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To the Editors:

Validity of Lateral Flow Immunochromatographic-Assays (LFIA) in diagnosis of leptospirosis

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Leptospirosis is an important public health problem in Sri Lanka. Clinically relevant rapid diagnostic tests are a high priority but they are not freely available for routine diagnosis of leptospirosis in Sri Lanka [1]. Previously, we evaluated commercially available ELISA kits for rapid diagnosis, which showed poor sensitivity and specificity [2]. Rapid diagnostic tests such as Leptocheck based on immunochromatography methods were previously evaluated in Sri Lanka with promising results [3]. One of the limitations in the evaluation of Leptocheck was that it was based on locally available microscopic agglutination test (MAT) with limited serovars, and not the standard MAT with broad panel of serovars. Purpose of the present study was to evaluate the validity and utility of a commercially available lateral flow immunochromatographic assay (LFIA) method for diagnosis of leptospirosis in Sri Lanka with standard MAT as the comparison.

The present study was carried out in the University Teaching Unit of Teaching Hospital, Anuradhapura. Sample for this analysis was selected from a large fever surveillance study conducted in the hospital from June 2012 to May 2013. For the original study, we included undifferentiated febrile patients as eligible for the study. All these patients were screened for dengue and leptospirosis. We used less stringent clinical criteria for leptospirosis case detection to get patients with wide range of clinical presentations, which required for a validation study [4]. Febrile patients with headache or myalgia with or without clinical features of severe disease were included. Case confirmation was done in the WHO Collaborating Center for Leptospirosis in France, using Microscopic Agglutination Test (MAT) with a broad panel of 21 serovars. A confirmed case was defined as having a single high titre ($>1/400$), seroconversion or fourfold rise in antibody titre in acute and convalescent samples in a suspected case of leptospirosis. A Lateral Flow Immuno-

Assay (LFIA) (Immunemed Leptospira rapid, Korea) was used as a bed side or point of care diagnostic test for this evaluation. Written informed consent was obtained from all patients prior to enrolment and the ethical clearance for the study was obtained from the Ethics Review Committee of Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka.

Serum samples from 78 patients were analyzed for this validation study. The sample size was based on the availability of paired sera. Paired sera obtained at least one week apart were available for all. To estimate the power of the test, 95% confidence intervals were calculated for all estimates.

Table 1. Test characteristics of LFIA in comparison to MAT, for diagnosis of leptospirosis

Test Parameter	Estimate (95% CI)
Sensitivity	95.6% (85.2-98.8)
Specificity	63.6% (46.6-77.8)
Positive Predictive Value	78.2% (65.6-87.0)
Negative Predictive Value	91.3% (73.2-97.6)
Diagnostic Accuracy	82.0% (72.1-89.0)
Likelihood ratio of a Positive Test	2.63 (2.23 - 3.10)
Likelihood ratio of a Negative Test	0.0698 (0.0248 -0.196)
Diagnostic Odds	37.6 (7.7 - 183.6)

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The mean age of the sample was 42.6 (SD 13.4) years. The sample included 63(80.8%) males and 15 (19.2%) females. Majority (n=57;73.1%) were unskilled laborers or unemployed.

The sample included 45 patients with confirmed leptospirosis and 33 patients who were negative. Leptospirosis negative patients were patients with fever who were diagnosed with other illnesses, and their serum samples showed no reactivity in MAT panel in both acute and convalescent phase. The 45 confirmed cases included 20 patients with seroconversion, 9 patients with fourfold rise in acute and convalescent serum, and 16 patients with a single high titre. Of the patients with a single high titre, only 3 were confirmed at a titre of 1/400. Others had titres ranging from 1/800 to 1/6400. Of the 45 cases, 43 (95.6%) were detected by LFAI, with 12 false positives (total 55 LFIA positive). Of the 33 negative cases, 21 (63.6%) were negative for LFIA. The LFIA had a very high sensitivity with low specificity with a diagnostic accuracy of 82.0% (Table 1).

Local evaluation of diagnostic or screening tests is important in leptospirosis; specially because of the availability of different serovars which do not have laboratory reported sensitivity and specificity. This preliminary study showed that LFIA had a high sensitivity as a screening test for leptospirosis. High negative predictive value of 91% is also important in clinical practice, as this informs the clinician to look into other causes of leptospirosis like illness, which is common in the tropics. Compared to low sensitivity and specificity reported for ELISA based commercial kits, the LFIA has a very high utility in Sri Lankan setting. This investigation can be done with minimal resources as a point of care diagnostic test. In Sri Lanka, MAT is only carried out at the Medial Research Institute (MRI), Colombo. There are logistical issues regarding sending blood samples from various part of the country to MRI and to get back reports on time. The MAT panel available in the MRI is neither a regionally optimized panel including Sri Lankan isolates nor the standard broad panel of serovars recommended for leptospirosis diagnosis. Therefore, alternative and rapid diagnostic methods are required for clinicians to improve their patient care. Since the LFIA can only detect positive antibodies on day 5 and after (similar to ELISA and MAT), diagnosis of leptospirosis in the very early stages is not possible with LFIA. Only option to confirm diagnosis up to day 5 is molecular or antigen based methods, which require sophisticated laboratory facilities. However previous studies show that patients with leptospirosis prenet to hospital around 4 to 5 days after onset of fever. Therefore, LFIA is an ideal investigation to assist clinicians working in peripheries of Sri Lanka to screen fever patients suspected of leptospirosis.

We used MAT with a broad panel of serovars as the standard comparison in this study. However, MAT is an imperfect gold standard test and low sensitivity of MAT was clearly demonstrated previously in several studies in Sri Lanka as well as in other countries [4, 5]. In these studies, sera which were positive in culture and molecular based methods showed no reactivity in MAT. Hence, the specificity of 63.6% observed in FLIA may be an underestimation in this analysis. Some of the LFIA

positive cases could have been detected, if molecular based methods were also used in case confirmation. Further, a sample of normal people (as compared to clinically suspected leptospirosis patients who were MAT negative used in this study) is required for proper evaluation of specificity.

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Conflict of interest

Authors declare that there are no conflicts of interest.

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