TESTING OF THE BORDIER TAENIA SOLIUM ELISA

FINAL REPORT

Prepared by Hector H. Garcia, MD, PhD
Universidad Peruana Cayetano Heredia, Lima, Peru.
Telephone +51 1 3287360
E-mail hgarcia@jhsph.edu

July 2018

SUMMARY

The performance of the Bordier ELISA test for antibodies to *Taenia solium* was tested using archive samples from patients with viable, subarachnoid and calcified neurocysticercosis (NCC), as well as samples from the most common cross reactive cestodes *Echinococcus granulosus* and *Hymenolepis nana.* From a sample battery used in a prior study, we increased the numbers and groups of samples and replaced those not anymore available. A total of 240 samples were selected and 15 were excluded because there was no archive serum available anymore, in order to complete five groups of 45 samples each. The sensitivity of the test was 71% (32/45) in sera from individuals with viable NCC, 98% (44/45) in subarachnoid NCC, and 40% (18/45) in individuals with calcified NCC. Thirty nine and 13 samples from patients with hydatidosis and hymenolepiasis tested positive. When applying the adjusted cut off (1.6 times the weak control), sensitivity for viable NCC dropped to 51%, subarachnoid NCC was 96%, and calcified NCC dropped to 16%. Cross reactions decreased from 39 to 31 and from 13 to 4 samples.

INTRODUCTION

Taenia solium cysticercosis is endemic in most developing countries, and results from the infection of humans or pigs with the eggs of the cestode that then invade the tissues and establish as larval cysts. Invasion of the nervous system (neurocysticercosis, NCC) causes one-third of seizures and epilepsy cases in endemic regions. NCC is also seen with some frequency in developed nations because of immigration and travel to endemic regions.

Diagnosing human NCC requires brain imaging and supports on serological assays. Neuroimaging uses using computed tomography (CT), magnetic resonance imaging (MRI), or both. CT or MRI machines are scarce in endemic areas, and also in many cases the imaging findings are not conclusive for the diagnosis. Immunodiagnosis complements the results of neuroimaging by detecting antibodies to the parasite, or antigens of the parasite. The sensitivity of antigen detection is in general higher, but antibodies may result from exposure to the parasite or resolved infections and thus it does not necessarily reflect current infection. The reference assay for the detection of antibodies to *T. solium* is the electro immunotransfer blot with lentil-lectin purified glycoprotein antigens (LLGP-EITB). This assay, however, is technically complicated and not widely available. Several ELISA tests are commercially available. We have recently evaluated two selected kits, with poor results. Here we provide data on the performance of the Bordier assay.

PROCEDURES

Study samples. Anonymized archive serum samples from patients with either viable parenchymal brain cysticercosis (n=45, positive on LLGP-EITB for cysticercosis to two or more antibody bands except for one sample that was positive to two antibody bands only), subarachnoid NCC (n=45, positive on LLGP-EITB for cysticercosis to three or more antibody bands) or resolved, calcified NCC (n=45, positive on LLGP-EITB for cysticercosis to only one [n=12], two [n=31], or three [n=2] antibody bands), and with other cestode parasites (hymenolepiasis, n=45, and cystic hydatid disease, n=45, all negative on LLGP-EITB), were evaluated using the Bordier *Taenia solium* antibody detection ELISA kit. In addition, all NCC samples had neuroimaging confirmation. Departing from the sampling framework used in a previous similar study, 15 samples were replaced because of lack of available serum volume, and a group of patients with subarachnoid NCC was added. Samples were collected under written informed consent specifically authorizing future use for diagnostic studies. The studies and informed consents were revised and approved by the main IRB of the Universidad Peruana Cayetano Heredia in Lima, Peru (IRB Code 51070, FWA 00002541).

Enzyme-linked immunosorbent assay (ELISA) test. The Bordier *Taenia solium* antibody detection ELISA kit was used following strictly the indications in the enclosure. All samples were run in duplicate. In the final plate we ran one sample that was missing in the hydatid group (this sample was ran in four wells for extra precaution), and repeated 13 samples whose coefficient of variation (difference between the OR readings of both wells) was higher than 9%, and also re-ran 12 samples whose readings were close to the "weak" reference reading for the plate where they were originally processed.

Statistical analysis. Descriptive statistics were calculated by sample group including mostly proportions, medians and interquartile (IQR) ranges. Positive rates and their corresponding 95% confidence intervals (CI) were calculated in samples of cases with viable, subarachnoid, and calcified NCC as a surrogate for sensitivity as compared to LLGP-EITB as the reference gold standard in these subgroups. In addition, the frequencies of cross-reactions in the subgroups with hydatid disease and *Hymenolepis* were also calculated.

RESULTS

The sensitivity of the test was 71% (32/45) in sera from individuals with viable NCC, 98% (44/45) in subarachnoid NCC, and 40% (18/45) in individuals with calcified NCC. Thirty nine and 13 samples from patients with hydatidosis and hymenolepiasis tested positive, thus resulting in 87% and 29% of cross reactions respectively. When applying the adjusted cut off (1.6 times the weak control), sensitivity for viable NCC dropped to 51%, for subarachnoid NCC it was 96%, and for calcified NCC it dropped to 16%. Cross reactions decreased from 39 to 31 (69%) in samples from patients with hydatidosis and from 13 to 4 (9%) in samples from patients with hymenolepiasis.

The frequency of positive results for each subgroup with its 95% confidence interval, as well as the median and interquartile range for the OD values in the subgroup are presented in Table 1 and Figure 1.

Table 1. Characteristics of the study population (n=45 in each group).

	Viable	Subarachnoid	Calcified	Hydatid	Hymenolepis
Sex = M	29 (64%)	24 (53%)	17 (38%)	19 (42%)	29 (64%)
Age (median, IQR)	34 (26 -40)	41 (32 – 51)	29 (24.5 – 48)	30 (21 – 53.5)	21 (14 – 30)
Cysticercosis EITB results (antibody bands, median, range)	Positive 5 (2 to 7)	Positive 7 (4 to 7)	Positive 2 (1 to 3)	Not performed	Not performed

Table 2. Frequency of positive antibody detection by ELISA in diverse types of neurocysticercosis and other cestode infections (n=45 in each group).

	Viable	Subarachnoid	Calcified	Hydatid	Hymenolepis
Positive by standard cut off	32 (71%) 95% CI 57% – 82%	44 (98%) 95% CI 88% – 100%	18 (40%) 95% CI 27% – 55%	39 (87%) 95% CI 74% – 94%	13 (29%) 95% CI 18% – 43%
Positive by adjusted cut off	23 (51%) 95% CI 37% – 65%	43 (96%) 95% CI 85% – 99%	7 (16%) 95% CI 8% – 29%	31 (69%) 95% CI 54% – 80%	4 (9%) 95% CI 4% – 21%
Median OD	1.175	2.832	0.564	1.898	0.455
OD IQR	0.704 – 1.993	1.995 – 3.171	0.386 – 0.785	0.773 - 2.482	0.379 – 0.709

Repeats. None of the 13 samples repeated because of high variation between wells changed its result (two were always negative and 11 were always positive). The overall variation in the mean OD between one processing and the next was 8.34%. In regard to the samples close to the borderline (n=12), half of them changed their results. These were five that moved from positive to negative (two in the calcified NCC group, two in the hydatid group, and one in the *Hymenolepis* group) and one from negative to positive (belonging to the calcified NCC group). The overall variation in the mean OD between one processing and the next in this subgroup with low ODs was 15.61%.

DISCUSSION

The Bordier assay performed along the expected yield for an antibody ELISA, and the obtained sensitivity and specificity values seem superior to other commercially available assays.

While it is difficult to compare tests that have been assessed using different sample batteries, Carod *et al.* in 2012 assessed five commercial cysticercosis antibody-detection ELISA assays (DRG™, Ridascreen™, Novatech™, Cypress™, and IVD™) and concluded that the Ridascreen™ and Novatech™ assays performed better than the other three. Sensitivity of the assays was evaluated with 14 archive serum samples of patients with defined cysticercosis, confirmed by imaging and positive to EITB, and specificity was evaluated with 99 negative samples from French blood donors and 60 sera from patients with heterologous infections living in countries not endemic for cysticercosis. The best sensitivity was obtained by the Ridascreen™ assay and it was only 71.4%. This assay, however, had the lower specificity (74.2%). The Novatech™ assay had the best specificity (95.6%), with a poor sensitivity (42.9%).

In a further study, our group examined the two best-performing commercially available ELISA kits according to Carod`s work, Novalisa™ and Ridascreen™. This study used

archive serum samples from patients with viable or resolved, calcified NCC, and heterologous infections (hymenolepiasis and hydatid disease), 45 samples in each group. The performance of both the Novalisa™ and Ridascreen™ assays was poor. Their sensitivity to detect specific antibodies in patients with viable NCC was low (44.4% and 22.2%), and cross reactions with cystic hydatid disease were highly frequent in both ELISA assays (38/45, 84.4%; and 25/45, 55.6%). Cross reactions with hymenolepiasis were present at lower rates (five cases, 11.1% in one of the assays, and only one sample, 2.2%, with the second assay).

The evaluation reported here is even more comprehensive and included a group of individuals with subarachnoid cysticercosis. This variety of NCC is known to course with strong antibody responses and high antigen levels. As expected, the proportion of seropositivity in this group was close to 100%. On the other hand, interpreting the proportion of positive results in people with calcified NCC is less intuitive since NCC patients may remain antibody-positive for months or years after parasitologic (neuroimaging) evidence of cure. The 71% sensitivity is appropriate, and in this evaluation the use of an adjusted cut-off did not improve the overall performance of the test.

APPENDIXES

Appendix 1 – Data per plate

Plate	Positive	Weak	Negative	Blank
1	3.007	0.554	0.217	0.161
2	3.134	0.639	0.228	0.160
3	3.550	0.731	0.282	0.178
4	3.605	0.768	0.262	0.188
5	3.035	0.618	0.252	0.165
6	3.558	0.788	0.251	0.180
7	3.458	0.821	0.275	0.178

Appendix 2 – Raw data

Attached in Excel file (DataFinalReporteElisaParaBordier.xls).