Development of an Immunochromatographic Test To Detect Antibodies against Recombinant Em18 for Diagnosis of Alveolar Echinococcosis

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An immunochromatographic test (ICT) for the rapid detection of antibodies to Echinococcus multilocularis was developed. The ICT showed a sensitivity of 94% and a specificity of 95.4%. High degrees of agreement were observed between the ICT and an enzyme-linked immunosorbent assay (ELISA) and an immunoblot analysis (IB) by using recombinant E. multilocularis 18-kDa antigen (Em18), the breakdown product of ezrin-radixin-moesin-like protein (2) that is also known as EM10 (8), EM II/3 (7, 9), or EM4 (10) by the cysteine peptidase, and demonstrated that these two tests have a high potential for differentially diagnosing AE (1, 12, 16, 18). However, these two methods are time-consuming and require special materials and equipments, which make them not suitable for clinical applications. In contrast, an immunochromatographic test (ICT) is a simple, rapid, and reliable method for detection of specific antibodies to infectious agents. In the present study, we developed an ICT with rEm18 antigen for diagnosis of AE and compared ICT with ELISA and IB.

The rEm18 was expressed in a bacteria system as described previously (16) with some modifications. Briefly, a DNA fragment encoding the Em18 was amplified by PCR with the primers 5′-GGGAATTCAGGAGCTGACTTACCGGA T-3′ and 5′-TTTGGATCTTGGTTGCTTATCCATATGG TG-3′. The PCR products were digested with EcoRI and BamHI and cloned into bacterial expression vector pTWIN-1 (New England Biolabs, Beverly, MA) for producing a fusion protein with chitin binding domain/mini-inteins. The cloned plasmid was transfected into Escherichia coli ER2566 strain and expression of the recombinant protein was induced by the addition of 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) to the culture. The expressed rEm18 was purified by using a chitin column (New England Biolabs) according to the manufacturer’s instructions. The purified rEm18 did not have the fusion partner, because rEm18 was released by intein activity of the fusion partner itself during purifications (6). The purified rEm18 (1 mg/ml) and anti-goat immunoglobulin G (IgG) antibody (1 mg/ml) were sprayed onto a nitrocellulose membrane in a 1-mm-wide line as test and control lines, respectively. The nitrocellulose membrane with rEm18 and anti-goat IgG antibody, absorbent pad, and substrate reservoir pad were assembled on a laminated membrane card, and the assembled sheet was cut into strips 5 mm in width. The strip was placed into a plastic assay device (Mitsubishi Chemical Medience, Tokyo, Japan) with windows for applying a serum sample and

where imaging techniques are not readily available (4, 9, 11). Previously, we have reported an enzyme-linked immunosorbent assay (ELISA) and an immunoblot analysis (IB) by using recombinant E. multilocularis 18-kDa antigen (Em18), the breakdown product of ezrin-radixin-moesin-like protein (2) that is also known as EM10 (8), EM II/3 (7, 9), or EM4 (10) by the cysteine peptidase, and demonstrated that these two tests have a high potential for differentially diagnosing AE (1, 12, 16, 18). However, these two methods are time-consuming and require special materials and equipments, which make them not suitable for clinical applications. In contrast, an immunochromatographic test (ICT) is a simple, rapid, and reliable method for detection of specific antibodies to infectious agents. In the present study, we developed an ICT with rEm18 antigen for diagnosis of AE and compared ICT with ELISA and IB.

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FIG. 1. Examples of ICT tests with negative and positive sera. (A) Result with a negative serum (one blue band at control line in the result window); (B) result with a positive serum (two blue bands at control and test lines in the result window). The inscriptions “Sample” and “Buffer” represents the positions for loading of sample and of substrate solution, respectively.

a substrate solution. For assay, first, 10 μl of serum sample was mixed 20 μl of a serum dilution buffer containing 0.1 mg of alkaline phosphatase-conjugated anti-human IgG antibody (Dako, Tokyo, Japan)/ml in a tube, and the mixed serum sample was applied into the sample window of the plastic device. Soon after application of the serum sample (within 30 s), 200 μl of the substrate solution was loaded onto the substrate reservoir pad, and the result was evaluated after 20 min. BCIP (5-bromo-4-chloro-3-indolylphosphate) was used for color development. As shown in Fig. 1, a sample was considered positive if two color lines corresponding to rEm18 and anti-goat IgG antibody appeared in the result window, and a sample was considered as negative if one color line corresponding to anti-goat IgG antibody appeared in the result window. In cases where there was no appearance of a colored anti-goat IgG antibody line, the assay was invalid even if a colored rEm18 line appeared. ELISA and IB were performed as described previously (16), except using the rEm18 prepared in the present study.

A total of 94 serum samples, including 50 serum samples from AE patients, 24 serum samples from cystic echinococcosis (CE) patients, and 20 serum samples from healthy persons, were examined by ICT, ELISA, and IB. Each diagnosis of AE and CE had been carried out by imaging techniques, clinical findings, histological observations (if feasible), and/or serology of IB with recombinant Em18 (16) or EmAgB8/1 (13). As shown in Table 1, 47 AE and 2 CE patient sera were determined to be positive by ICT, and none of sera from healthy persons showed positive reactions; thus, the sensitivity and specificity of ICT were 94.0% and 95.4%, respectively. There were no significant differences in sensitivity and specificity among ICT, ELISA, and IB (P > 0.1, Pearson chi-square test).

Two CE patient sera, determined to be positive by ELISA and/or IB, were also positive by ICT. This is not an incomprehensible result, because it is known that a few CE patient sera react to rEm18 even though rEm18 is highly specific antigen for AE (11, 12, 16, 18). These results indicated that the ICT is a sensitive and specific method for the diagnosis of E. multilocularis infection.

The results obtained by ICT were compared to those of previously established ELISA and IB with rEm18 (Table 2). All ELISA-positive samples, except one from AE patient, were ICT positive. Two ELISA-negative samples with the optical density values 0.068 and 0.079 close to the cutoff optical density value of 0.093 at 405 nm were ICT positive, and both were also positive by IB (data not shown). All IB-positive samples, except for one from a CE patient, were ICT positive, and none of the IB-negative samples was ICT positive. The degrees of agreement between ICT and ELISA and between ICT and IB were estimated by kappa analysis (17). A kappa statistics value of >0.75, 0.40 to 0.75, or <0.4 represents excellent agreement, good to fair agreement, and poor agreement, respectively. High degrees of agreement were observed between ICT and ELISA (κ = 0.93) and between ICT and IB (κ = 0.97), which indicated that ICT is reliable.

In conclusion, we developed a rapid, simple, sensitive, and specific ICT with rEm18 for detection of specific antibodies to E. multilocularis infection. Although ICT, ELISA and IB with rEm18 show similarities to each other with regard to both sensitivity and specificity, ICT has the following advantages: (i) expertise, experience, and special equipment are not required; (ii) 20-min incubation is enough to detect specific antibodies; and (iii) it is more economical than ELISA and IB. These advantages suggest a high diagnostic potential for the ICT in clinical practice in providing immediate and proper treatments and in mass-screening programs in areas of endemicity as a

![Table 1](image.png)

![Table 2](image.png)

* Results with a total 94 sera shown in Table 1 were used for comparisons.
primary screening tool. Further analysis on stability of ICT and a large-scale evaluation might be necessary.

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REFERENCES


