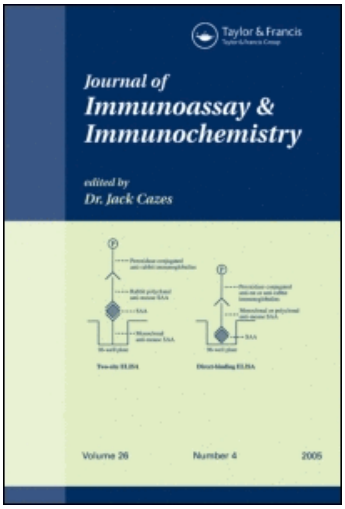

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

DEVELOPMENT AND EVALUATION OF PORCINE CYSTICERCOSIS QUICKELISA™ IN TRITURUS® EIA ANALYZER

Sukwan Handali[ab]; Sowmya Pattabhi[b]; Yeuk-Mui Lee[b]; Maria Silva-Ibanez[b]; Victor A. Kovalenko[c]; Andrew E. Levin[c]; Armando E. Gonzalez[d]; Jacquelin M. Roberts[b]; Hector H. Garcia[e]; Robert H. Gilman[f]; Kathy Hancock[b]; Victor C. W. Tsang[b]
[a] Atlanta Research and Education Foundation, [b] Parasitic Diseases Branch, Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA [c] Immunetics, Inc., Boston, Massachusetts [d] School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Salamanca de Monterrico, Lima, Perú [e] Cysticercosis Unit, Instituto de Ciencias Neurológicas, Lima, Perú and Departments of Microbiology and Pathology, Universidad Peruana Cayetano Heredia, San Martin de Porras, Lima, Perú [f] Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland

Online publication date: 30 December 2009

To cite this Article Handali, Sukwan , Pattabhi, Sowmya , Lee, Yeuk-Mui , Silva-Ibanez, Maria , Kovalenko, Victor A. , Levin, Andrew E. , Gonzalez, Armando E. , Roberts, Jacquelin M. , Garcia, Hector H. , Gilman, Robert H. , Hancock, Kathy and Tsang, Victor C. W.(2010) 'DEVELOPMENT AND EVALUATION OF PORCINE CYSTICERCOSIS QUICKELISA™ IN TRITURUS® EIA ANALYZER', Journal of Immunoassay and Immunochemistry, 31: 1, 60 — 70
To link to this Article: DOI: 10.1080/15321810903405068
URL: http://dx.doi.org/10.1080/15321810903405068

Journal of Immunoassay and Immunochemistry, 31:60–70, 2010

ISSN: 1532-1819 print/1532-4230 online
DOI: 10.1080/15321810903405068

Taylor & Francis
Taylor & Francis Group

DEVELOPMENT AND EVALUATION OF PORCINE CYSTICERCOSIS QUICKELISA™ IN TRITURUS® EIA ANALYZER

Sukwan Handali,[1,2] Sowmya Pattabhi,[2] Yeuk-Mui Lee,[2] Maria Silva-Ibanez,[2] Victor A. Kovalenko,[3] Andrew E. Levin,[3] Armando E. Gonzalez,[4] Jacquelin M. Roberts,[2] Hector H. Garcia,[5] Robert H. Gilman,[6] Kathy Hancock,[2] and Victor C. W. Tsang[2]

[1]*Atlanta Research and Education Foundation*
[2]*Parasitic Diseases Branch, Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA*
[3]*Immunetics, Inc., Boston, Massachusetts*
[4]*School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Salamanca de Monterrico, Lima, Perú*
[5]*Cysticercosis Unit, Instituto de Ciencias Neurológicas, Lima, Perú and Departments of Microbiology and Pathology, Universidad Peruana Cayetano Heredia, San Martin de Porras, Lima, Perú*
[6]*Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland*

□ *We evaluated three diagnostic antigens (recombinant GP50, recombinant T24H, and synthetic Ts18var1) for cysticercosis and found that all three performed well in detecting cysticercosis in humans and pigs in several assay formats. These antigens were adapted to a new antibody detection format (QuickELISA). With one single incubation step which involves all reactants except the enzyme substrate, the QuickELISA is particularly suited for automation. We formatted the Quick-ELISA for the Triturus EIA analyzer for testing large numbers of samples. We found that in Quick-ELISA formats rGP50 and rT24H have better sensitivity and specificity than sTs18var1 for detecting porcine cysticercosis.*

Keywords assay development, cysticercosis, ELISA, pig, serological diagnosis, *Taenia solium*

Correspondence: Sukwan Handali, Centers for Disease Control and Prevention, 4770 Buford Highway, Bld. 23, Room 1001, Chamblee, GA, 30341. E-mail: ahi0@cdc.gov

INTRODUCTION

Serological diagnosis of cysticercosis is based on lentil lectin purified glycoproteins (LLGP) from cysticerci used in Western blot, also known as the enzyme-linked immunoelectrotransfer blot assay (EITB).[1] Although very valuable as a diagnostic test, this format is not quantitative, technically challenging, and unsuitable for field use. To overcome these limitations, we cloned, expressed, and evaluated the three major diagnostic antigens from LLGP. These 3 major diagnostic antigens (recombinant GP50 = rGP50, recombinant T24H = rT24H, and synthetic Ts18var1 = sTs18var1) performed well in detecting cases with cysticercosis in Western blot format and in FAST-ELISA.[2–7] Although these diagnostic antigens work well in Western blot and FAST-ELISA, we need a format that allows for automation to deal with large sample sizes, such as in a control program. We pursued a further improvement of diagnostic testing through application of a QuickELISA™ format, a simplified antibody detection method that significantly outperforms conventional approaches of solid-phase immunoassay utilizing secondary class-specific antibody-enzyme conjugates.

The principle of the method is based on detection of only the specific complexes between antigen conjugates and antibodies that contain at least two antigen-combining sites. One antigen conjugate contains a detector label (horse radish peroxidase enzyme in current application). In the second conjugate, the antigen is conjugated with a component of a high-affinity binding pair (streptavidin). The second component of this high affinity binding pair (e.g., biotinylated carrier protein or another polymer) is immobilized on the solid phase at high density, providing high binding capacity for its affinity partner (streptavidin), and in a manner that significantly reduces non-specific binding effects during serological testing. The assay is species independent reaction, and fast. According to this scheme, only the label involved in specific complex formation is captured and detected, making the test insensitive to most factors related to various non-specific reactions, especially, these related to antibody adsorption on solid phase. There is no interference in this method from excess non-specific antibodies in the sample. In many cases, undiluted or several-fold diluted serum (plasma) can be used for testing contrary to conventional tests which require dilution of sera up to several hundred folds in separate tubes to reach low non-specific signal, related to non-specific antibody adsorption. Because the immunological reaction is started at conjugate addition, multiple samples and controls can be conveniently added directly into coated wells without worries for time differences between adding samples and controls. All classes of serum antibodies (IgG, IgM, IgE and IgA) can be detected by this method.

Because antibody-specific reagents are not required for detection, QuickELISA test can be applied for testing human and animal materials. This attribute is especially important in the case of cysticercosis, a disease with two hosts, human and pig. One kit can be applied for testing samples from both species. Non-specific reactions related to physical adsorption of immunoglobulins on solid phase, which creates problems for conventional tests, are completely eliminated. Many cross-reactive reactions related to presence of bacterial contaminations in solid phase with immobilized antigens are also significantly reduced in the new method. One of the advantages of the described approach is that the immunological reaction between antigen and antibody takes place in solution where antigen is easily accessible for antibody and no conformational changes are induced by immobilization of antigen on solid phase.

The assay protocol for QuickELISA is quite simple and contains fewer steps than conventional ELISA protocols for antibody detection. The sample and diluent are added directly into wells coated with biotinylated carrier protein and followed by the addition of a mixture of two antigen conjugates. The concentration of antigens in the two conjugates is optimized to allow for simultaneous binding of both conjugates to the identical antigen-binding sites in antibody molecules. The triple immune complex thus formed (antibody plus two antigen conjugates) is captured through high affinity interaction between components of the binding pair (streptavidin-biotin). After a relatively short incubation (10–60 min), with agitation or stationary, and following washing to remove non-immobilized components, enzyme substrate is added to wells and after another short incubation the reaction is stopped and products of the chromogenic reaction are detected. The assay thus has a single incubation step and a single wash step. The whole ELISA test may be completed within 35 min. All these characteristics make QuickELISA very easy for adaptation for automated analysis.

Though the assay is easily performed by hand on the laboratory benchtop, for the purpose of a cysticercosis control program to test large numbers of samples, automated versions of ELISA tests have an obvious advantage. Triturus® is an open platform and completely automated ELISA immunoassay analyzer which can perform a variety of different tests on a series of samples and process up to 4 batches simultaneously. These features allow for handling large numbers of samples (roughly 8 plates of 96 well per 8 hours per Triturus).

Although we have three diagnostic antigens for porcine cysticercosis, it is important to determine the performance of these antigens in a QuickELISA format and to understand if these diagnostic antigens behave similarly or not in defining the clinical condition of the subject. In this study, we report the QuickELISA assay performance of the three major

cysticercosis diagnostic antigens on several sets of defined pig sera using Triturus® EIA analyzer.

EXPERIMENTAL

Serum Samples

Four sets of defined pig sera were used for the study. Defined porcine cysticercosis (set 1, n = 34 sera) and other parasitic infections (set 2, n = 29) serum samples were collected at the School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Perú. The definitive diagnosis of porcine cysticercosis and other parasitic pig diseases was based on necropsy analysis of the pigs. Defined normal US (set 3, n = 88) pig serum samples were collected from a local abattoir in Calhoun, Georgia and considered free of porcine cysticercosis. The last set of pig sera, from an oxfendazole treated study (set 4) has been described previously.[6,8–10] In the oxfendazole study, 19 pigs with cysticercosis were treated with a single oral dose of oxfendazole and then sera were collected ~biweekly for nearly 52 weeks.

Preparation of Antigen Conjugates

The concentration of recombinant proteins was estimated spectrophotometricaly using A280 and the theoretical molar absorption coefficients calculated from content of tyrosine, tryptophan and phenylalanine, which are 1.677, 1.023, and 1.82 for rGP50, rT24H, and sTs18var1, respectively.

Streptavidin concentration was calculated based on absorbance at 280 using $A_{280nm,\ 1cm,\ 1mg/mL} = 3.0$. Horse Radish Peroxidase (HRP) concentration was calculated based on absorbance at 403 nm using coefficient $A_{403nm,\ 1cm,\ 1mg/mL} = 2.26$.

The primary conjugation strategy is based on maleimide/thiol reactions using primary amino groups of rGP50 and rT24H antigens for modification with bifunctional reagents or introduction of sulfhydryl groups. sTs18var1 antigen was conjugated with maleimide activated proteins through two existing reactive cysteine residues. Reaction conditions for preparation of maleimide activated proteins using N-[g-MaleimidoButyryloxy]-succinimide ester (GMBS) or thiolated proteins (with latent thiol groups) through modification with S-acetylthioacetate anhydride (SATA) were conducted at conditions where only limited number of existing amino groups in antigens or streptavidin were modified (typically 3–4 amino groups for rGP50, rT24H or streptavidin).

In a typical modification by GMBS experiment, the reaction was performed in 50 mM sodium phosphate buffer, containing 0.5 mM EDTA,

pH 7.6 at protein concentration 2–5 mg/mL for recombinant proteins and 5–8 mg/mL for streptavidin. GMBS solution in DMSO (2 mg/mL) was added into protein solution in a ratio 6–8 moles GMBS per mole of protein. Reaction time at room temperature was 30–45 min with subsequent dialysis at 4°C against 50 mM phosphate buffer, 0.5 mM EDTA, pH 6.5. Modification of HRP with GMBS containing only 2–3 amino groups per molecule was performed with 10–20 molar excess of modification reagents at protein concentration 12–15 mg/mL. The amount of maleimide groups in modified proteins was estimated by determination of ability to react with a known amount of cysteine as determined by 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB) test using 0.1–0.2 mg protein per test with a final volume of reaction mixture 0.6 mL. The molar extinction of TNB^- anion used for calculation was 1.415×10^4 at 412 nm. The reaction proceeds in 0.1 M TRIS-HCl buffer, pH 8.0 at DTNB concentration 0.66 mg/mL.[11]

Modification with SATA was performed in the same buffer and under similar conditions as described for modification with GMBS using SATA dissolved in DMSO (2 mg/mL). Modified proteins were then dialyzed against 50 mM sodium phosphate buffer, pH 6.5 containing 0.5 mM EDTA.

For releasing of sulfhydryl groups, SATA-modified proteins were treated with hydroxylamine by adding 1 M hydroxylamine hydrochloride solution in 0.5 M sodium phosphate buffer, 10 mM EDTA, pH 7.6 to SATA-modified proteins to a concentration 50 mM. After 15–20 min reaction at room temperature, the reaction mixtures were dialyzed for 1–1.5 hour against 50 mM sodium phosphate, 0.5 mM EDTA, pH 6.5.

Conjugation of maleimide-activated Streptavidin and HRP with sTs18var1 antigen was done at a molar ratio of protein : antigen as 1:1 or 2:1. The reaction mixture was prepared by quick mixing maleimide and thiol containing conjugation partners and incubating during 20–30 min at room temperature. At the end of conjugation, reaction residual maleimide groups were inactivated by adding 10 mM beta-mercaptoethanol solution in 5 mM EDTA, pH 6.5 at a ratio of approximately 3–4 moles per mole of original maleimide groups added into reaction mixture.

Conjugation of maleimide-activated rGP50 and rT24H with thiolated Streptavidin and HRP was done also at a molar ratio of 1:1 or 2:1. Reaction mixtures containing conjugated proteins were then treated with low concentration of N-ethylmaleimide (2–3 moles over moles of originally added thiol groups) and then beta-mercaptoethanol (4–5 moles over total moles of added maleimide groups) to inactivate residual sulfhydryl and maleimide groups in conjugates.

All conjugates were analyzed using gel filtration on HPLC columns (Sorbax F250) and stored at −20°C after dilution in storage buffer containing 50% glycerol in StabilZyme Select (SurModics).

Preparation of Capture Reagent for Plate Coating. Modification of BSA with Biotin-PEG

Bovine Serum Albumin (BSA) was biotinylated with Biotin-PEG-N-hydroxysuccinimide (MW 3,400) at weight ratio BSA/Biotin-PEG-NHS of 2:1 (at molar ratio PEG/BSA 10:1) and BSA concentration of 50–60 mg/mL mL in 0.1 M sodium phosphate, pH 8.1. Biotinylated BSA preparations were purified by dialysis against PBS-sodium azide using dialysis membrane with cut-off 25,000 D or by preparative HPLC. No differences were found for Biotin-PEG-BSA purified using these two different methods.

Preparation of Coated Plates

Polystyrene plates (Costar) were coated with 250 μL/well of Biotin-PEG-BSA [4 μg/mL] in PBS - sodium azide overnight at 4°C. Plates were blocked by two incubations with PBS-0.05% Tween-20 during 2 hours and finally dried at 45°C for 45 min. Plates were stored in bags with desiccants.

Working Conjugate Solutions

Conjugates of antigens with Streptavidin and HRP were prepared in immunoassay stabilizer StabilZyme Select containing protease inhibitor cocktail (Sigma, Cat. # P5726) and dye (Orange G, 0.0005%). Streptavidin and HRP conjugates solutions were stored separately as Conjugate solution A (Streptavidin conjugate) and Conjugate solution B (HRP conjugate). Equal volumes of conjugate solutions A and B were mixed on the day of testing for preparation of working conjugate solution.

Protocol for Automated QuickELISA

To ensure comparability of day-to-day measurement, we prepared a standard curve using serum pool from the sera of five Peruvian highly positive cysticercosis pigs.[6] One positive control serum (chosen with value within the linear range of the standard curve) was used to set-up the range for validation of the run. The QuickELISA steps were as follows: 96-well plate coated with biotinylated BSA was soaked 15 second with 300 μL of PBS/0.1% Tween-20 and aspirated. A total of 5 μL serum/plasma was dispensed into each well, diluted 1:12 in StabilZyme Select (SurModics, cat. # SZ03–2000); Well with 60 μL of StabilZyme Select only was used as assay negative control/blank. Immediately after dispensing sera samples, 60 μL/well working conjugate for each antigen was added. The plate was incubated for 40 min at room temperature (20–24°C) on a shaking platform. The plate was washed

4 times with 300 µL of PBS/0.1% Tween-20, and 120 µL/well SureBlue™ TMB Microwell Peroxidase Substrate (1 component) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was added. The incubation was for 5 min, at room temperature, and on a shaking platform. The reaction was stopped by adding 120 µL/well of 450 nm Stop Reagent for TMB Microwell (BioFX Laboratories, Owing Mills, MD; Product # STPR-1000–01). The absorbance of sample at 450 nm (with reference filter at 600 nm) was read using the Triturus system reader and Triturus software version 3.00b (Diagnostic Grifols, S.A.). All samples were tested in single for two times, and if the results are varied widely, a third run was carried out.

Coefficient of Variation of Triturus® EIA Analyzer

We ran 5 aliquots of the positive control, put into several different well positions on a plate, for 10 times. The mean and standard deviation of the result was used to determine the coefficient of variation (CV) of the run in Triturus system. The mean ±2 standard deviations was used as the range for validation of the run of the batch. If the value of the positive control was beyond the validation range, the results from that run were discarded and the samples were tested again.

Data Analysis

The QuickELISA – for rGP50, rT24H, and sTs18var1 – performance index (Youden J index) was determined as previously described.[6] A receiver operating characteristic curve (ROC) was also used to evaluate the ability of the three antigens to distinguish infected or uninfected groups.[12] The area under the ROC Curve (AUC) was used as a measure of test accuracy.

A repeated-measures two-factor ANOVA was used to test the oxfendazole data for differences in antibody values by antigen (rGP50, rT24H, and sTs18var1) over time. All calculations were performed on log transformed data. One was added to each antibody value in order to derive logs on zero values. Descriptive statistics were calculated by antigen and day for the oxfendazole data set. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC). Statistical significance was set at alpha = 0.05.

RESULTS

Test Reproducibility

Using the positive control sera, we tested the inter-assay CV of the QuickELISA performed using the Triturus® in Atlanta and in Lima, Peru,

TABLE 1 Sensitivity and Specificity of the rGP50, rT24H, and sTs18var1 QuickELISAs

Antigen	Cut-off (Units/μL)	J-index	Sensitivity for 2 or more Viable Cysts (%)	Sensitivity for Single Viable Cyst (%)	Specificity (%)
rGP50	0.58	0.90	94.1 (32/34)	50 (5/10)	95.7 (112/117)
rT24H	0.22	0.86	97.1 (33/34)	70 (7/10)	88.9 (104/117)
sTs18var1	0.96	0.63	73.6 (25/34)	50 (5/10)	88.9 (104/117)

for rGP50 and sTs18var1. We also tested the variability in another machine in Lima, Peru for rGP50, rT24H, and sTs18var1. The CVs we obtained were 8.21% and 8.6% (Atlanta) and 8.8 and 8.93% (Lima, Peru), for rGP50 and sTs18var1, respectively. In another study in Lima, the CVs for rGP50, rT24H, sTs18var1 were 3.8%, 3.6%, and 3.4%, respectively. The intra-assay variability for rGP50 and sTs18var1 was 5.48% and 7.3%, respectively and in another study, the intra-assay variability for rGP50, rT24H, and sTS18var1 was 3.0%, 3.9%, and 4.2%, respectively. Based on these results, we decided to use a single well per sample.

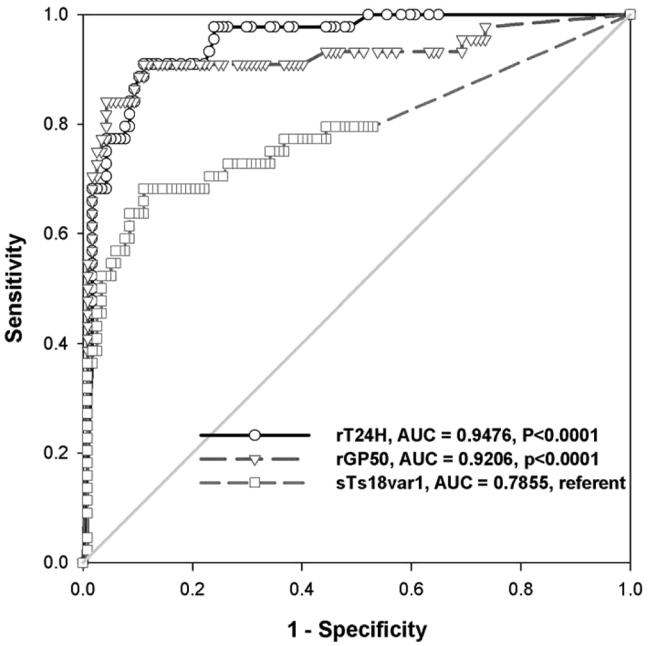

FIGURE 1 ROC Curves of rT24H, rGP50, and sTs18var1. ROC curves for rT24H, rGP50, and sTs18var1 that were developed based on sensitivity and (1-specificity) data pairs calculated for each possible cut-off point. AUC = Area Under Curve.

Test Performance

The performance of QuickELISA assays (based on defined pig sera set 1–3) for rGP50, rT24H, and sTs18var1 is shown in Table 1. The ROC Curves of these three QuickELISA can be seen in Figure 1. The ROC Curve for rT24H, rGP50, and sTs18var1 QuickELISA yielded AUCs of 0.9476, 0.9206, and 0.7855, respectively. There was no statistical difference between rT24H and rGP50 AUC; but, both rT24H and rGP50 were statistically significantly different from sTs18var1 ($p < 0.0001$).

Test Performance in Oxfendazole Treated Pig Sera

When cysticercosis positive pigs were treated with a single dose of oxfendazole, antibodies against rGP50, rT24H, and sTs18var1 increased for the first 9 weeks and then gradually decreased (Fig. 2). There was no difference in the amount of antibody responses against rGP50 and rT24H across the time, but antibody responses against these two antigens were significantly different from the antibody response against sTs18var1 ($p < 0.0001$). Although there were different amounts of antibody responses across time,

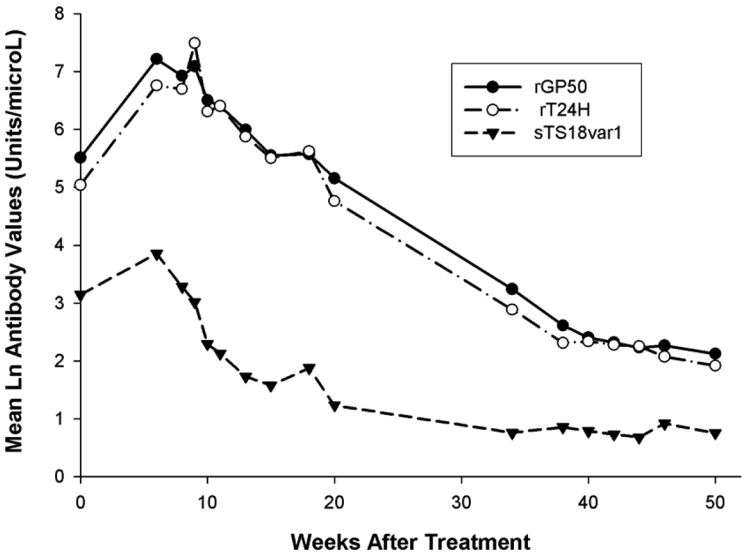

FIGURE 2 Follow-up of Antibody Responses in Oxfendazole Treated Pigs. Nineteen pigs with cysticercosis were treated with a single oral dose of oxfendazole and sera were collected weekly for nearly 52 weeks. Sera were assayed for their antibody responses against rGP50, rT24H, and sTs18var1 using the QuickELISA. Each sample was run in a single well, the A_{450nm} was converted into units of activity based on a standard curve. Mean Ln Antibody Value = the average of natural logarithm of the units of antibody response from 19 pigs.

there were no clinically important differences (such as pattern differences) in antibody responses against those three antigens.

DISCUSSION

Monitoring porcine cysticercosis in a cysticercosis/taeniosis control program is important and this means that a large number of pig serum samples should be monitored regularly. This condition sets up a need for an automated, fast, and reliable porcine cysticercosis test.

In this study, adaptation of QuickELISA to automation worked well and the whole reaction could be carried out within 1 h. Triturus EIA Analyzer performs well for the QuickELISA and it gives a very good intra- and inter-assay variations. As reliability and well-to-well difference is below 10%, it is possible to run each test sample in a single well. By setting-up a tighter protocol on running the assay (such as the time to take out the substrate TMB to ensure reaching room temperature before running the assay), the study could achieve lower inter- and intra-assay variations in Lima, Peru compared to the study conducted in Atlanta. By allowing low inter- and intra-assay variations, QuickELISA on Triturus EIA Analyzer allows more samples to be tested in a faster and reliable way.

Performance of the QuickELISA for detecting porcine anti-cysticercosis antibodies in the Triturus EIA Analyzer is quite good. The sensitivity and specificity with the rGP50 QuickELISA is 94% and 96%, respectively; for rT24H QuickELISA it is 97% and 89%, respectively. For sTs18var1 QuickELISA, the specificity is 89% but the sensitivity drops to 74%.

Based on these performance data and on the data that showed all three antigens detected the same clinical condition (Fig. 2), we eliminated sTs18var1 QuickELISA from further use. The rGP50 and rT24H QuickELISA perform well and the performance difference is not statistically significant. As it is costly to perform two assays for the same conditions, it is very important to test the performance of rGP50 and rT24H QuickELISA in a larger set of samples. If again the performance of both of these assays is statistically not significant, then, the decision to use either one depends on practical considerations such as the cost of producing the antigen and the ease of production of the QuickELISA test.

CONCLUSIONS

For the purpose on conducting serological surveys in a cysticercosis control program setting, there is a need for an assay that is automated, fast, and reliable. rGP50 and rT24H QuickELISA perform well on Triturus EIA Analyzer platform. The decision to use either one depends on practical

considerations the cost such as the cost of producing the antigen and the ease of production of the QuickELISA test.

REFERENCES

1. Tsang, V.C.; Brand, J.A.; Boyer, A.E. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J. Infect. Dis. **1989**, *159*, 50–59.
2. Bueno, E.C.; Scheel, C.M.; Vaz, A.J.; Machado, L.R.; Livramento, J.A.; Takayanagui, O.M.; Tsang, V.C.; Hancock, K. Application of synthetic 8-kD and recombinant GP50 antigens in the diagnosis of neurocysticercosis by enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. **2005**, *72*, 278–283.
3. Hancock, K.; Khan, A.; Williams, F.B.; Yushak, M.L.; Pattabhi, S.; Noh, J.; Tsang, V.C. Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. J. Clin. Microbiol. **2003**, *41*, 2577–2586.
4. Hancock, K.; Pattabhi, S.; Greene, R.M.; Yushak, M.L.; Williams, F.; Khan, A.; Priest, J.W.; Levine, M.Z., Tsang, V.C. Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. Mol. Biochem. Parasitol. **2004**, *133*, 115–124.
5. Hancock, K.; Pattabhi, S.; Whitfield, F.W.; Yushak, M.L.; Lane, W.S.; Garcia, H.H.; Gonzalez, A.E.; Gilman, R.H.; Tsang, V.C. Characterization and cloning of T24, a *Taenia solium* antigen diagnostic for cysticercosis. Mol. Biochem. Parasitol. **2006**, *147*, 109–117.
6. Handali, S.; Gonzalez, A.E.; Hancock, K.; Garcia, H.H.; Roberts, J.M.; Gilman, R.H.; Tsang, V.C. Porcine antibody responses to *Taenia solium* antigens rGp50 and sTs18var1. Am. J. Trop. Med. Hyg. **2004**, *71*, 322–326.
7. Scheel, C.M.; Khan, A.; Hancock, K.; Garcia, H.H.; Gonzalez, A.E.; Gilman, R.H.; Tsang, V.C. Serodiagnosis of neurocysticercosis using synthetic 8-kD proteins: comparison of assay formats. Am. J. Trop. Med. Hyg. **2005**, *73*, 771–776.
8. Gonzalez, A.E.; Falcon, N.; Gavidia, C.; Garcia, H.H.; Tsang, V.C.; Bernal, T.; Romero, M.; Gilman, R.H. Treatment of porcine cysticercosis with oxfendazole: a dose-response trial. Vet. Rec. **1997**, *141*, 420–422.
9. Gonzalez, A.E.; Falcon, N.; Gavidia, C.; Garcia, H.H.; Tsang, V.C.; Bernal, T.; Romero, M.; Gilman, R.H. Time-response curve of oxfendazole in the treatment of swine cysticercosis. Am. J. Trop. Med. Hyg. **1998**, *59*, 832–836.
10. Gonzalez, A.E.; Verastegui, M.; Noh, J.C.; Gavidia, C.; Falcon, N.; Bernal, T.; Garcia, H.H.; Tsang, V.C.; Gilman, R.H.; Wilkins, P.P. Persistence of passively transferred antibodies in porcine *Taenia solium* cysticercosis. Cysticercosis Working Group in Peru. Vet. Parasitol. **1999**, *86*, 113–118.
11. Aitken, A.; Learmonth, M. Estimation of disulfide bonds using Ellman's Reagent. In *The Protein Protocol Handbook*, Walker, J.M., Ed.; Humana Press, Inc: Totowa, N.J., **1996**, 487–488.
12. Zweig, M.H.; Campbell, G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin. Chem. **1993**, *39* (4), 561–577.