

# Evaluation of a user-friendly test device (*AcQuick<sup>Dx</sup>*) for detection of specific antibodies to human angiostrongyliasis

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Short Communication

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## ABSTRACT

This study was conducted to evaluate the reliability of a recently developed *AcQuick<sup>Dx</sup>* Test based on lateral flow immunochromatographic assay to detect the 31-kDa specific antibody against *Angiostrongylus cantonensis* for rapid serodiagnosis of angiostrongyliasis in patient-derived sera from the parasite endemic areas of northeast Thailand. The diagnostic performance of the assay was evaluated with a total of 184 serum samples from clinically diagnosed patients with eosinophilic meningitis due to *A. cantonensis* infection (n=98), and individuals at risk of infection with no evidence of eosinophilic meningitis (n=86). The serological *AcQuick<sup>Dx</sup>* results were then compared with previous performance results based on dot-immunogold filtration assay (*Ac-DIGFA*) and dot-blot enzyme-linked immunosorbent assay (dot-blot ELISA) to determine its validity. The overall positivity rate of *AcQuick<sup>Dx</sup>* in 98 clinically diagnosed cases from three highly endemic districts in Khon Kaen province was 39.79%. Among the 86 sera of subjects at risk of infection with *A. cantonensis*, 24.41% were positive by *AcQuick<sup>Dx</sup>*. The positivity rates were comparable with the results obtained previously in both groups of defined serum samples: *Ac-DIFGA*, 39.79% and 24.41% respectively; and dot-ELISA, 37.75% and 23.25% respectively. This study demonstrated that *AcQuick<sup>Dx</sup>* is equally sensitive and specific as *Ac-DIGFA* and dot-blot ELISA for clinically confirmed eosinophilic meningitis caused by *A. cantonensis* infection. In addition to rapidity and ease of performance, the dry-format lateral flow assay in *AcQuick<sup>Dx</sup>* Test allows shipment and storage without requiring a cold chain and is thus more suitable for use in remote and resource-limited settings.

**Keywords:** *Angiostrongylus cantonensis*; angiostrongyliasis; food-borne zoonotic disease, lateral flow immunochromatographic assay; evaluation; 31-kDa antigen; rapid test

## INTRODUCTION

Eosinophilic meningitis caused by infection with *Angiostrongylus cantonensis*, the rat lungworm, is the most common food-borne zoonotic disease in Thailand and other tropical and subtropical regions of the world (Cross and Chen, 2007; Wang et al., 2012; Eamsobhana, 2015). The range of rat lungworm infection is expanding into more temperate regions due to increasing international travel that facilitates further spread of infected intermediate snail and definitive rodent hosts of *A. cantonensis*. (Eamsobhana, 2015; Baratt et al., 2016). Humans are usually infected with *A. cantonensis* through consumption of undercooked infected snails and the presentation of disease is often severe. In Thailand, detection of *A. cantonensis*-specific antibodies with domestically prepared enzyme-linked immunosorbent assay (ELISA) or immunoblotting have been used to support clinical diagnosis due to difficulty in recovering the parasite larvae from infected patients (Eamsobhana and Yong, 2009; Eamsobhana, 2015). In recent years, a more rapid flow-through, gold-based assay, *Ac*-DIGFA, had been developed and proven to be useful in the parasite endemic regions in Thailand (Eamsobhana et al., 2014; 2015). Although the use of visible gold-conjugated antibody in DIGFA instead of enzyme conjugates in ELISA makes the test rapid and simple, the colloidal gold detector reagent needs refrigeration and is therefore not suitable for large-scale field applications. Recently, an easier and rapid lateral flow immunochromatographic test format (*Ac*Quick<sup>Dx</sup> Test) with long shelf-life of the test device at ambient temperature has been successfully established (Eamsobhana et al., 2018). The present study tested archived serum samples from eosinophilic meningitis patients and individuals at risk of infection with *A. cantonensis* from three most endemic districts in Khon Kaen province to evaluate the usefulness of *Ac*Quick<sup>Dx</sup> Test for the detection of specific 31-kDa antibodies against *A. cantonensis* infection.

## MATERIALS AND METHODS

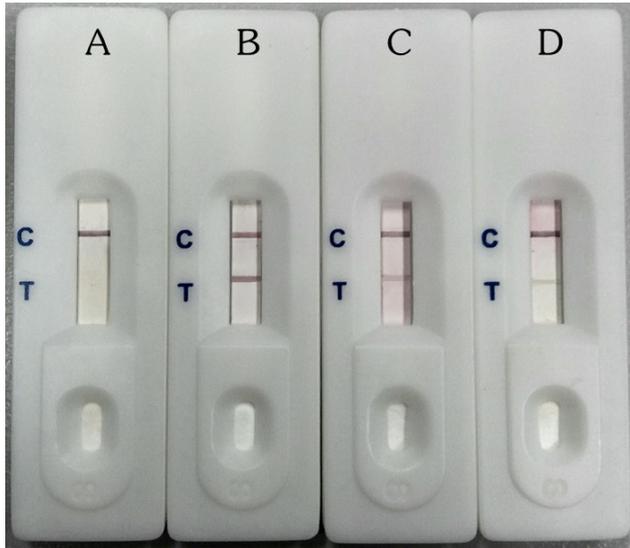
One hundred eighty-four archived serum samples, collected from three most endemic districts (Phu Wiang, Waeng Yai, and Phra Yuen) for *A. cantonensis* in Khon Kaen province (Table 1), were used in

this study to evaluate *Ac*Quick<sup>Dx</sup> Test for detection of the 31-kDa specific antibody against *A. cantonensis*. They were from clinically diagnosed patients with eosinophilic meningitis (n=98), and individuals at risk of infection with *A. cantonensis* (n=86). The inclusion criteria for clinically diagnosed angiostrongyliasis cases were patients with fever, headache, stiff neck and painful paresthesia, more than 10% of eosinophils of the total cerebrospinal fluid (CSF) white blood cell count, and a history of eating raw freshwater snails, freshwater prawns, frogs, monitor lizards or raw vegetables within the past three months, while the individuals at risk of infection with *A. cantonensis* were cases who had a history of eating similar or the same uncooked snails dish along with the patients but with no evidence of eosinophilic meningitis and clinical symptoms. The serum samples were sent to the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Bangkok, for specific antibody testing by a dot-blot ELISA to detect the specific antibody (31 kDa) against *A. cantonensis* (Chaiyaseth et al., 2002). This set of archived serum samples kept at -80°C had been retrieved and tested by *Ac*-DIGFA (Eamsobhana et al., 2015) and was re-tested in the present study using *Ac*Quick<sup>Dx</sup> Test to detect the 31-kDa specific antibody against *A. cantonensis*.

Purified 31-kDa antigen of *A. cantonensis*, prepared as described previously (Eamsobhana et al., 2001), was used in the immunochromatographic strip for the detection of specific antibody to *A. cantonensis*. The immunochromatographic test device, designated *Ac*-Quick<sup>Dx</sup> (Eamsobhana et al., 2018), prepared as per the standard method by Serve Science Co., Ltd., Bangkok, Thailand, was assembled and housed in a protective plastic cassette, and stored in a desiccated sealed foil-package.

The test was performed as previously reported (Eamsobhana et al., 2018). In the detection test, the appearance of a red band at the T line and a red band at the C line within 15 min indicates a positive reaction; the absence of such a red band at the T line and appearance of a red band at the C line indicates a negative reaction (Figure 1). Each serum sample was tested twice to confirm the reproducibility of the re-

sults. Results were evaluated semi-quantitatively based on the color intensity (Figure 1). The results were compared with those previously obtained by dot-immunogold filtration assay (*Ac*-DIGFA) and enzyme-coupled dot-blot ELISA using the 31-kDa *A. cantonensis* antigen (Chaiyaseth et al., 2002; Eamsobhana et al., 2015).



**Figure 1.** Interpretation of the results of *AcQuick*<sup>Dx</sup> test for detection of *A. cantonensis*-specific antibody. A, negative (only 1 red band at the control (C) line area); B, C and D, positive (2 red bands in the read-out zone; 1 in the control (C) line and 1 in the test (T) line areas). The different positive reactions with *AcQuick*<sup>Dx</sup>: strong (B), moderate (C), and weak (D).

## RESULTS AND DISCUSSION

The overall positivity rate of *AcQuick*<sup>Dx</sup> Test for *A. cantonensis* infection in the sera from 98 clinically diagnosed cases with eosinophilic meningitis from three highly endemic districts in Khon Kaen province, *i.e.* Phu Wiang (n=39), Waeng Yai (n=26), and Phra Yuen (n=33), was 39.79% (39/98) (Table 1). Among the 86 sera of subjects at risk of infection with *A. cantonensis* – Phu Wiang (n=37), Waeng Yai (n=27), and Phra Yuen (n=22) – 24.41% (21/86) were positive by *AcQuick*<sup>Dx</sup> Test (Table 1). The assay reproducibility proved good since identical positive results were obtained with two consecutive tests.

There was no significant difference between *AcQuick*<sup>Dx</sup>, *Ac*-DIGFA and dot-blot ELISA on positive and negative rates of detection (Table 1). The positivity rates of *AcQuick*<sup>Dx</sup> were comparable with the results obtained in both groups of defined serum samples with *Ac*-DIGFA (39.79% and 24.41% respectively) and dot-ELISA (37.75% and 23.25% respectively). Furthermore, the visual grading of *AcQuick*<sup>Dx</sup>, *Ac*-DIGFA and dot-blot ELISA showed good correlation in both groups of defined sera (data not shown). The intensity of color bands reflected the antibody activity level of immune serum (Eamsobhana et al., 2014). Strong and weak bands indicate respectively high and low antibody level (Chaiyaseth et al., 2002; Eamsobhana et al., 2015).

**Table 1.** Comparison of *AcQuick*<sup>Dx</sup>, *Ac*-DIGFA and dot-blot ELISA for the detection of specific 31-kDa antibody against *A. cantonensis* infection in sera from eosinophilic meningitis (EoM) patients and subjects at risk of infection (Data of *Ac*-DIGFA and dot-blot ELISA were from Eamsobhana et al., 2015)

Khon Kaen province (endemic district)	Positivity rate (%)					
	EoM patient			Individual at risk		
	<i>AcQuick</i> <sup>Dx</sup>	<i>Ac</i> -DIGFA	Dot-blot ELISA	<i>AcQuick</i> <sup>Dx</sup>	<i>Ac</i> -DIGFA	Dot-blot ELISA
Phu Wiang	46.15 (18/39)	46.15 (18/39)	43.58 (17/39)	40.54 (15/37)	40.54 (15/37)	40.54 (15/37)
Waeng Yai	38.46 (10/26)	42.30 (11/26)	38.46 (10/26)	14.81 (4/27)	14.81 (4/27)	11.11 (3/27)
Phra Yuen	30.30 (10/33)	30.30 (10/33)	30.30 (10/33)	9.09 (2/22)	9.09 (2/22)	9.09 (2/22)
Total	39.79 (39/98)	39.79 (39/98)	37.75 (37/98)	24.41 (21/86)	24.41 (21/86)	23.25 (20/86)

Effective diagnostic is an essential tool in the control, elimination and eradication of eosinophilic meningitis caused by *A. cantonensis*, an emerging infectious disease now recognized as a serious global health issue (Graeff-Teixeira, et al., 2018). In this communication, *AcQuick*<sup>Dx</sup> using parasite specific (31-kDa) antigen demonstrated good performance with archived serum specimens from eosinophilic meningitis patients in parasite endemic areas in Khon Kaen province, Thailand. Its diagnostic sensitivity to detect the specific 31-kDa antibody is consistent, showing agreement with those previously obtained by *Ac-DIGFA* and dot-blot ELISA for performance under field situations (Chaiyaseth et al., 2002; Eamsobhana et al., 2015).

Our dry-format lateral flow assay, *AcQuick*<sup>Dx</sup> test device can be shipped and stored conveniently at ambient temperature with shelf life of at least one year (Eamsobhana et al., 2018). Although *AcQuick*<sup>Dx</sup> and *Ac-DIGFA* can be performed anywhere by paramedical personnel without instrumentation or special training, drying the conjugate onto the pad improves the usefulness of *AcQuick*<sup>Dx</sup> as a field-friendly test over *Ac-DIGFA* that requires a liquid conjugate. As the *AcQuick*<sup>Dx</sup> test needs fewer number of steps to carry out the assay and does not require a cold chain, so it is more suitable in remote and resource-poor settings for mass screening survey to monitor the endemic status of *A. cantonensis* infection toward the surveillance and intervention control of the disease.

Additionally, other common food-borne parasitic helminthes (e.g. *Gnathostoma spinigerum*, *Paragonimus heterotremus* and *Taenia solium* metacestodes) that may invade the central nervous system and produce eosinophilic meningitis, also exist in Thailand. Since the performance of dry reagent assay indicated excellent field-robustness, the improvements/extensions of *AcQuick*<sup>Dx</sup> (single test format) to also differentiate other clinically related parasites in a multiplex test format would broaden its applicability especially in angiostrongyliasis co-endemic areas in northeast and north Thailand, where cerebral gnathostomiasis and neurocysticercosis cases have been reported. Development of a dry-format lateral flow test, utilizing

multiple antigens, either on the same lateral flow strip or a simple multiple channel device running multi-antigen strips from a single sample in parallel that can differentiate common parasitic causes of eosinophilic meningitis in Thailand will make such rapid device even more attractive.

## CONCLUSIONS

In summary, the rapid, dry-format field applicable test, *AcQuick*<sup>Dx</sup> is as sensitive as the rapid *Ac-DIGFA* and the dot-blot ELISA for confirming eosinophilic meningitis due to *A. cantonensis*, with advantages of low-tech and robust assay that allow storage and shipment of the test devices at ambient temperature. The *AcQuick*<sup>Dx</sup> Test is thus very suitable for application in resource poor-settings in the field.

## CONFLICT OF INTERESTS

None of the authors have any conflict of interest in this study.

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