

Evaluation of dot immunogold filtration assay (DIGFA) for rapid serodiagnosis of eosinophilic meningitis due to *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea)

Eamsobhana, P.^{1*}, Prasartvit, A.², Gan, X.X.³ and Yong, H.S.⁴

¹Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

²Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand

³Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences, Hangzhou, PR China

⁴Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

*Corresponding author email: praphathip.eam@mahidol.ac.th

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Abstract. *Angiostrongylus cantonensis* is the most frequent cause of eosinophilic meningitis in humans in Thailand and worldwide. Because of difficulty of recovering the *Angiostrongylus* larvae from infected patients, detection of parasite-specific antibodies is used to support clinical diagnosis. This study tested serum samples from eosinophilic meningitis patients and individuals at risk of infection with *A. cantonensis* to evaluate a recently developed simple and rapid dot-immunogold filtration assay (DIGFA) for detection of specific antibodies against *A. cantonensis*. Purified 31-kDa glycoprotein of *A. cantonensis* and protein A colloidal gold conjugate were employed to detect the 31-kDa anti-*A. cantonensis* antibody in patients sera from the parasite endemic areas of northeast Thailand. The results were compared with those obtained by dot-blot enzyme-linked immunosorbent assay (ELISA) with 31-kDa *A. cantonensis* antigen. The overall positivity rate of DIGFA and dot-blot ELISA for *A. cantonensis* infection in 98 clinically diagnosed cases from three highly endemic districts in Khon Kaen province were 39.79% and 37.75%, respectively. Among 86 sera of subjects at risk of infection with *A. cantonensis*, 24.41% were positive by DIGFA and 23.25% by dot-blot ELISA. There were good correlation between the visual grading of DIGFA and dot-blot ELISA in both groups of defined sera. DIGFA is as sensitive and specific as dot-blot ELISA for confirming eosinophilic meningitis due to *A. cantonensis* infection, with advantages of simplicity, rapidity and without the use of specific and expensive equipment, and can be used in field settings.

INTRODUCTION

Eosinophilic meningitis caused by infection with the rat lungworm *Angiostrongylus cantonensis* is a public health problem in Thailand and many parts of the world (Wang *et al.*, 2012; Eamsobhana, 2013). The disease is prevalent and widely distributed in Thailand, especially in the northeastern and northern regions of the country. Cases of *A. cantonensis* associated-eosinophilic meningitis have mostly been reported in Thai farmers with a history of eating a popular undercooked snail dish “koi-hoi”. It can also be contracted by eating raw freshwater

snails, especially *Pila* spp., as well as other paratenic hosts (Chaiyaseth *et al.*, 2002; Karnjanapiboonwong, 2010).

Immunological tests are crucial for the diagnosis of infection, since the parasite is rarely found in cerebrospinal fluid (CSF) samples obtained for identification. Serological assays to detect parasite-specific antibodies are supportive evidence for the clinical diagnosis. The current specific immunoblot test based on enzyme-coupled technique to detect the 31-kDa anti-*A. cantonensis* antibody in infected individuals, although sensitive and specific, is time-consuming and requires well-trained

personnel and special instruments, which limit its application as an initial screening method in parasite endemic areas (Eamsobhana, 2006; Eamsobhana & Yong, 2009).

Recently, a more rapid and simple DIGFA for detection of *A. cantonensis* antibodies against the 31-kDa *A. cantonensis* antigen has been established and tested in a hospital setting with high sensitivity and specificity (Eamsobhana *et al.*, 2014). It has been demonstrated to be comparable to immunoblot for specific diagnosis of eosinophilic meningitis caused by *A. cantonensis* (Eamsobhana *et al.*, 2014). The use of visible gold-conjugated antibody in DIGFA instead of enzyme conjugates in ELISA makes the test rapid and simple.

In order to pre-qualify our newly developed diagnostic test for future field trials, archived serum specimens from three most endemic districts in Khon Kaen province were re-tested by the rapid DIGFA test, and the results compared to those previously obtained with dot-blot ELISA for the detection of the 31-kDa specific antibody against *A. cantonensis*.

MATERIALS AND METHODS

A total of 184 archived serum samples (Table 1) from clinically diagnosed patients with eosinophilic meningitis (n=98), and individuals at risk of infection with *A. cantonensis* (who had a history of eating similar or the same uncooked snails dish

along with the patients) but with no evidence of eosinophilic meningitis (n=86) were tested by DIGFA for detection of the 31-kDa specific antibody against *A. cantonensis*.

These serum samples were collected from three most endemic districts for *A. cantonensis* in Khon Kaen province, i.e. Phu Wiang, Waeng Yai, and Phra Yuen and 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). These archived serum samples kept at -80°C were retrieved and tested by DIGFA for detection of the 31-kDa specific antibody against *A. cantonensis*. The use of stored left-over clinical samples for this study was approved by the Director of Siriraj Hospital, Faculty of Medicine Siriraj Hospital, Mahidol University.

Electroeluted, purified 31-kDa antigen of *A. cantonensis* was used in rapid flow-through immunofiltration test (Eamsobhana *et al.*, 2014). Purified antigen (1.0 mg/mL) was dotted (1 µL/dot) onto the nitrocellulose membrane (pore size 0.45 µm) and laid on the top of water-absorbing pads in a flow through module (30 mm x 40 mm x 6 mm) with a test hole (10 mm) in the center of the lid. The gold-labeled protein A conjugate was prepared as described previously and was stored at 4°C (Eamsobhana *et al.*, 2014). The DIGFA was performed as previously reported (Eamsobhana *et al.*, 2014). The appearance of a well-defined pink color spot in the hole-opening indicated a positive reaction; the

Table 1. Comparison of DIGFA and dot-blot ELISA for the detection of specific 31-kDa antibody against *A. cantonensis* infection in sera from patients with clinically diagnosed eosinophilic meningitis (EoM) and subjects at risk of infection with *A. cantonensis* from three highly endemic districts in Khon Kaen province

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

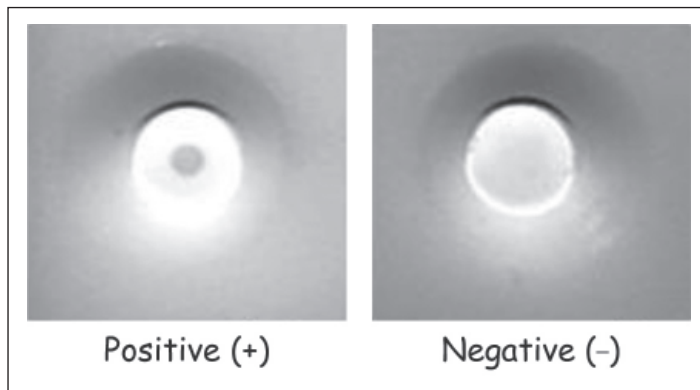


Figure 1. Positive and negative DIGFA for *A. cantonensis* infection.

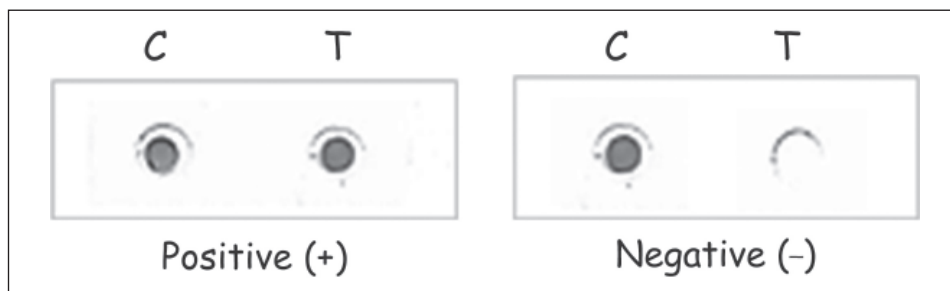


Figure 2. Positive and negative dot-blot ELISA for *A. cantonensis* infection. C: positive control; T: test.

absence of such a spot indicated a negative reaction (Figure 1). The results were compared with those previously obtained by enzyme-coupled dot-blot ELISA (Figure 2) using the 31-kDa *A. cantonensis* antigen (Chaiyaseth *et al.*, 2002).

RESULTS

The overall positivity rate of DIGFA and dot-blot ELISA 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), were 39.79% (39/98) and 37.75% (37/98), respectively (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) and 23.25% (20/86)

were positive by DIGFA and dot-blot ELISA, respectively (Table 1).

There was no significant difference between DIGFA and dot-blot ELISA on positive and negative rates of detection. The visual grading of DIGFA and dot-blot ELISA showed good correlation in both groups of defined sera.

DISCUSSION

Our recently developed DIGFA using the 31-kDa antigen of *A. cantonensis* has been shown to be rapid, simple to use and reliable without extra expensive equipment except handheld micropipettes and micro-pipette tips. The use of purified antigen in DIGFA eliminated nonspecific immunological reaction (Gan *et al.*, 2007; Eamsobhana *et al.*, 2014). It allows reliable detection of the anti-31 kDa antibody of *A. cantonensis* from a

small volume of serum sample (20 µL), within 3-5 min and with sensitivity and specificity similar to that of the 3-hr immunoblot test (Eamsobhana *et al.*, 2014). It is simple to be used by paramedical personnel without special training. This rapid flow-through immune filtration test, as a ready-to-use disposable device, will have high potential applicability in mass screening survey for monitoring the endemic status of *A. cantonesis* infection towards the surveillance and control of the disease.

With the advantage of rapid and ease of use over ELISA format, DIGFA using parasite specific antigen showed good performance with archived serum specimens from eosinophilic meningitis patients in parasite endemic areas in Khon Kaen province. Its diagnostic sensitivity to detect the specific 31-kDa antibody is consistent with those previously obtained by the 3-h, dot-blot ELISA. Based on the detection of an antigen-antibody complex on nitrocellulose membrane, the colloidal gold-labelled protein A used in DIGFA appears to have a similar sensitivity as the enzyme-conjugated immunoglobulins used in dot-blot ELISA because color signal was obtained with both assays. However, in addition to long reaction time and multiple steps involved, the enzyme-coupled method has potential disadvantage of employing a poisonous colorimetric substrate to generate the read-out signal (Chaiyaseth *et al.*, 2002; Eamsobhana *et al.*, 2014). Thus, the rapid and simple procedure, with the visual interpretation of results and reagent stability will make DIGFA particularly suitable for field testing and epidemiological surveys. Nevertheless, in this study, a technical problem with membrane permeability was encountered with several serum samples showing slow membrane penetration. Such sera were centrifuged to remove fibrin clots or blood lipids and re-tested in order to prevent false positive or false negative results. Fibrin clots and lipids in serum samples will frequently interfere with the drawing rate on nitrocellulose membranes and reaction of antigen and antibody bindings

(Mansour *et al.*, 2009; Eamsobhana *et al.*, 2014).

Additionally, other common food-borne parasitic helminthes (e.g. *Gnathostoma spinigerum*, *Paragonimus westermani* and *Taenia solium* metacestodes) that may invade the central nervous system, and produce eosinophilic meningitis can be individually distinguished using a variety of specific immunological tests (Tapchaisri *et al.*, 1991; Gan *et al.*, 2006; Wang *et al.*, 2007). As DIGFA is promising in term of future diagnostic test kit for angiostrongyliasis in Thailand, improvements 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 gnathostomiasis and neurocysticercosis cases are also reported. With the rising need for more economical, reliable, and rapid diagnostic tools, the development of a multi-antigen DIGFA that can differentiate parasitic causes of eosinophilic meningitis is underway at our laboratory.

In conclusion, the 3-min DIGFA is as sensitive and specific as the 3-h dot-blot ELISA for confirming eosinophilic meningitis due to *A. cantonensis*, with advantages of simplicity, rapidity and without the use of specific and expensive equipment. It is therefore very suitable for large-scale field applications and clinical detection.

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