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# Performance Assessment of a Lateral Flow Rapid Test (SsRapid®) Compared with Two Commercial ELISAs in Detecting *Strongyloides* Infection

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*Abstract.* Approximately 600 million people worldwide are infected with *Strongyloides stercoralis*. Many diagnostic laboratories use serology to detect the infection. SsRapid® is a prototype IgG4 lateral flow test based on NIE recombinant protein. We compared SsRapid with two commercial IgG-ELISAs (Bordier and Euroimmun) using five sera groups (G1–5, $N = 170$). Healthy individuals from nonendemic areas (G1, $N = 33$) and *Strongyloides* larvae-positive patients (G2, $N = 20$) showed no significant difference ($P > 0.05$) among the three assays. The group of other parasitic infections (G3) showed that SsRapid results were more concordant with Bordier ELISA (73%, 35/48) than Euroimmun ELISA (65%, 31/48). In corticosteroid-treated cancer patients who were polymerase chain reaction–positive for *Strongyloide*s (G4, $N = 25$), SsRapid showed a higher detection rate (28%, 7/25) than both ELISAs (4%, 1/25). Meanwhile, SsRapid showed significantly less infection prevalence among the endemic area population (G5) than the ELISAs. Overall, SsRapid showed good performance in detecting *Strongyloides* infection compared with the commercial IgG-ELISAs.

*Strongyloides stercoralis* is a neglected tropical disease that infects approximately 600 million people worldwide.[1] Approximately 2.6 billion people risk acquiring the infection.[2] The parasite can maintain decades of chronic, asymptomatic autoinfection in humans. This phenomenon poses a problem for immunosuppressed patients, especially those under corticosteroid treatment, because the low-level infection in such individuals may become a potentially fatal hyper and disseminated disease.[3]

Detecting *Strongyloides* larvae in stool during chronic infection is challenging due to intermittent and low larvae output. Thus, serological tests are used in many laboratories to help diagnose suspected patients, and researchers often use them for epidemiological studies. Commercial kits are ELISAs, with most of them detecting anti-*Strongyloides* IgG antibodies. Examples of commonly used commercial ELISA include Bordier IgG-ELISA (Bordier Affinity Products SA, Crissier, Switzerland), Euroimmun IgG-ELISA (Euroimmun, Lubeck, Germany), and IVD *Strongyloides* Serum Antibody Detection Microwell ELISA (IVD Research Inc., Carlsbad, CA).

SsRapid® is a commercial prototype point-of-care test that uses recombinant NIE protein as the test line, with 97% sensitivity and 94.5% specificity and is performed as described previously.[4] The aim of this study is to compare the SsRapid with two commercial IgG-ELISAs, one from Bordier and another from Euroimmun. The commercial ELISAs use parasite lysates, *S. ratti* in Bordier's kit and *S. papillosus* in Euroimmun's kit. They were performed and interpreted according to the manufacturer's instructions. The diagnostic sensitivity and specificity of Bordier ELISA were reported to be 89.5% and 98.3%, respectively,[5] and Euroimmun ELISA showed 90.6% sensitivity and 87.7% specificity.[6]

The assay comparison was performed using 170 samples divided into five sera groups (G1–G5). G1 was healthy individuals from *Strongyloides* nonendemic areas ($N = 33$), G2 was *Strongyloides* larvae-positive patients ($N = 20$), and G3 was individuals with other parasitic infections; most were from *Strongyloides*-endemic areas ($N = 48$). G4 was corticosteroid-treated cancer patients who were *Strongyloide*s-positive via real-time polymerase chain reaction (PCR), cycle threshold $\leq 35$ ($N = 25$). The sera were sampled at different times after corticosteroid treatment; we deemed it acceptable because even short-term corticosteroid treatment can initiate *Strongyloides* hyperinfection.[7] G5 were residents of a *Strongyloides* endemic area ($N = 44$). The use of the sera samples was approved by the Human Research Ethics Committee at Universiti Sains Malaysia; the approval reference for G1–G3 and G5 sera is USM/JEPeM/20040230 and for G4 sera is USM/JEPeM/20050254.

We used Graph Pad Prism version 8.0.2 (GraphPad Software, San Diego, CA) to perform a one-way analysis of variance to compare the three assays and a *t* test to compare between two assays; $P < 0.05$ was considered statistically significant. Cohen's kappa coefficient using IBM SPSS Statistics 28.0 (Armonk, NY) was used to analyze the agreement between assays. MedCalc statistical software version 20.115 (MedCalc Software Ltd, Ostend, Belgium) was used to perform the receiver operating curve (ROC) analysis.

Table 1 summarizes the results of assays tested on the five groups of serum samples. For GI (healthy) and G2 (*Strongyloides* larvae-positive), the three assays showed comparable performance with no significant difference in results ($P > 0.05$) among them. Healthy sera were from individuals living in *Strongyloides* nonendemic areas and thus were highly unlikely to be infected. Meanwhile, infected individuals were diagnosed based on finding *Strongyloides* larvae in their stools. Because both sera groups can be considered "defined" samples, the results of the three assays were thus accurate. Relative to the two ELISAs, the sensitivity and specificity of SsRapid were 94% and 100%, respectively. Furthermore, ROC analysis was performed using data from these two defined sera groups. It showed that SsRapid was 100%

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TABLE 1
Results of SsRapid, IgG-ELISA (Bordier), and IgG-ELISA (Euroimmun) tested on five sera groups

| Group | Assay type | Result | Frequency | Percent | Group | Assay type | Result | Frequency | Percent |
|---|---|---|---|---|---|---|---|---|---|
| Healthy individuals from *Strongyloides* non-endemic areas (G1, $N = 33$) | Bordier IgG-ELISA | Negative | 32 | 97.0 | Cancer patients positive for *Strongyloides* based on stool real-time PCR (G4, $N = 25$) | Bordier IgG-ELISA | Negative | 24 | 96.0 |
| | | Positive | 1 | 3.0 | | | Positive | 1 | 4.0 |
| | Euroimmun IgG-ELISA | Negative | 32 | 97.0 | | Euroimmun IgG-ELISA | Negative | 24 | 96.0 |
| | | Positive | 1 | 3.0 | | | Positive | 1 | 4.0 |
| | SsRapid | Negative | 33 | 100.0 | | SsRapid | Negative | 18 | 72 |
| | | Positive | 0 | 0.0 | | | Positive | 7 | 28 |
| *Strongyloides* larvae-positive patients (G2, $N = 20$) | Bordier IgG-ELISA | Negative | 2 | 10.0 | *Strongyloides* endemic area residents (G5, $N = 44$) | Bordier IgG-ELISA | Negative | 18 | 40.9 |
| | | Positive | 18 | 90.0 | | | Positive | 26 | 59.1 |
| | Euroimmun IgG-ELISA | Negative | 3 | 15.0 | | Euroimmun IgG-ELISA | Negative | 24 | 54.5 |
| | | Positive | 17 | 85.0 | | | Positive | 20 | 45.5 |
| | SsRapid | Negative | 1 | 5.0 | | SsRapid | Negative | 35 | 79.5 |
| | | Positive | 19 | 95.0 | | | Positive | 9 | 20.5 |
| Individuals with other parasitic infections (G3, $N = 48$) | Bordier IgG-ELISA | Negative | 39 | 81.3 | | | | | |
| | | Positive | 9 | 18.8 | | | | | |
| | Euroimmun IgG-ELISA | Negative | 29 | 60.4 | | | | | |
| | | Positive | 19 | 39.6 | | | | | |
| | SsRapid | Negative | 38 | 79.2 | | | | | |
| | | Positive | 10 | 20.8 | | | | | |

G = group; PCR = polymerase chain reaction.

sensitive and 97.1% specific, with an area under the curve (AUC) of 0.985. Bordier ELISA showed 94.74% sensitivity, 94.12% specificity, and an AUC of 0.944. Meanwhile, Euroimmun ELISA showed 94.44% sensitivity, 91.43% specificity, and an AUC of 0.929.

A recent study compared several *Strongyloides* ELISA kits using archived serum samples, which tested positive ($N = 45$) or negative ($N = 46$) for *Strongyloides* IgG based on an in-house ELISA and a composite reference standard. They found that Euroimmun and Bordier ELISAs showed comparable high values for positive percent agreements (100% and 95.8%), negative percent agreements (96.3% and 92.7%), overall percent agreements (97.2% and 93.4%), and Cohen's kappa indices ($\kappa$) (0.92 and 0.82), respectively. In our study, these two ELISA kits also showed good and comparable performance with the defined samples of Groups 1 and 2.[8]

For G3 (other infections), the assays' specificity (non-reactivity) rates were not expected to be very high since most samples came from *Strongyloides*-endemic areas; thus, some were possibly coinfected with the parasite. Cohen's $\kappa$ coefficient showed slight agreements between SsRapid and Bordier ELISA ($\kappa = 0.148$) and between SsRapid and Euroimmune ELISA ($\kappa = 0.194$). Higher concordance (percentage of concordant positive or negative results) of SsRapid was observed with Bordier ELISA (73%, 35/48) than with Euroimmun ELISA (65%, 31/48). The SsRapid® results differed significantly ($P < 0.05$) from the Euroimmun ELISA's. Meanwhile, there was a non-significant difference ($P > 0.05$) between the results of SsRapid and Bordier ELISA. It is consistent with a recent report in which SsRapid showed similar specificity with Bordier ELISA using sera from a migrant population.[9]

The corticosteroid-treated cancer patients positive for *Strongyloide*s via real-time PCR (G4) probably had chronic *Strongyloides* infection. They were neutropenic and advised to maintain good hygiene to prevent infections, thus were unlikely to have a recent *Strongyloides* infection. We also do not expect a high seropositive rate from these individuals due to their general immune-suppressed status. The results showed that the positive rate by the IgG4-SsRapid test was 28% (7/25), 7 times higher than the positives detected by the two IgG-ELISAs (4.0%, 1/25). There was a significant difference ($P < 0.05$) between SsRapid and the ELISAs, and Cohen's kappa coefficients showed no agreement between the rapid test and each of the ELISAs ($\kappa = 0.075$). During the early phase of *Strongyloides* infection, the level of IgG antibody is higher than IgG4 antibody. As the infection becomes chronic, both IgG and IgG4 antibody levels are elevated, and with time IgG4 level exceeds that of IgG due to the continuous antigenic stimulation of chronic infection.[10,11] Thus, a higher IgG4 level in chronic infection is consistent with the results of the G4 sera group.

The G4 results are also consistent with a recent report using sera from immunocompromised patients (HIV, blood, and solid organ cancers) at a hospital in Kuala Lumpur, Malaysia. SsRapid showed significantly higher ($P < 0.05$) seroprevalence (10.5%, 21/200) compared with Euroimmun ELISA (7.5%,15/200). Furthermore, the SsRapid-positive samples tested negative with the rapid test after sera adsorption with rNIE, indicating good specificity.[12]

Concerning G5 (endemic residents), Cohen's kappa coefficients showed slight agreements between SsRapid and Bordier ELISA ($\kappa = 0.138$) and between SsRapid and Euroimmun ELISA (kappa 0.183). There was a significant difference ($P < 0.05$) between SsRapid and each of the ELISAs. We were surprised to see many positive results with the ELISAs, that is, 40.9% (18/44) by Euroimmun ELISA and 59.1% (26/44) by Bordier ELISA. Meanwhile, 20.5% (9/44) of the G5 sera were positive by SsRapid, with six also positive by both ELISAs. In a previous report, Bordier ELISA's specificity was reduced from 97.2% ($N = 220$) to 88.7% ($N = 257$) after adding 37 filariasis sera to the samples tested.[13] Because G5 sera were collected from a *Brugia malayi* endemic area, we also tested the G5 samples for filariasis using the Brugia Rapid (BR) test (Reszon Diagnostics International, Selangor, Malaysia), a WHO-recognized test for *B. malayi* infection.[14] The results showed that eight G5 sera (18.2%) were positive by BR, with seven of the eight also positive by one of the three assays. If the seven sera were excluded (due to possible cross-reaction with filariasis), the *Strongyloides* seroprevalence by both ELISAs was still high: 40.5% (15/37) by Euroimmun and 51.4% (19/37) by Bordier.

G5 sera originated from a low-to-medium endemic region for *Strongyloides*. Thus, the high seroprevalence rate by the IgG-ELISAs in this study seemed perplexing. Some possible reasons could be the "excessive" sensitivity of the ELISAs, higher than expected cross-reactions with other helminth infections, or a significant number of "old" or nonactive infections detected by the IgG-ELISAs. In light of the G4 results in which the SsRapid showed a significantly higher detection rate among the chronically infected cancer patients than the IgG-ELISAs, we opined that the lower *Strongyloides* prevalence by SsRapid in G5 was not due to not detecting chronic infection. We hypothesize that the IgG-ELISAs detected IgG antibodies that remained after the infection was "cured." It will be necessary to perform a post-treatment follow-up study of *Strongyloides* patients using the three serological assays to confirm the hypothesis. Related to this, the BR test (mentioned earlier), also an IgG4 rapid test, showed a significant decrease in filariasis detection posttreatment and is used for evaluating the success of the mass drug administration in brugian filariasis areas.[15,16]

A limitation of our study is the small number of samples, especially G2 and G4. In conclusion, the SsRapid showed an overall good performance compared with the two commercial IgG-ELISAs. Its performance was comparable to the ELISAs with G1-G3 sera groups and better than the ELISAs with the G4 sera.

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## REFERENCES

1. Buonfrate D, Bisanzio D, Giorli G, Odermatt P, Fürst T, Greenaway C, French M, Reithinger R, Gobbi F, Montresor A, 2020. The global prevalence of *Strongyloides stercoralis* infection. *Pathogens 9:* 468.
2. Fleitas PE, Kehl SD, Lopez W, Travacio M, Nieves E, Gil JF, Cimino RO, Krolewiecki AJ, 2022. Mapping the global distribution of *Strongyloides stercoralis* and hookworms by ecological niche modeling. *Parasit Vectors 15:* 1–12.
3. Salvador F, Sulleiro E, Sánchez-Montalvá A, Saugar JM, Rodríguez E, Pahissa A, Molina I, 2014. Usefulness of *Strongyloides stercoralis* serology in the management of patients with eosinophilia. *Am J Trop Med Hyg 90:* 830–834.
4. Noordin R, Osman E, Kalantari N, Anuar NS, Gorgani-Firouzjaee T, Sithithaworn P, Juri NM, Rahumatullah A, 2022. A point-of-care cassette test for detection of *Strongyloides stercoralis. Acta Trop 226:* 106251.
5. Bisoffi Z et al., 2014. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Negl Trop Dis 8:* 38.
6. Buonfrate D, Marrone R, Silva R, Mirisola C, Ragusa A, Mistretta M, Perandin F, Bisoffi Z, 2021. Prevalence of strongyloidiasis in a cohort of migrants in Italy and accuracy of a novel ELISA assay for *S. stercoralis* infection, a cross-sectional study. *Microorganisms 9:* 401.
7. Ghosh K, Ghosh K, 2007. *Strongyloides stercoralis* septicaemia following steroid therapy for eosinophilia: report of three cases. *Trans R Soc Trop Med Hyg 101:* 1163–1165.
8. Lu Y, Ramachandran V, Mahanty S, Rawlinson WD, 2022. Comparison of *Strongyloides*-specific IgG enzyme immunoassays for laboratory detection of *Strongyloides stercoralis* infection. *Pathology 54:* 127–129.
9. Tamarozzi F, Longoni SS, Mazzi C, Rizzi E, Noordin R, Buonfrate D, 2022. Theaccuracy of a recombinant antigen immunochromatographic test for the detection of *Strongyloides stercoralis* infection in migrants from sub-Saharan Africa. *Parasit Vectors 15:* 1–7.
10. Arifin N, Hanafiah KM, Ahmad H, Noordin R, 2019. Serodiagnosis and early detection of *Strongyloides stercoralis* infection. *J Microbiol Immunol Infect 52:* 371–378.
11. Lillibridge JP, 1989. Prominence of IgG4 antibodies in the human responses to *Strongyloides stercoralis* infection. *J Infect Dis 160:* 692–699.
12. Osman E, Amin NA, Noon TPM, Lahat SNH, Rosli MS, Sham SF, Periyasamy PR, Ghazali N, Abd Manap SNA, Noordin R, 2022. Comparison of two serological assays in detecting *Strongyloides* infection in immunocompromised patients. *Am J Trop Med Hyg 107:* 636–639.
13. Van Doorn HR, Koelewijn R, Hofwegen H, Gilis H, Wetsteyn JCFM, Wismans PJ, Sarfati C, Vervoort T, Van Gool T, 2007. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of *Strongyloides stercoralis* infection in humans. *J Clin Microbiol 45:* 438–442.
14. Dewi RM, Tuti S, Ganefa S, Anwar C, Larasati R, Ariyanti E, Herjati H, Brady M, 2015. Brugia RapidTM antibody responses in communities of Indonesia in relation to the results of "transmission assessment surveys" (TAS) for the lymphatic filariasis elimination program. *Parasit Vectors 8:* 1–6.
15. Supali T, Djuardi Y, Lomiga A, Linda SN, Iskandar E, Goss CW, Miller JP, Weil GJ, Fischer PU, 2019. Comparison of the impact of annual and semiannual mass drug administration on lymphatic filariasis prevalence in Flores Island, Indonesia. *Am J Trop Med Hyg 100:* 336.
16. Djuardi Y, Jannah IF, Supali T, 2022. IgG4 antibodies against Bm14 as an evaluation tool of mass drug administration in a co-endemic area of *Brugia timori* and *Wuchereria bancrofti. Acta Trop 227:* 106278.