongyloides stercoralis* in 35 individuals (64.8%). Data shown are number and percentile of positive tests by serum and urine assays. Data shown for kappa are in agreement in proportions.

* Obtained from McNemar's chi-square test.

Based on similar approaches, the cutoff OD values for the InBios ELISA kit were 0.178 and 0.096 for serum and urine, respectively. The cutoff OD values for the SciMedx ELISA kit were 0.100 and 0.063 for serum and urine, respectively. MedCalc version 11.6.1.0 software (Ostend, Belgium) was used to establish the ROC curve.

SPSS version 21 (IBM, Chicago, IL) was used to calculate the diagnostic performance of serum and urine assays as well as for statistical tests by McNemar's chi-square (sensitivity, specificity, and positive infection rate), Kendall rank correlation, and agreement tests. Overall agreement, as determined by the kappa value (κ), was interpreted as follows: almost perfect, 0.81–1.0; substantial, 0.61–0.80; moderate, 0.41–0.60; fair, 0.21–0.40; slight, 0–0.20; and poor, < 0 .¹³

From the original 180 intended participants, 54 individuals provided a completed set of clinical samples with urine, serum, and feces. The participants comprised 32 men and 22 women with a mean age (standard deviation [SD]) of 60.04 (10.2) years. The combined results of fecal examinations showed that 35 of 54 individuals (64.8%) were infected with *S. stercoralis*, and nine cases had a mixed infection with other parasites (*Opisthorchis viverrini* six, minute intestinal flukes 2, and *Taenia* spp. 1). The positive rates by serum IgG assay were higher than those of the urine but the statistical significance was observed only in SciMedx ELISA kit ($P < 0.05$) (Table 1).

In the diagnostic agreement tests between the serum and urine assays, the in-house ELISA showed a moderate agreement ($\kappa = 0.615$), whereas the SciMedx and InBios ELISA kits had a fair agreement ($\kappa = 0.232$ and 0.207, respectively).

When fecal examination was used as a reference standard, the measurement of IgG in serum gave the sensitivity of 82.9–97.1% and specificity of 42.1–63.2% (Table 2). By serum assay, the in-house ELISA had the highest sensitivity (97.1%), followed by the SciMedx ELISA kit (91.4%) and the InBios ELISA kit (82.9%). The specificity was moderately high for

both the in-house and InBios ELISA kit and lower for the SciMedx ELISA kit.

When using fecal examination as a reference standard, the diagnostic values of urine IgG assays were slightly lower than those of serum. The highest sensitivity was seen in the in-house ELISA (82.9%), and both kits showed a similar sensitivity of 62.9% but with a lower specificity (38.9–47.4%).

In a comparison between serum and urine ELISA, the sensitivity and specificity of three ELISA were similar, except for SciMedx ELISA kit that the sensitivity for serum was significantly higher than that for urine ($P = 0.004$), and the specificity of InBios ELISA kit for serum was significantly higher than that for urine ($P = 0.046$) (Table 2).

In the quantitative comparison between the serum and urine assays, the levels of IgG in the serum were significantly positively correlated with the in-house ELISA values ($r = 0.670$, $P < 0.0001$) and those for the InBios ELISA kit ($r = 0.533$, $P < 0.0001$). The correlation between serum and urine was less strong in case of the SciMedx ELISA kit ($r = 0.272$, $P = 0.0469$).

The finding that seropositive rates by IgG measurement in urine were comparable to those of sera as detected by our in-house ELISA and the commercial kits indicates that urine IgG is a reliable method for the diagnosis of strongyloidiasis in clinical specimens. The performance of our in-house ELISA was in accordance with previously published results obtained from the same endemic area.⁹ The use of the InBios and SciMedx ELISA kit has never been reported in Thailand or elsewhere in Southeast Asia. Both showed that *Strongyloides*-specific IgG could be detected in serum and urine. When using parasitological diagnosis as a reference method, the in-house ELISA provided a high concordance ($\kappa = 0.6$), whereas the two diagnostic kits gave relatively lower concordances ($\kappa = 0.2$). Considering the diagnostic performance parameters, serum ELISA from the in-house and the commercial kits showed high sensitivities (82–97%) and moderate specificities (42–63%).

When considering the urine assays, a greater diagnostic performance, in terms of sensitivity and specificity, was provided by our in-house ELISA, whereas relatively lower diagnostic values were seen for the commercial kits. This was probably due to the availability of full optimization of the protocol for the in-house ELISA. By contrast, the commercial kits were designed and optimized for serum testing and were not intend for urine assay. Thus, future work should aim for full optimization of assay protocol for urine analysis.

There are limitations in this study, such as cross-reactivity with other parasitic infections is not comprehensive particularly for *Trichinella spiralis* and filarial infection. Also, analysis of a more diverse sample populations from different geographical areas with different pattern of parasitic infection is required.

TABLE 2

Diagnostic performances of our in-house ELISA and two commercial ELISA kits for the serodiagnosis of strongyloidiasis using matched pairs urine and serum with reference to parasitological methods (agar plate culture and FECT) as a standard

ELISA	Serum assay			Urine assay			P-value*	
	Sens†	Spec†	AUC	Sens†	Spec†	AUC	Sens	Spec
In-house	97.1 (83.4–99.9)	63.2 (38.6–82.8)	0.833	82.9 (65.7–92.8)	47.4 (25.2–70.5)	0.753	> 0.05	> 0.05
InBios strongy detect	82.9 (65.7–92.8)	57.9 (40.0–78.9)	0.666	62.9 (44.9–78.0)	38.9 (10.1–51.4)	0.563	> 0.05	0.046
SciMedx <i>Strongyloide</i>	91.4 (75.8–97.8)	42.1 (21.1–66.0)	0.645	62.9 (44.9–78.0)	42.1 (21.1–66.0)	0.517	0.004	> 0.05

AUC = area under the curve; ELISA = enzyme-linked immunosorbent assay; FECT = formalin-ethyl acetate concentration technique; Sens = sensitivity; Spec = specificity. Data shown are diagnostic accuracy values (%).

* Obtained from McNemar's chi-square test (sens and spec).

† % (95% CI).

In conclusion, we show that the use of a urine assay for the measurement of anti-*Strongyloides* IgG for diagnosis of strongyloidiasis is possible. The ELISA protocols consisted of different sources of antigen, including *S. ratti*, NIE recombinant, and *S. stercoralis* crude antigen with comparable qualitative and quantitative results being obtained. Because of the ease of sample collection, the urine assay has a high potential for diagnosis and large-scale screening of strongyloidiasis in the surveillance and control program.

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Authors' addresses: Sirowan Ruantip and Chompunoot Wangboon, Faculty of Graduate School, KhonKaen University, Khon Kaen, Thailand, E-mails: sirowan.rtip@gmail.com and tookkiko@hotmail.com. Chatanun Eamudomkarn and Paiboon Sithithaworn, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, E-mails: paibsit@gmail.com and chatanune@yahoo.com. Anchalee Techasen, Cholangiocarcinoma Research Institute (CARI), Khon Kaen University, Khon Kaen, Thailand, and Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand, E-mail: anchaleetechasen@gmail.com. Jiraporn Sithithaworn, Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand, E-mail: jirapornsith@gmail.com. Jeffrey M. Bethony, Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, DC, E-mail: jeffreymbethony@gmail.com. Makoto Itoh, Department of Infection and Immunology, Aichi Medical University School of Medicine, Nagakute, Japan, E-mail: macfilaria@gmail.com.

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