Lateral Flow Test Using *Echinococcus granulosus* Native Antigen B and Comparison of IgG and IgG4 Dipsticks for Detection of Human Cystic Echinococcosis

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Abstract. Cystic echinococcosis (CE) caused by infection with *Echinococcus granulosus* is of major concern for humans in many parts of the world. Antigen B was prepared from *E. granulosus* hydatid fluid, and Western blots confirmed eight batches showing a band corresponding to the 8-/12-kDa subunit with positive serum and no low-molecular mass band (< 15 kDa) with negative serum. The batches were pooled and used to prepare lateral flow immunoglobulin G4 (IgG4) and IgG dipsticks. Diagnostic sensitivity was determined using serum samples from 21 hydatidosis patients, and diagnostic specificity was established using sera from 17 individuals infected with other parasites and 15 healthy people. IgG4 dipstick had a diagnostic sensitivity of 95% (20 of 21) and a specificity of 100% (32 of 32). The IgG dipstick had a sensitivity of 100% (21 of 21) and a specificity of 87.5% (28 of 32). Thus, both IgG and IgG4 dipsticks had high sensitivities, but IgG4 had greater specificity for the diagnosis of human CE.

INTRODUCTION

Cystic echinococcosis (CE) or hydatid disease is an important zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus* sensu lato.¹ Humans, sheep, cattle, goats, and other herbivorous mammals serve as intermediate hosts in the parasite lifecycle.² *E. granulosus*, the major causative agent of CE, is highly prevalent among dogs in many parts of the world, including the Middle East, Africa, Europe, Asia, and Central and South America.³

Diagnosis of hydatid disease is initially based on clinical signs followed by the imaging of suspected organs combined with serological tests. Disease diagnosis is hampered in humans owing to the absence of specific clinical signs, and imaging methods may not be able to differentiate between hydatid cysts, tumors, and other lesions.^{4,5} Therefore, immunodiagnosis remains an important modality in the diagnosis of hydatid disease. Unfortunately, serological tests currently used for the diagnosis of hydatid disease have several limitations, including cross-reactivity with other infections and altered diagnostic sensitivity because of cyst characteristics (location, stage, and size) and antigen type.⁶ A successful immunodiagnostic test depends on the use of highly specific and sensitive antigens as well as the detection of the appropriate antibody class or subclass. A variety of techniques, including enzyme-linked immunosorbent assays (ELISAs), indirect immunofluorescence, immunoelectrophoresis, and immunoblotting, have been used for the serodiagnosis of CE. However, high proportions of false-negative (< 25%) and false-positive results have been reported for currently available assays.^{6,7}

During the developmental stages of *E. granulosus*, different antigens are produced that modulate the host immune response and promote parasite survival and development.⁸ Hydatid cyst fluid is the major source of antigenic material used for the serodiagnosis of CE, with most immunodiagnostic studies detecting hydatid cyst fluid-specific antibodies.⁹ How-

ever, assay sensitivity and specificity are variable owing to the different antigens and methods used.^{6,10,11} Human hydatid cyst fluid is deemed unsuitable for diagnostic purposes because of the presence of human proteins, such as immunoglobulin G (IgG). In addition to the parasite protein, some human IgG may also bind to the solid support in an immunoassay and potentially react with the anti-human IgG secondary antibody. Thus, hydatid cyst fluid of infected sheep has been a primary source of antigen for the immunodiagnosis of human CE.¹²

Antigen B (AgB) purified from hydatid cyst fluid has a diagnostic sensitivity of > 90% and is the most commonly used antigen in immunodiagnostic assays.¹³ AgB is a strongly immunogenic polymeric lipoprotein with a molecular mass of 120–160 kDa.¹⁴ Under sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) reducing conditions, AgB dissociates into three bands of molecular masses 8 or 12, 16, and 24 kDa, suggesting that it comprises polymers of 8-kDa subunits.¹⁵ The 8-/12-kDa subunit has been shown to be the most valuable target for diagnosis.¹⁶ Thus, in this study, only AgB batches yielding a distinct 8-/12-kDa antigenic band with positive serum and no low-molecular mass band (< 15 kDa) with negative serum on Western blots were selected for dipstick development.

Immunological tests, such as the ELISA and immunoblotting, have been the predominant assays used for the detection of human *E. granulosus*.¹⁰ However, these tests are time-consuming and difficult to perform. In recent times, lateral flow immunochromatographic dipstick tests have proven popular as a diagnostic tool because of their simplicity to use. Lateral flow dipstick tests have been developed for the rapid diagnosis of parasitic diseases, such as malaria,¹⁷ strongyloidosis,¹⁸ lymphatic filariasis,¹⁹ and schistosomiasis.²⁰ Thus, this study aimed to produce lateral flow tests using *E. granulosus* native AgB and compare IgG and IgG4 dipsticks for the rapid diagnosis of human CE.

MATERIALS AND METHODS

Patients and serum samples. CE patients were recruited from pathology laboratories in Tehran and Nemazi and Faghihi Hospitals in Shiraz, Iran. Initial diagnosis was performed by the hospitals and based on clinical symptoms, imaging (ultrasound, X-ray, or computed tomography [CT])

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scan), and serology. The patients had not received drug therapy before surgery. Most patients had single liver cysts (approximately 49×36 mm), whereas some had multiple liver cysts (each approximately 35×70 mm). Cyst stage was not determined. Confirmation by the pathologists was based on histopathology performed on the hydatid cysts. Post-surgery, a patient with a ruptured cyst was treated with albendazole (50–100 mg/kg body weight) for a period of 3 months. DNA was extracted from the protoscolex or germinal layer of cysts from four patients, and restriction fragment length polymorphism (RFLP) and sequencing of the DNA confirmed that all of the samples were of G1 genotype.

Venous blood samples were collected from consenting CE patients by trained nurses. Sera from 17 patients with other parasitic diseases and 15 healthy people were obtained from sera banks at Universiti Sains Malaysia and Shiraz University of Medical Sciences. The Research Ethics Committee at Shiraz University of Medical Sciences reviewed the proposal and approved the collection and use of the patients' samples (ref. nos. 83-2094 and 83-2272). The Research Ethics Committee at Universiti Sains Malaysia permitted the use of previously banked serum samples at Institute for Research in Molecular Medicine (INFORMM) for diagnostic specificity determination.

Our laboratory tested all serum samples using the DRG Echinococcus IgG Enzyme Immunoassay Kit (DRG International Inc., Mountainside, NJ), a qualitative and semiquantitative assay for the determination of IgG class antibodies to *Echinococcus* in human serum and plasma. The manufacturer states that the kit has a diagnostic sensitivity of 97% and a diagnostic specificity of 100%.

The serum samples were divided into three categories: groups I, II, and III. Group I sera was collected from CE patients with liver cysts who were diagnosed based on clinical symptoms, imaging, serodiagnosis, and pathology reports ($N = 21$). Testing by our laboratory confirmed that group I was positive for anti-*Echinococcus* IgG. Group II patients were healthy individuals with no history of CE who were negative for anti-*Echinococcus* IgG ($N = 15$). Group III patients were diagnosed with other diseases (malaria, toxocariasis, strongyloidiasis, ascariasis, fascioliasis, and teniasis) and negative for anti-*Echinococcus* IgG ($N = 17$). Laboratory diagnosis of parasitic diseases from group III was based on microscopic findings, with the exception of toxocariasis, which was based on serology using *Toxocara canis* IgG ELISA (IBL International GMBH, Hamburg, Germany). The collected sera samples were aliquoted at $50 \mu\text{L}/\text{tube}$ and stored at -20°C .

Preparation of native AgB. Hydatid cysts were collected from the livers of three groups of sheep (i.e., groups A, B, and C with three, one, and five animals, respectively). The nine sheep were slaughtered in Shiraz, Fars Province, Iran. A minimum of five liver cysts was collected per sheep, and the eosin exclusion test confirmed that the protoscolexes in the cysts were mostly viable. Genotyping established that the cysts were of G1 genotype. Hydatid fluid (HF) was pooled from each group of sheep, aliquoted into 27 batches, and AgB-purified from each batch.

Three technicians prepared the AgB as described previously.²¹ Briefly, the protoscolexes were removed, and the fluid was dialyzed against 5 mM acetate buffer (pH 5) overnight at 4°C and pelleted by centrifugation at $50,000 \times g$ for 30 minutes. The pellet was dissolved in phosphate-buffered saline (PBS; $100\text{-}\mu\text{g}$ pellet to 1 mL buffer), an equal volume of

saturated ammonium sulfate was added, and the sample was agitated on a shaker (100 rpm) at room temperature for 15 minutes. The suspension was centrifuged at $4,000 \times g$ for 30 minutes to remove contaminating host IgG, and the supernatant was collected and dialyzed against PBS for 24 hours to remove the ammonium sulfate. The sample was boiled for 15 minutes and centrifuged at $50,000 \times g$ for 1 hour to separate heat-stable AgB in the supernatant from other components in the pellet.^{11,22} AgB concentration was determined using a commercially available protein assay (Bio-Rad, Hercules, CA) based on the method by Bradford.²³ Purified AgB was stored at -80°C until required.

Western blotting of native AgB. SDS-PAGE and Western blotting were performed as described previously.²⁴ Each AgB batch was mixed 1:4 with $5 \times$ sample buffer (0.3125 M Tris-HCl [pH 6.8], 10% SDS, 50% glycerol, 0.005% bromophenol blue, and 25% 2-mercaptoethanol), and $20 \mu\text{g}$ sample/well was electrophoresed in a 12% (vol/vol) SDS-PAGE gel in fresh running buffer (0.3% Tris, 1.44% glycine, and 0.1% SDS [pH 8.3]) at a constant current of 100 V for 110 minutes. Proteins were transferred from the gel onto a nitrocellulose membrane using the Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio Rad) at a constant current of 12 A for 30 minutes. The success of the protein transfer was confirmed by Ponceau S solution (Sigma, St. Louis, MO). Unbound sites on the nitrocellulose membrane were blocked for 1 hour at room temperature with a working dilution of blocking buffer (Roche Diagnostics, Mannheim, Germany) and washed three times in Tris-buffered saline with 0.05% Tween-20 (TBS-T) for 10 minutes. The membranes were cut into strips, and each strip was incubated with serum diluted 1:100 in blocking buffer overnight at 4°C . Each batch of AgB was tested with a positive serum sample (pooled from three CE patients), and a serum sample was pooled from three healthy individuals. After three washes with TBS-T, the strips were incubated with horseradish peroxidase (HRP)-conjugated polyclonal mouse anti-human IgG antibody (Invitrogen, Carlsbad, CA) at a dilution of 1:2,000 for 1 hour. Blots were developed using the enhanced chemiluminescence (ECL) blotting reagent (Roche Diagnostics).

Protein concentration and buffer exchange. Western blot profiles were used to select batches of hydatid fluid AgB, which were then pooled and concentrated using a Vivaspin filter (Sartorius, Gottingen, Germany) with a 3,000-Da molecular mass cutoff. Equal volumes of PBS were added and buffer-exchanged by centrifugation at $3,000 \times g$ until a final volume of $200 \mu\text{L}$ was reached; this procedure was performed two times. Final protein concentration was determined using the Bio-Rad protein assay.

Preparation of lateral flow dipstick. This procedure was similar to our previous report on a dipstick test for the detection of extraintestinal amoebiasis.²⁵ AgB, as the test line, was jetted linearly onto a Hi-Flow Plus 90 Membrane Card (Millipore, Billerica, MA) using an IsoFlow Dispenser (Image Technology, Hanover, NH). Various AgB concentrations (1, 1.2, 1.5, and 2 mg/mL) were dispensed onto separate membrane cards. The control lines for the IgG4 and IgG dipsticks were goat anti-mouse IgG (Invitrogen) and rabbit anti-goat IgG (Sigma), respectively. These antibodies served as controls for the integrity of the gold-conjugated antibodies. The antibodies were dispensed at 0.5 mg/mL at approximately 5 mm above the test line. After the lined reagents had dried,

the membrane card was gently wetted with ELISA blocking solution (Roche) and dried overnight at 37°C. An absorbent pad was added to the top of the membrane card, and the card was cut into 5-mm strips using an automated cutter (Index Cutter-I; A-Point Technologies, Gibbstown, NJ).²⁶ The strips were stored in a dry cabinet (< 20% humidity) at room temperature until required.

Conjugation of anti-human IgG and IgG₄ to gold nanoparticles. Colloidal gold nanoparticles were prepared using the seeding growth method as previously described.²⁷ Monoclonal mouse anti-human IgG₄ antibody (Invitrogen) and polyclonal goat anti-human IgG antibody (Invitrogen) were conjugated to the gold nanoparticles separately. The pH values of the gold nanoparticles were adjusted to pH 7 and pH 9 in accordance to the isoelectric points of anti-human IgG₄ and anti-human IgG, respectively, using 0.2 M K₂CO₃. The optimum amounts of anti-human IgG₄ and anti-human IgG were each added to 10 mL gold nanoparticles (optical density [OD] = 1.0) at 80 and 20 µg, respectively, which was obtained by observing the lowest amount of antibody that did not change color after addition of 10% NaCl. The antibody was gently mixed with the colloidal gold nanoparticles and incubated for 1 hour at room temperature. For every milliliter of the colloidal gold antibody conjugate, 100 µL 1% bovine serum albumin (BSA; Sigma) in water was added and then vortexed. The solution was centrifuged at 7,400 × g for 10 minutes, and the pellet was resuspended with 100 µL 1% BSA and then made up to a final volume of 1 mL with water. The above steps helped to remove the excess non-conjugated antibody and block the remaining unbound sites on the gold nanoparticles. After another centrifugation, the final pellet was resuspended in 1% BSA at 30 µL/mL colloidal gold antibody conjugate. The approximate optical densities (ODs) of the conjugates were OD = 20 for both colloidal gold antibody conjugates. The conjugation of the antibody with colloidal gold nanoparticles was confirmed using a UV-VIS-NIR spectrophotometer (UV-3600; Shimadzu, Kyoto, Japan). The colloidal gold-conjugated antibodies were then stored at 4°C.

Test procedure. A volume of 10 µL serum sample was diluted 1:2 in PBS with 0.05% Tween 20 (PBS-T; pH 7) and dispensed in the wells of a 96-well microtiter plate (Costar, Lowell, MA). The dipstick was placed in the well, and the sample was allowed to move up the dipstick by capillary action. After the sample had reached the top of the strip, the dipstick was dipped into an adjacent well containing colloidal gold conjugated to monoclonal mouse anti-human IgG₄ (IgG₄ dipstick) or colloidal gold conjugated to polyclonal goat anti-human IgG (IgG dipstick). Both gold-conjugated antibody solutions were diluted in PBS at an OD of 8. After the lines were well-developed, the dipstick was dipped in a well containing PBS-T to wash away excess colloidal gold conjugate. The result was interpreted within 15 minutes. Tests were recorded as positive when two red/purplish lines (control and test lines) were observed or negative when only a single red/purplish line (control line) was visible. The diagnostic sensitivity and specificity of the tests were determined using groups I–III serum samples.

RESULTS

Western blot profile analysis of AgB. The Western blot profiles for 8 of 27 AgB batches had a distinct band corre-

FIGURE 1. Representative Western blot profiles of different batches of AgB. Batches 1 and 2 showed a distinct band corresponding to the 8-/12-kDa subunit, whereas Batch 3 did not have the band. Molecular mass maker (10–250 kDa; Bio-Rad). Lanes 1, 3, and 5 show pooled positive serum samples from CE patients, and lanes 2, 4, and 6 show pooled healthy serum samples. MW = molecular mass.

sponding to the 8-/12-kDa subunit when probed with pooled CE serum. With pooled healthy serum, the Western blot did not show any low-molecular mass bands (< 15 kDa), thus indicating no cross-reaction with the 8-/12-kDa subunit. The eight batches were pooled, concentrated, and buffer-exchanged into PBS. Figure 1 shows Western blots for two AgB batches with the 8-/12-kDa band and an AgB batch lacking this band.

Lateral flow dipstick test. The optimum concentration of hydatid fluid AgB for use as the test line was 2 mg/mL. The diagnostic sensitivities and specificities of the lateral flow dipsticks are shown in Table 1; 20 of 21 hydatidosis patients (group I) were positive with the IgG₄ dipstick (95% sensitivity), and all sera (*N* = 32) from groups II and III were non-reactive (thus, 100% diagnostic specificity). The IgG dipstick had a diagnostic sensitivity of 100% (21 of 21) and a diagnostic specificity of 87.5% (28 of 32). For group III, two sera from toxocarasis and fascioliasis patients gave false-positive results with the IgG dipstick. Representative results are shown in Figures 2 and 3.

DISCUSSION

In a previous Western blot study using confirmed CE patient sera, the 8-/12-kDa subunit had the highest diagnostic sensitivity (80%) compared with 72.5% sensitivity for the 16- and 24-kDa subunits.¹⁴ Thus, as a quality check and to ensure that the highest diagnostic sensitivity was achieved, Western blot analysis was first performed on all batches of AgB in this study; 8 of 27 batches had a band corresponding to the 8-/12-kDa subunit with positive serum, and with negative serum, there was no low-molecular mass band (< 15 kDa). The variability in the quality of the AgB batches could be attributed to a number of factors as outlined below. Rather than using a single pool of hydatid fluid, the hydatid fluid Ag was recovered from three pools of hydatid fluid (each from a different sheep) and aliquoted into 27 batches for its purification. Although the cysts contained predominantly viable protoscoleces, the percentages of viable to non-viable protoscoleces were expected to vary from one cyst to another, potentially contributing to variability among AgB batches. According to the experiences of S.M.S., the greater

TABLE 1
Diagnostic sensitivity and specificity of AgB lateral flow dipsticks for the diagnosis of CE

Colloidal gold-conjugated anti-human antibodies	Lateral flow dipstick test result	Reactivities with different groups of serum samples	
		Group I* [diagnostic sensitivity]	Groups II† and III‡ [diagnostic specificity]
IgG	Positive	21 (21/21) [100%]	4
	Negative	0	28 (28/32) [87.5%]
IgG4	Positive	20 (20/21) [95%]	0
	Negative	1	32 (32/32) [100%]

*Group I: CE patients diagnosed based on clinical symptoms, serodiagnosis, and pathology reports ($N = 21$).

†Group II: Healthy individuals who are seronegative ($N = 15$).

‡Group III: Patients with other infections who are seronegative ($N = 17$).

the number of viable protoscoleces in a cyst, the higher the hydatid fluid protein concentration and the greater the quality of AgB. Another possible source of variability may have arisen from differences in the technical skills of the three technicians who prepared the AgB.

The diagnostic sensitivity of native or synthetic peptide AgB for human CE was between 63% and 92%.²⁸ Compared with native antigens, synthetic peptides and recombinant proteins are considered superior for immunodiagnostic purposes, because they are well-defined and can be produced in large quantities.^{29,30} However, the low diagnostic sensitivities and specificities of some recombinant proteins remain a significant challenge in the serodiagnosis of CE.³¹ For example, 34 CE samples, which were positive with native Ag5, showed diagnostic sensitivities of 65% and 21% when tested with rAg5 and a 38-kDa subunit of rAg5, respectively.³²

Virginio and others³³ investigated six *E. granulosus* recombinant antigens identified by screening complementary DNA (cDNA) expression libraries with the sera of CE patients. A recombinant AgB subunit (AgB8/2) was shown to be of

potential diagnostic value and had a much higher diagnostic sensitivity (93%) compared with native AgB (60%). All other recombinant antigens had diagnostic sensitivities between 58.6% and 89.7%. The lower diagnostic sensitivity of native AgB in relation to AgB recombinant subunits AgB8/1 and AgB8/2 was most probably owing to the presence of other subunits.³³

Rapid tests are simple, efficient, and easy to apply, they do not require a reader device or expensive equipment, and they can be performed by an untrained person.^{34,35} Al-Sherbiny and others³⁶ used camel hydatid fluid to develop a rapid dipstick assay for the diagnosis of hydatidosis with 100% diagnostic sensitivity and 91.4% specificity. However, this test was not based on immunochromatographic techniques and required incubation with serum and conjugate solutions.³⁶ Feng and others³⁷ developed a 3-minute rapid dot immunogold filtration assay (DIGFA) for the diagnosis of human hydatid cyst disease using four native antigen preparations. Like for the dipstick assay, the DIGFA incorporates a simple eye-read color change, with a diagnostic sensitivity of 80.7% when evaluated in a hospital diagnostic setting.³⁷ However, DIGFA

FIGURE 2. Representative AgB dipsticks probed with colloidal gold-conjugated anti-human IgG4. Lanes 1–3 show positive test results with serum samples from individual CE patients. Lane 4 shows a negative test result with a serum sample from a healthy individual. Lanes 5 and 6 show negative test results with serum samples from other diseases. C = control line; NEG = negative; POS = positive; T = test line.

FIGURE 3. Representative native AgB dipsticks probed with colloidal gold-conjugated anti-human IgG. Lanes 1–3 show positive test results with serum samples from individual CE patients. Lane 4 shows a negative test result with a serum sample from a healthy individual. Lane 5 shows a negative test result with a serum sample from a patient with teniasis. Lane 6 shows a false-positive test result with a serum sample from a patient with toxocariasis. C = control line; NEG = negative; POS = positive; T = test line.

differs from the current test, because it is a flow-through assay and not a lateral flow assay, and it uses only IgG for detection. In another study, lateral flow rapid dipstick test using sheep hydatid fluid and protein A-gold conjugate was reported to show 94.87% sensitivity and 85.71% specificity for the diagnosis of hydatidosis.³⁸ However, this study did not use gold-conjugated IgG4 antibody like in this study.³⁸

Several commercial kits for the immunodiagnosis of CE are currently available, most of which are ELISAs. However, these assays are time-consuming, and they are neither easy to perform nor easy to interpret. Vircell (Granada, Spain) has commercialized an IgG lateral flow test (VIRapid HYDATIDOSIS) for the rapid diagnosis of human CE using high-pressure liquid chromatography (HPLC) -purified *E. granulosus* native AgB and Ag5 with 94.7% sensitivity and 99.5% specificity (http://en.vircell.com/products/rapid_tests/). However, a recent study reported that test sensitivity was 86.8% and specificity was 94.4%.³⁹ The same study also compared the kit with an IgG dipstick test developed using recombinant AgB 8/2, and Hernandez Gonzalez³⁹ found that the former had lower diagnostic specificity (57.8%) compared with the latter (81.1%) and that both tests had similar sensitivities (approximately 95%) for the detection of active and transitional cysts.

Native and recombinant AgB showed good reactivity for IgG4 antibodies in the serum of patients diagnosed with CE.^{40,41} IgG4 antibody levels were reported to either increase or remain unaltered in patients with active cysts.^{42,43} However, IgG4 antibody levels decreased in patients with calcified hydatid cysts and became negative in patients after the surgical removal of the cyst.^{43,44} The IgG4 response seems to be associated with hydatid cyst development, growth, and disease progression or active disease, and as such, it has been used to monitor and evaluate treated CE cases.⁴² A previous study by our laboratory with *E. granulosus* protoscolex antigen showed an increased sensitivity for the detection of CE antigens when IgG4 was used compared with IgG.⁴⁵ The results of this study reaffirmed the high potential for an IgG4 assay to be used in CE diagnosis.

In this study, the IgG4 dipstick had a diagnostic sensitivity of 95% (20 of 21) and a specificity of 100% (32 of 32), whereas the IgG dipstick had a diagnostic sensitivity of 100% (21 of 21) and a specificity of 87.5% (28 of 32). However, because of the small sample size, the results may be considered as preliminary. Serological cross-reactions were expected to occur with serum samples from patients with other cestode and trematode (e.g., *Taenia* spp. and *Schistosoma* spp.) infections.^{46,47} Additionally, antibodies to other tissue helminthes responsible for toxocariasis, fascioliasis, and strongyloidiasis were also considered potentially cross-reactive.^{48–50} Although examples of most of these sera were included in this study, their numbers were limited; thus, additional studies should ensure inclusion of larger numbers of these potentially cross-reactive samples. Other important samples to be included in future studies would be sera from patients with hydatid cysts at various body locations and at different stages (active, transitional, and calcified), patients infected with different genotypes of *Echinococcus*, and post-treatment patients.

This dipstick used a wet conjugate solution without a sample pad. However, for a multicenter evaluation or commercialization, the conjugate needs to be dried on a conjugate pad and attached to one of the adhesive sections on the membrane card before cutting the card into strips. In addition, a sample pad for serum and/or whole-blood samples could also be

attached. The whole assembly could be placed in a cassette housing to make it physically more robust.

A high protein concentration derived from native antigen was required for the dipstick assay; hence, there needs to be a constant and sufficient supply of hydatid fluid for the test to be commercially viable. Thus, antigen production needs to be located near or at an endemic location. However, in the long term, the production of recombinant or synthetic peptide forms of the antigen may be necessary.

In conclusion, this study indicated that an IgG4 dipstick test using native AgB has strong potential for the rapid diagnosis of CE. In addition, we also showed that Western blot analysis of AgB can be used to confirm the quality of the antigen before use in a dipstick test.

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