

# Evaluation of a new immunochromatographic assay for the detection of antibodies against *Echinococcus granulosus*

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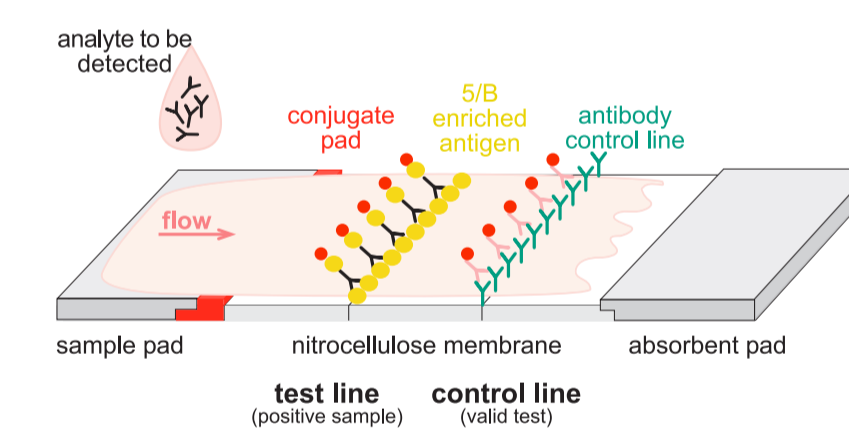
## Introduction and Purpose

*Echinococcus granulosus* is the causal agent of human hydatidosis. The adult phase parasitizes the small intestine of canids (definitive hosts), while the larva affects sheep and cattle (intermediate hosts) or accidentally, other animals, including humans. The larval phase develops as a hydatid cyst in the internal organs of intermediate host, most often locating in the liver (mainly in the right lobule), followed by the lung and other organs, such as the brain and bones. The intensity of the immune response depends on the location and integrity of the cyst. Cysts in liver and bone are more reactive than those in lung, brain or spleen. Serologic assays, together with imaging techniques, are most frequently used for diagnosis.

A new immunochromatographic assay (ICA) for total *E. granulosus* antibodies, VIRAPID® HYDATIDOSIS, has been evaluated.

### ICA description and test procedure

VIRAPID® HYDATIDOSIS is a single strip assay with two lines, test and control, based on a HPLC-purified antigen prepared from a 5/B enriched fraction obtained from hydatid fluid. The antigen is adsorbed on both the conjugate and the test line. So, when the specimen is added along with the running solution, there is a first immunological reaction between the specific antibodies of the serum/plasma and the protein coupled to the gold particles. These complexes move along the membrane to the test line, and a coloured band will appear if *E. granulosus* antibodies are present (see Figure 1). A control line is included for the validation of the assay.



### Test procedure

Sample volume: 30 µl  
 Running solution: 2 drops (ca 70 µl)  
 Reaction time: 20-30 min

## Material and methods

### Sera/plasma samples and reference methods

Two main groups of samples were analyzed to check the test performance: 199 negative specimens from two Spanish hospitals (Jaén and Almería), and 77 positive samples from different geographic areas. Two CE-marked commercial assays for hydatidosis, an ELISA IgG (Vircell, Spain) and an indirect haemagglutination (Fumouze, France) were used as reference methods.



Figure 1. Examples of VIRAPID® HYDATIDOSIS results: negative and positive (low, clear and high) samples.

## Results

73 out of 77 positive sera showed a distinct red test line in the ICA. Results of the ICA and the two reference tests are presented in Table 1. In order to perform the reading of the test and to determine the positivity of the samples, an intensity card (see Figure 2) has been used. 4 levels of colour intensity ranging from 0.5 to 3 can be read. When the intensity is lower than 0.5, the result is considered negative. When the intensity is higher than or equal to 0.5, the reading is considered positive.

198 out of 199 negative sera showed no reactivity on the test line in the ICA. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for the new VIRAPID® HYDATIDOSIS assay as 94.7% (86.5- 98.3%), 99.5% (96.8- 99.9), 98.6% (91.7- 99.9%) and 98.0% (94.7- 99.4%), respectively (95% confidence intervals shown in parentheses).

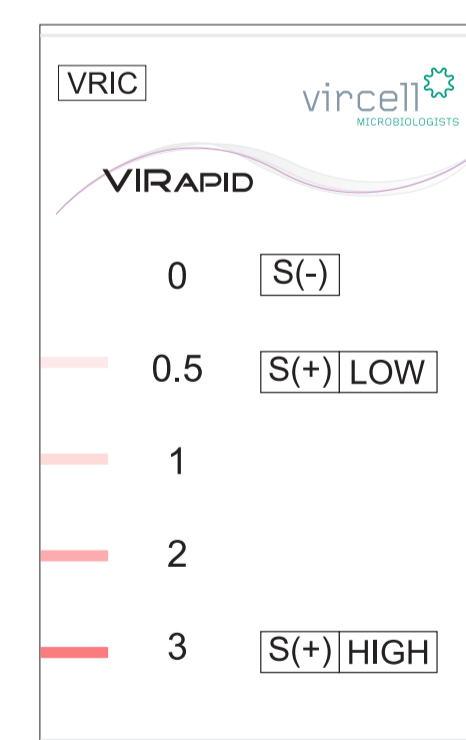


Figure 2. Reading intensity card

## Conclusions

The new test has proved to be able to detect anti-hydatid antibodies in human serum samples with good sensitivity and specificity values. This assay offers a new diagnostic tool that brings together good performance characteristics with the advantages of rapid tests (easy to run, easy to interpret and easy to store). It suits the demand of both laboratories from developed areas with low prevalence of the disease and those from developing regions, with more limitations in their material equipment.

Serum #	Origin	ELISA Index (cutoff=10)	Indirect haemagglut.	ICA Reading	ICA Interpret.
1	Spain	25	>10240	1	Positive
2	Spain	31	5120	3	Positive
3	Spain	30	5120	3	Positive
4	Spain	28	5120	3	Positive
5	Spain	31	>10240	3	Positive
6	Spain	32	>10240	2	Positive
7	Spain	20	>10240	3	Positive
8	Spain	22	640	3	Positive
9	Spain	33	>10240	3	Positive
10	Spain	34	5120	3	Positive
11	Spain	21	10240	2	Positive
12	Spain	17	10240	1	Positive
13	Spain	11	1280	0,5	Positive
14	Spain	35	>10240	2	Positive
15	Spain	28	>10240	2	Positive
16	Spain	27	1280	1	Positive
17	Spain	28	2560	1	Positive
18	Spain	27	2560	3	Positive
19	Spain	21	1280	1	Positive
20	Spain	27	640	1	Positive
21	Spain	29	2560	3	Positive
22	Spain	16	>10240	3	Positive
23	Spain	28	>10240	3	Positive
24	Spain	29	>10240	1	Positive
25	Spain	21	5120	1	Positive
26	Spain	14	5120	1	Positive
27	Spain	20	640	1	Positive
28	Spain	22	1280	<0,5	Negative
29	Spain	24	>10240	2	Positive
30	Spain	16	5120	2	Positive
31	Spain	26	5120	2	Positive
32	Spain	19	5120	1	Positive
33	Spain	18	10240	1	Positive
34	Spain	16	640	0	Positive
35	Spain	13	1280	1	Positive
36	Spain	13	320	<0,5	Negative
37	Spain	14	1280	<0,5	Negative
38	Spain	16	5120	1	Positive
39	Spain	17	10240	2	Positive

Serum #	Origin	ELISA Index (cutoff=10)	Indirect haemagglut.	ICA Reading	ICA Interpret.
40	Spain	14	640	2	Positive
41	Spain	19	5120	3	Positive
42	Spain	19	10240	3	Positive
43	Spain	19	10240	3	Positive
44	Spain	25	10240	3	Positive
45	Spain	29	2560	3	Positive
46	Spain	14	>640	1	Positive
47	Spain	26	1280	2	Positive
48	Spain	16	1280	2	Positive
49	Turkey	29	2560	1	Positive
50	Spain	33	>10240	3	Positive
51	Spain	34	>10240	3	Positive
52	Turkey	32	>10240	3	Positive
53	Spain	26	>10240	2	Positive
54	Spain	28	>10240	<0,5	Negative
55	Tunisia	21	1280	3	Positive
56	Tunisia	26	>2560	3	Positive
57	Tunisia	28	>2560	3	Positive
58	Tunisia	14	>1280	0,5	Positive
59	France	30	>2560	3	Positive
60	France	34	>2560	3	Positive
61	France	29	>2560	3	Positive
62	France	26	>2560	3	Positive
63	France	35	>2560	1	Positive
64	France	33	2560	3	Positive
65	France	25	2560	3	Positive
66	France	18	2560	1	Positive
67	France	14	2560	2	Positive
68	France	23	1280	1	Positive
69	France	28	1280	2	Positive
70	France	27	1280	3	Positive
71	France	20	1280	3	Positive
72	France	22	1280	1	Positive
73	France	19	640	1	Positive
74	France	18	640	1	Positive
75	France	19	640	2	Positive
76	Spain	25	640	3	Positive
77	Spain	24	640	3	Positive

Table 1. Results in the ICA and the reference tests for the positive samples. ICA reading was carried out with the help of the interpretation card shown in figure 2.