

1 The usefulness of four different *Echinococcus granulosus* recombinant
2 antigens for the serodiagnosis and follow-up of hydatid patients.

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Abstract

Four different recombinant antigens derived from *Echinococcus granulosus*, designated B1t, B2t, E14t and C317, were tested with ELISA for the detection of specific IgG in unilocular hydatid disease (UHD) patients. The results were compared with those obtained with hydatid fluid and were subjected to receiver operator characteristic (ROC) analysis. The diagnostic performance of the above proteins was defined in respect to their specificity, sensitivity, predictive values (PV), the influence of cyst location, and usefulness in the follow-up and detection of surgically cured UHD patients. The best diagnostic approach was obtained with anti-B2t IgG ELISA, with 91.2% sensitivity, 93% specificity and high positive and negative PV (89.4 and 94.2, respectively). In addition, this diagnostic tool proved to be useful for the follow-up of surgically-treated UHD patients. Anti-B2t IgG ELISA may find application in the serodiagnosis of UHD in clinical laboratories.

Keywords: Hydatid disease; Serodiagnosis; Follow-up; Recombinant antigens.

1. Introduction

Human unilocular hydatid disease (UHD) is caused by the larval stage of the tapeworm *Echinococcus granulosus*. The diagnosis of UHD is usually approached applying an imaging technique. In 2003, the World Health Organization Informal Working Group on Echinococcosis (28) proposed the use of the ultrasonography (US) as the imaging technique of choice in order to promote uniform standards of UHD diagnosis and follow-up. It was also pointed out that suspected UHD images from US should be examined by alternative diagnostic methods, e.g. serological techniques (28). In addition, for pulmonary UHD US is unhelpful in most cases, unless the cysts are close to the pleural surface (12). Currently, preferred UHD immunodiagnostic techniques used in clinical practice detect serum IgGs against crude parasite extracts (mainly hydatid fluid; HF), most commonly by haemagglutination and enzyme-linked immunosorbent assay (ELISA; 26). However, HF cannot be standardized and gives rise to relatively frequent false-positive and -negative results (rev. in 15, 3). Moreover, HF is not useful for the follow-up of UHD, since the levels of specific (IgG) antibodies against HF remain high over long periods of time after curing (19). Many authors have attempted to find alternative antigens to improve UHD serodiagnosis and follow-up. Thus, purified, recombinant antigens and synthetic peptides -mainly derived from two parasite molecules (AgB and Ag5)- have been applied, with greater or lesser success. Specifically, antigens derived from AgB have been shown to be good candidates for UHD serodiagnosis (3). AgB is formed by several subunits (6), of which two (AgB1 and 2) have been produced as recombinant molecules. Both AgB1 and 2 have been tested for the detection of specific antibodies, although different authors disagree about the diagnostic performance of these two subunits (20, 27, 11). Thus, the standardization

of currently available antigen preparations and the characterization of new candidate antigens remain to be undertaken to improve UHD diagnosis.

In this study, we set up an enzyme-linked immunosorbent assay (ELISA) to perform an extensive serological survey. With this ELISA, we first compared the diagnostic performance of the N-terminal-truncated recombinant B1 and B2 antigens. We also obtained and assayed for the first time the usefulness of two additional recombinant antigens (E14t; 23, 25, and C317; 5) as diagnostic molecules, and this was compared with the usefulness of HF by ELISA. In addition, a comparison of the performance of these antigens was made regarding cyst location. Finally, we attempt to define a new tool for the follow-up and detection of cured patients after surgery.

2. Materials and methods

2.1. Antigens

Crude hydatid fluid (HF), obtained from fertile hydatid cysts from sheep at a slaughterhouse, was a kind gift from Dr. Jiménez (Servicio de Seguridad Alimentaria y Sanidad Ambiental. Consejería de Salud de La Rioja, Spain). HF was centrifuged at 1,000 g for 5 min and the protein concentration in the supernatant was measured with the Micro BCATM Protein Assay kit (Pierce, IL, USA). Both the supernatant and the sediment (hydatid sands) were stored at -80°C until use.

Four recombinant antigens were used in this study: the carboxy-terminal portions of the secretory antigens B1 and B2 (B1t and B2t; see below), and the E14t (23, 25) and C317 (5) somatic proteins. The B1t and B2t antigens were obtained as follows: total RNA from *E. granulosus* hydatid sands (mentioned above) was extracted with the Rneasy Protect Mini kit (Qiagen, Spain) and reverse-transcribed with the First Strand cDNA Síntesis kit (Roche Farma, Spain). The resulting cDNA was used for PCR performed with the primer pairs B1Fwd (5'-GATGATGGCCTCACCTCG) plus B1Rev (5'-GTATTGGCACCTCTATTC) and B2Fwd (5'-AAAGATGAGCCAAAAGCAC) plus B2Rev (5'-AGGCAAATCATGTGTCCCG), designed on the nucleotide sequences from antigens B1 and B2, and available at GenBank (accession numbers AF143813 and U15001, respectively). Primers were designed to amplify the sequence encoding the C-terminal portion of antigens B1 and B2 (the last 65 and 70 amino acids of the full-length sequences, respectively) in order to eliminate the coding region for the putative signal peptides from both sequences (Figure 1). These were predicted with the SIG-Pred internet facility at http://www.bioinformatics.leeds.ac.uk/prot_analysis/Signal.html as the most likely signal peptide in each sequence. The absence of signal peptide sequences will prevent the potential secretion of the corresponding recombinant proteins

during their expression in *Escherichia coli* (see below). PCR was performed in 30 cycles of 40 sec at 94°C; 40 sec at 48°C, and 1 min at 72°C and should result in PCR products of 210 and 241 base pairs (bp) for B1t and B2t sequences, respectively. PCR products were electrophoresed in Tris borate EDTA (TBE) 1% agarose gels and stained with ethidium bromide. Amplification products were then visualized in a UV transilluminator (UVP, Cambridge, UK), and purified with the StrataPrep DNA Gel Extraction kit (Stratagene, Spain). The purified fragments were cloned in the pCR 2.1 TOPO vector (Invitrogen, Spain), and the cloning reaction was used to transform DH5α *E. coli* cells (Invitrogen, Spain). Plasmids extracted from the transformants with the Strataprep Plasmid Miniprep kit (Stratagene, Spain) were automatically sequenced at the Automated Sequencing Service (Universidad de Salamanca), and digested with *EcoRI* (Roche Farma, Spain) and electrophoresed, and the resulting products were subcloned in the pGEX-4T1 expression vector (GE Healthcare Lifesciences, Spain) with T4 DNA ligase (Roche Farma, Spain). The ligation products were used to transform the BL21 CodonPlus RIL *E. coli* strain (Stratagene, Spain). Plasmids extracted from the transformants were automatically sequenced as described above to check the open reading frames. Selected transformants were grown in 2 x yeast extract tryptone (2xYT) medium overnight, with gentle shaking. The resulting cultures were diluted 1:100 in 2xYT medium and further grown for 3 hours under the same conditions. Protein expression was induced with 0.1 mM isopropyl thiogalactoside (IPTG; Applied Biosystems, UK) for 3 hours under the same conditions. The corresponding recombinant proteins were extracted from the resulting pellet cells, purified, and thrombin cleaved, as described elsewhere (27).

E14t recombinant protein was obtained as described elsewhere (25), and the C317 recombinant polypeptide (accession number BI244032) was a kind gift from Dr.

Fernández and Dr. Osinaga (Cátedra de Inmunología and Departamento de Inmunobiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay).

The concentration of the B1t, B2t, E14t and C317 recombinant proteins was measured with the Micro BCA™ Protein Assay kit (Pierce, IL, USA). The purity and integrity of the recombinant proteins and the HF was checked in 15% polyacrylamide gels stained with Coomassie-blue. All proteins were stored at -80°C until use.

2.2. Sera

A total of 265 sera were collected and classified as follows: 102 sera from surgically confirmed unilocular hydatid disease (UHD) patients, 68 sera from patients with other parasitic diseases (10 alveolar echinococcosis, 20 schistosomosis, 10 teniosis, 12 cysticercosis, 8 dirofilariosis, 4 fasciolosis and 4 mixed infections –fasciolosis and schistosomosis-), confirmed by parasitological and/or serological diagnosis, and 95 sera from healthy donors from the Canary Islands, an area free of UHD. From the 102 UHD sera, the cyst location was known in 100 patients, and hence divided into 86 samples from liver and 14 from lung hydatid cyst patients.

Finally, 71 sera from 35 UHD patients subjected to surgical intervention and cured were recovered to follow up the level of specific UHD antibodies over eleven years. These sera were classified in six groups as follows: before surgery (n=35); 6 months after surgery (as; n=10); 1 year as (n=7); 3 years as (n=8); 7 years as (n=5), and 11 years as (n=6). All patients were followed up by an imaging technique (ultrasonography) over 11 years.

2.3. Enzyme linked immunosorbent assay

The ELISA technique for the detection of total IgG was used for the comparison of the diagnostic performance of the four recombinant antigens and the HF as follows: 96 wells polystyrene plates (Corning, Spain) were incubated at 4°C overnight with 100 µl/well B1t, B2t, E14t, C317 (0.5 µg/ml) or HF (5 µg/ml) in carbonate buffer, pH 9.6. Plates were then washed six times with phosphate-buffered saline (PBS), pH 7.4, - Tween 0.05% (washing buffer) and incubated 1h 30 min at 37°C with 200µl washing buffer plus 1% bovine serum albumin (BSA, Sigma Aldrich, Spain) (blocking buffer). Sera were then added in duplicate (100 µl/well) at 1:200 dilution (or 1:100 for the C317 antigen) in blocking solution and the plates were incubated for 1 h at 37°C. After washing as above, the secondary antibody (peroxidase-labelled rabbit anti-human IgG, Sigma Aldrich, Spain) was added (100 µl/well) at 1:2,000 diluted in blocking buffer and the plates were incubated for 1 h at 37°C. After washing as above, the reaction was developed in the darkness with 100 µl/well of citrate buffer, pH 5, plus orthophenylene diamine (0.28 mg/ml, Sigma Aldrich, Spain) and hydrogen peroxide (0.4 µl/ml, Sigma). The reaction was stopped with 50 µl/well of 3N sulphuric acid and plates were read at 492 nm in an ELISA reader (EAR 400 FT, SLT Labinstruments, Germany).

2.4. Statistics

The serological index (SI) was calculated for each optical density and used to establish a common cut-off for all the ELISAs performed for each antigen as follows: SI (%) = [(NC)-(S)/(NC)-(PC)] x 100, where NC and PC represent the negative and positive controls, respectively, and S stands for each serum.

Receiver operator characteristic (ROC) analysis was used to determine the diagnostic value of each antigen (10). Levels of sensitivity were plotted against the levels of one minus specificity at each cut-off point on a ROC curve. The threshold

(cut-off) values used for the four antigens were those giving the highest sum of sensitivity (%) and specificity (%) for each antigen. The area under the ROC curve (AUC) was used to define the discriminatory values of the antigens (between subjects with and without the disease). The non-parametric X^2 test was used to test differences between AUCs. Positive and negative predictive values were calculated as described elsewhere (17).

All statistical calculations were done with the Epidat-3.1 statistics program, available at <http://dxsp.sergas.es/default.asp>.

3. Results

3.1. Comparative performance of B1t and B2t recombinant subunits in ELISA

Owing to the disagreement among several authors regarding the comparative diagnostic performance of the B1 and B2 recombinant antigen subunits (20, 27, 11), and to the differences in the sequence composition between the B1t and B2t antigens (Fig. 1) and those used by the above-mentioned authors, we first compare the diagnostic usefulness of B1t and B2t in ELISA IgG using a selected panel of sera (Fig. 2). Our results showed that the B2t antigen afforded better values of AUC, sensitivity (86.7%) and specificity (97.5%) in comparison with the corresponding values from the B1t recombinant protein (83.3% and 87.5%, respectively; Fig. 2). We therefore discarded the B1t antigen in ensuing experiments, also because of the limitation to the volume of each serum available and the low efficiency in B1t production obtained under our experimental conditions.

3.2. Usefulness of B2t, E14t and C317 recombinant antigens for the serodiagnosis of hydatid disease

Two additional recombinant proteins, E14t and C317, were tested individually and compared in ELISA with the B2t and HF antigens, since the combination of new recombinant antigens with the B2t molecule could improve the overall sensitivity and specificity of the serological test; for this comparison, a defined panel of sera was used (Fig. 3). The best AUC value was obtained with antigen B2t (0.949), with consecutive decreasing AUC values for the HF (0.898), C317 (0.714) and E14t (0.611) antigens (Fig. 3). Comparison of the AUCs from the four above-mentioned antigens with the non-parametric X^2 test revealed significant differences ($P<0.05$) among them, demonstrating that these differences were solely due to the antigens used in the study

and not to other parameters. The antigen B2t also gave rise to the best sensitivity (91.2%), specificity (93%), PP, and NP values (89.4% and 94.2%, respectively; Fig. 3).

Finally, the sera giving a positive ELISA score with the C317 and E14t antigens were also positive in the B2t-ELISA test.

3.3. Comparison of specific antibody levels against the HF, B2t, E14t and C317 antigens in patients with liver or lung hydatid cysts

Two AUCs from the respective ROC curves were calculated for each antigen (HF, B2t, E14t and C317), representing the comparative usefulness of the above-mentioned antigens for the detection of hydatid patients, depending on cyst location (liver or lung). The AUCs from the four antigens were slightly higher for lung cysts as compared with the respective AUCs obtained for patients with liver cysts (Fig. 4). Comparison of the liver and lung AUCs from each antigen with the non-parametric X^2 test did not uncover significant differences ($P<0.05$) between them (Fig. 4).

3.4. Usefulness of the B2t recombinant antigen for the follow-up of hydatid patients

The usefulness of the B2t recombinant antigen for the follow-up of cured patients after surgical cyst removal was studied using ELISA in a group of 35 patients. This was compared with the ELISA results obtained with HF using the same sera. The 35 sera proved to be positive (100%) against both HF and B2t antigens just before surgery (Fig. 5). During the follow-up period (up to 11 years), the number of positive sera against the B2t molecule began to decrease 1 year after surgery, 25% and 0% of the sera being B2t-positive at 3 years and 7 years after surgery, respectively (Fig. 5). In contrast, IgG antibodies against HF persisted in 100% of patients at 7 years after surgery, decreasing to 67% of patients at 11 years after surgery (Fig. 5).

4. Discussion

The diagnosis and follow-up of patients with unilocular hydatid disease (UHD) by means of serology remains a limited support in clinical practice, despite the advent of several recombinant and peptide antigens that are potentially useful for the detection of specific antibodies. This is mainly due to the lack of standardization of both the antigens and the techniques applied. The characterization of new antigens that might improve the serodiagnosis of UHD is also an important task.

This study reports an assessment of two recombinant subunits of the antigen B, B1t and B2t, and the use of two new recombinant antigens, E14t and C317, in ELISA for the detection of specific antibodies in UHD patients. For this study, we selected a well characterized and extensive panel of sera, including sera from patients with UHD (n=102) confirmed by surgery; from patients with different parasitic diseases (n=68) especially those related to *E. granulosus* at antigen composition level (alveolar hydatid disease, cysticercosis, teniosis, schistosomosis, fasciolosis and nematodes; 18, 7, 30), and healthy donors (n=95). This latter group of individuals was chosen from an area free of *E. granulosus* because healthy donors from endemic areas could give rise to false positive results from individuals who have been in contact with the parasite without developing the disease (rev. in 32).

After setting up the ELISA technique with the recombinant antigens, we first compared the diagnostic performance of the B1t and B2t antigens. This comparison was undertaken owing the equivocal results obtained by other authors pointing to conflict as regards the values of sensitivity and specificity attained when the B1 or the B2 antigens, as well as defined peptides derived from the above-mentioned sequences, were used in the ELISA test. Similar to Rott et al. (20) and Virginio et al. (27), our results showed that the B2 subunit provides better diagnostic performance than the B1 antigen. Those

252 authors found values of 84-93.1% for sensitivity and 98-99.5% for specificity for the B2
253 antigen and 55-84.5% for sensitivity and 80-91.2% for specificity for the B1
254 polypeptide. In contrast, Lorenzo et al. (11) found higher sensitivity and specificity
255 values for the B1 recombinant antigen (72.9% and 89.5%, respectively) than for B2
256 (66.7% and 71.2%, respectively). These performances match those obtained with the
257 corresponding derived peptides (rev. in 3). Surprisingly, Lorenzo et al. (11) attributed
258 the best diagnostic performance to hydatid fluid, in comparison with the B1 and B2
259 antigens, especially with defined sera from specific geographical areas. These
260 differences could be partially attributed to the panel of sera used by each author, but
261 also to slight sequence variations in the recombinant proteins, which could affect their
262 antigenicity. Thus, the lack of the signal peptide sequence in the B1t and B2t
263 recombinant proteins could enhance the diagnostic value of the B2 subunit in
264 comparison with the B1 antigen, being then the present results an advancement over
265 previous work.

266 We therefore chose the B2t antigen for subsequent experiments. Nevertheless,
267 the diagnostic values reach with this antigen could be improved, e.g., though its
268 combination with new antigens that react with otherwise false negative sera.
269 Consequently, we tested two additional antigens in our ELISA: namely, E14t and C317,
270 located in *E. granulosus* protoscoleces (24) and the cyst germinal layer (5), respectively,
271 both therefore representing biologically well characterized somatic parasite antigens.
272 Under our ELISA conditions, both the E14t and the C317 molecules provided relatively
273 low specificities and sensitivities when compared with the respective values for the HF
274 and B2t antigens. Furthermore, E14t and C317 positive sera were also found to be
275 positive against the Bt2 antigen and hence did not add any further diagnostic advantage
276 to this recombinant protein. The B2t antigen showed the best scores of sensitivity

(91.2%), specificity (93%), and positive predictive (89.4%) and negative predictive (94.2%) values, followed by the HF, C317 and Et14 antigens. Additionally, the statistical analysis confirmed that the diagnostic differences among the four antigens tested here were solely due to them, and not to other factors (e.g., the panel of sera used). It is noteworthy that the B2t antigen did not cross-react with sera from *E. multilocularis* patients. This could be an additional advantage over the HF and other *E. granulosus* antigens, especially in areas of the world where *E. granulosus* and *E. multilocularis* co-exist. Nevertheless, a higher number of sera should be tested to draw a statistical conclusion.

Several authors have tested different somatic proteins for the detection of specific antibodies in sera from UHD patients. Among them, the best characterized are malate dehydrogenase (EgcMDH), assessed in ELISA (27, 11), and rEpC1-GST, tested in immunoblot (9). Regarding the EgcMDH antigen, the results described (95.1% sensitivity and 89.7% specificity; 27) are no better than those reported here for the B2t antigen. In addition, a high variability in specificity and sensitivity in comparison with the above-mentioned results has been observed by other authors on applying the same antigen in ELISA (71.8% sensitivity and 62.2% specificity; 11). Similar results have been described for the vast majority of somatic molecules applied in UHD-ELISA (rev. in 3). The rEpC1-GST antigen, in contrast, seems to have a better performance than the B antigen (92.2% vs. 84.5% sensitivity), although the comparison was done by immunoblot and the antigen B represented a purified fraction of native HF (9); such factors could influence the comparative data obtained by those authors. Thus, and like other authors, we failed to observe that the newly tested somatic antigens improved the results obtained with the B (B2t) recombinant antigen. The low sensitivities reported here for the E14t and C317 antigens could be due to the lack of recognition of the native

antigens by UHD patients. These results and those obtained by others suggest that somatic antigens that are not directly in contact with the host would not be useful for the serodiagnosis of UHD.

Regarding cyst location and specific antibody levels, it has been reported that the intensity of the immunological response to hydatid cysts may differ, depending on the anatomical location of the parasite. In general, it has been postulated that the location of cysts in the liver will elicit higher antibody levels than in the lung (e.g., 1) and this will therefore affect the sensitivity of a given serological test. Nevertheless, it has not been clearly stated whether these differences would be due to the antigen used in the test or to other factors (e.g., technique, antibody isotype, etc.). In this respect, it has been reported that IgE detection gives rise to lower sensitivity rates for pulmonary cysts than for hepatic lesions, regardless of the technique applied for diagnosis (13, 14, 31). The opposite results are obtained when the detected isotype is IgM (13). Additionally, by use of two different antigens (HF and native antigen B; 13, 14, 22) the detection of total IgG by ELISA has been shown to be location-independent. This is in agreement with our own results, since we failed to find significant differences in antibody levels depending on cyst location with any of the antigens used during our study. Thus, the detection of specific IgGs in UHD patients with ELISA should provide similar results regardless of cyst location, independently of the antigen used for the diagnosis.

The risk of recurrences in human UHD after surgery varies from 3 to 16% (29), but today's imaging methods are not fully functional for the detection of new cysts, cured patients, or small changes in cyst morphology (19). In addition, serological testing has its own drawbacks because specific antibodies may persist in patients' sera for several years after recovery, especially those against HF (16). Thus, the follow-up and detection of cured UHD patients also remains a problem to be solved. In this work, we

assessed the usefulness of long-term serological monitoring by antibody (IgG) detection against the B2t antigen, in comparison with IgG anti-HF, in the detection of cured UHD patients. A series of 35 patients was studied, all of whom had undergone surgical treatment and had been subjected to an imaging follow-up lasting eleven years. Our findings confirm that HF specific IgGs remained elevated in the majority of cured patients throughout the study period. In contrast, anti-B2t specific IgG began to decrease 1 year after surgery, all patients being negative against this antigen at between 3 and 7 years after cure.

The results obtained by other authors have shown that IgG is the most common isotype in UHD patients, normally persisting over prolonged time periods after their cure (14, 31, 19). The levels of additional antibody isotypes such as IgE, IgA and IgM seem to decline 2 to 4 years after the cure, as reported elsewhere (e.g., 2, 21, 4, 8). Unfortunately, only a limited number of UHD patients show detectable levels of the above isotype antibodies (e.g., 21). In addition, these changes in antibody levels have been demonstrated both with crude and purified native antigens, but not with recombinant or peptide-derived parasite proteins. Only one *E. granulosus* derived recombinant antigen has been tested regarding its usefulness in the follow-up of UHD patients, unfortunately with unsuccessful results since specific antibody levels persisted against this recombinant protein 6 years after the cure (rEpC1-GST; 9). Thus, our results demonstrate for the first time that a defined *E. granulosus* recombinant antigen - B2t- could be useful for the detection of cured UHD patients after surgery, although its usefulness in the follow-up of pharmacologically treated patients needs to be further investigated.

350 In conclusion, we have defined a new serodiagnostic tool, ELISA-B2t for the
351 detection of specific IgG, with high specificity and sensitivity and that is potentially
352 applicable for the follow-up and detection of cured UHD patients.

ACCEPTED

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Figure legends

Figure 1. Recombinant proteins B1t and B2t. (a) The coding regions of these proteins were obtained by RT-PCR on *Echinococcus granulosus* cDNA, with primers designed on the nucleotide sequences from antigens B1 and B2 available in GenBank (accession numbers AF143813 and U15001, respectively) to amplify the sequence encoding the C-terminal portion of antigens B1 and B2 (white boxes), not including the coding region for the putative signal peptides from both sequences (black boxes). Numbers indicate amino acid positions. (b) The resulting RT-PCR products encoding the proteins B1t and B2t were subcloned in the pGEX-4T1 vector and expressed in *Escherichia coli* BL21 CodonPlus RIL cells. (c) The purified proteins after thrombin cleavage are shown in a 15% acrylamide Coomassie Blue stained gel. 1, Molecular weight markers; 2, B1t and 3, B2t proteins. Relative molecular weights are shown on the left of the panel. kDa, kilodaltons.

Figure 2. Comparative analysis diagnostic performance of B1t and B2t recombinant proteins in IgG-ELISA with a panel of defined sera. (a) Number of positive and negative (+/-) sera against B1t or B2t antigens. (n) Indicates number of sera in each group. (b) ROC curves used to determine AUC and cut-off values for the B1t and B2t antigens. The calculated AUCs $\pm$ standard error (se), confidence intervals (CI), cut-offs, sensitivity and specificity for each antigen are shown in (c).

Figure 3. Applicability of B2t, E14t and C317 recombinant proteins for the diagnosis of UHD by IgG-ELISA as compared with HF with a panel of defined sera. (a) Number of positive and negative (+/-) sera against HF, B2t, E14t and C317 antigens. (n) Indicates number of sera in each group. (b) ROC curves used to determine AUC and cut-off

values for HF and the B2t, E14t and C317 antigens. The calculated AUCs±standard error (se), confidence intervals (CI), cut-offs, sensitivity and specificity for each antigen are shown in (c). Positive and negative predictive values (PPV and NPV, respectively) are also shown in (c).

Figure 4. Influence of cyst location on the diagnostic performance of HF, B2t, E14t and C317 antigens by IgG ELISA with a panel of defined sera. (a) Number of positive and negative (+/-) sera against HF, B2t, E14t and C317 antigens. (n) Indicates number of sera for each cyst location (liver or lung). (b) ROC curves used to determine AUC and cut-off values for HF and the B2t, E14t and C317 antigens in the detection of liver (dashed lines) or lung (black lines) cysts. The calculated AUCs±standard error (se), confidence intervals (CI) and statistical significances (*P*) are shown in (c). Differences are considered statistically significant when *P*<0.05.

Figure 5. Comparative performance of HF and B2t antigens in the follow-up and detection of surgically cured UHD patients by IgG ELISA. (a) Time intervals after surgery, number of sera for each time interval and positive/negative (+/-) sera against the above-mentioned antigens in each time interval. (b) Percentage of positive sera in each time interval after surgery against HF (dotted line) and B2t (black line) antigens.

FIGURE 1

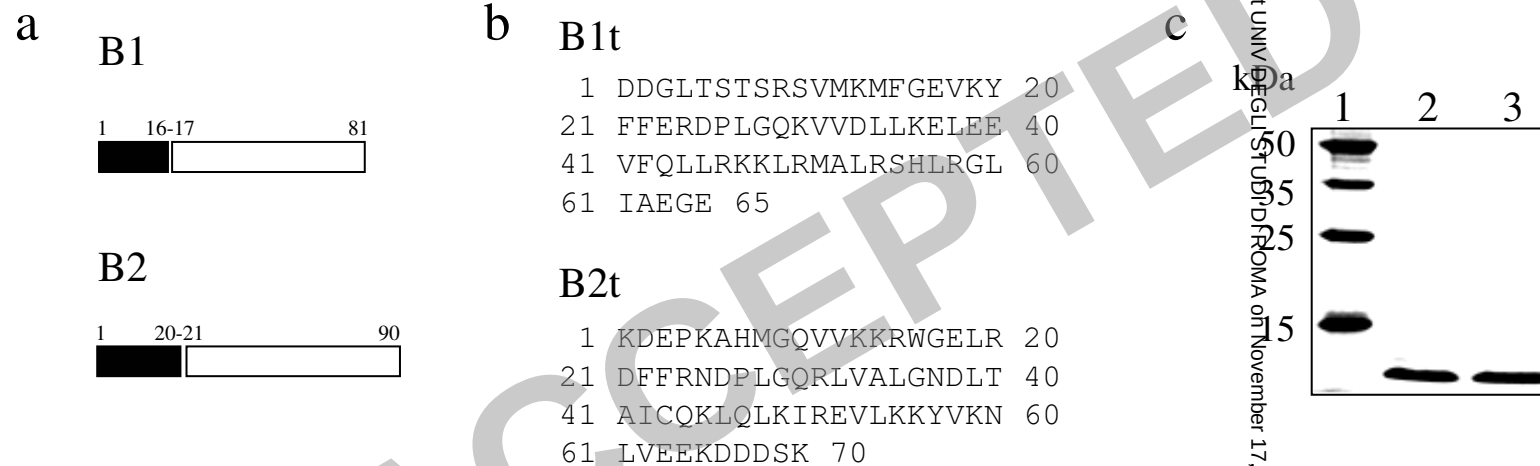
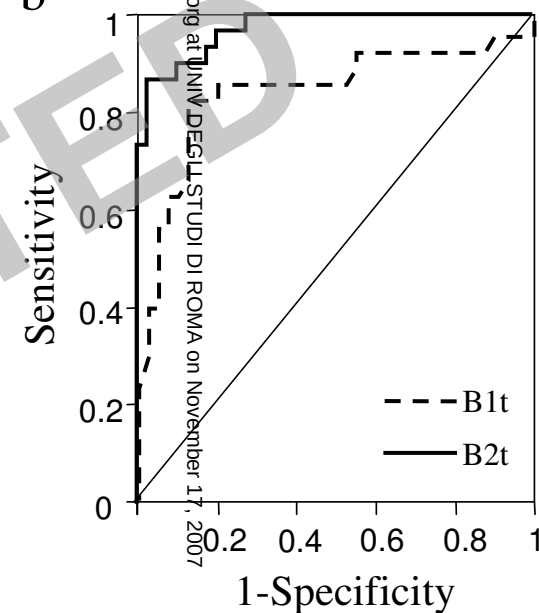


FIGURE 2

a

Sera (n)	Antigen B1t (+/-)	Antigen B2t (+/-)
Unilocular echinococcosis (30)	25/5	26/4
Alveolar echinococcosis (10)	1/9	0/10
Cysticercosis (2)	1/1	0/2
Teniosis (2)	0/2	0/2
Schistosomosis (2)	1/1	1/1
Fasciolosis (2)	1/1	0/2
Mixed infections (2)	0/2	0/2
Healthy donors (20)	1/19	0/20

b



c

Antigen	AUC $\pm$ se	CI (95%)	Cut-off	Sensitivity(%)	Specificity(%)
B1t	0.853 $\pm$ 0.052	0.752-0.954	78.36	83.3	87.5
B2t	0.972 $\pm$ 0.016	0.941-1.002	69.94	86.7	97.5

FIGURE 3

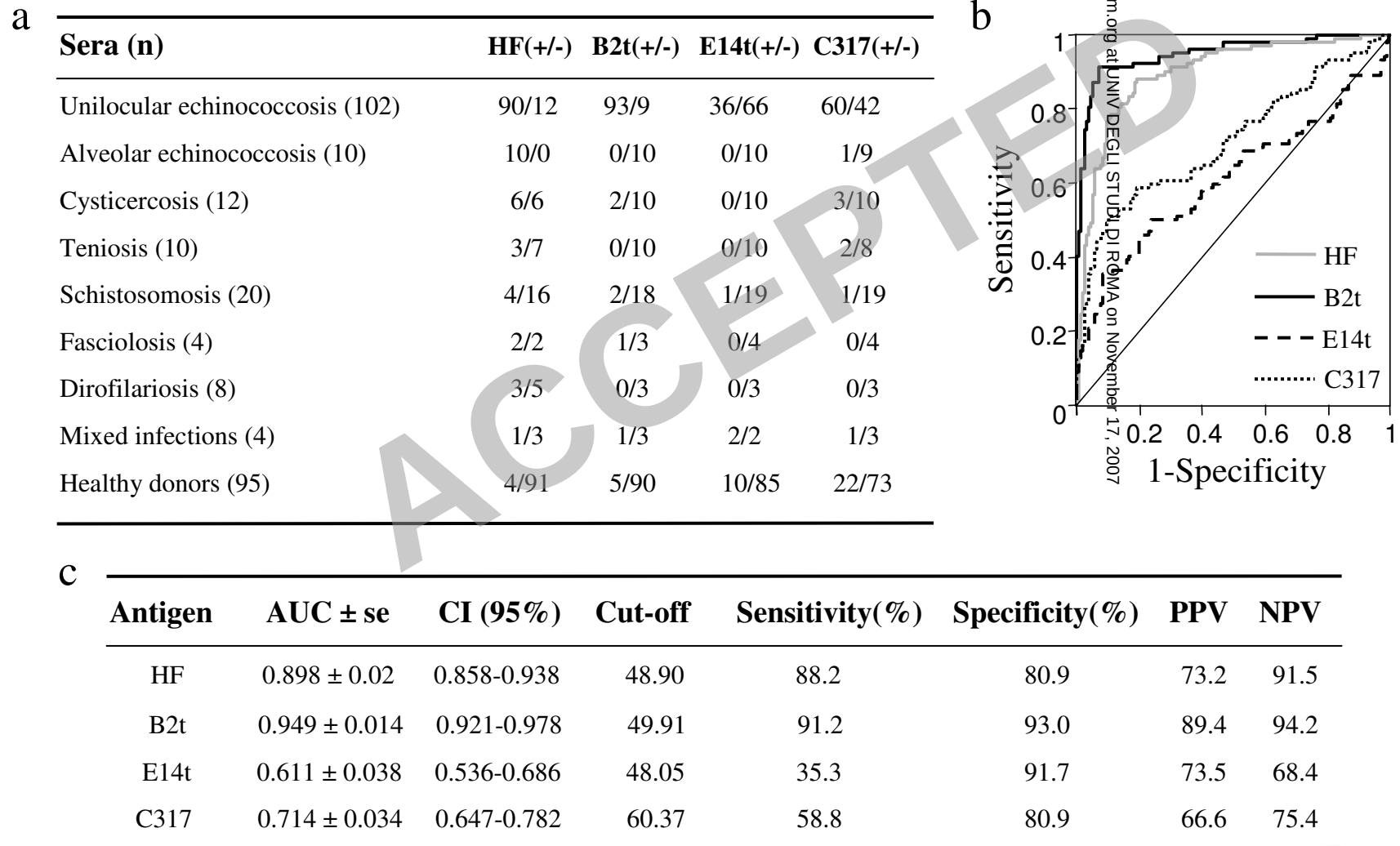


FIGURE 4

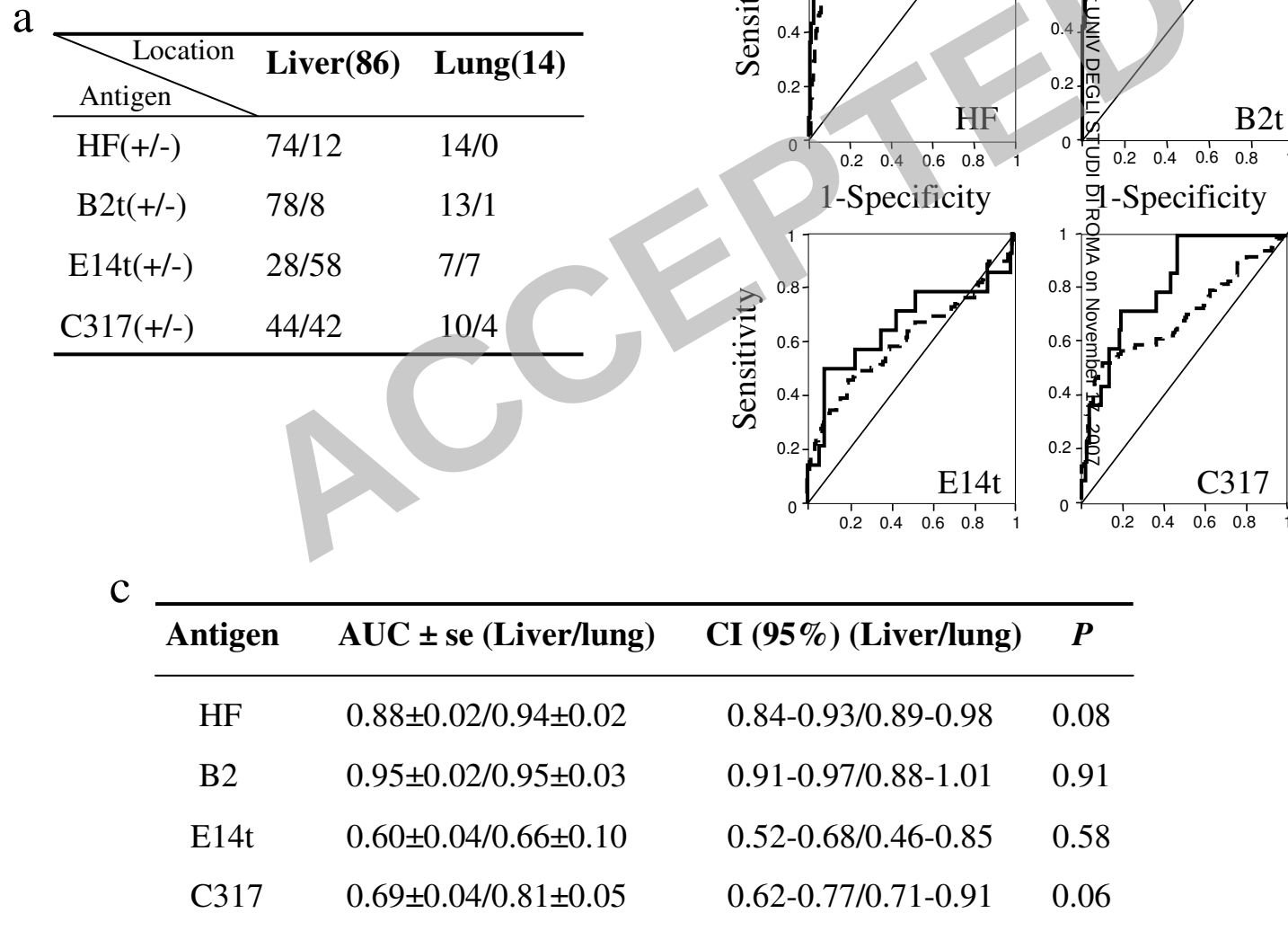


FIGURE 5

a

Time after surgery	Sera (n)	HF(+/-)	B2t(+/-)
0 days	35	35/0	35/0
6 months	10	10/0	10/0
1 year	7	7/0	6/1
3 years	8	8/0	2/6
7 years	5	5/0	0/5
11 years	6	4/2	0/6

b

