ECHINOCOCCUS Western Blot IgG is a qualitative Immunoblot Assay intended for:

- *E.granulosus* and *E.multilocularis* serology confirmatory testing on human sera previously tested with classical serology screening tests (IHA, IFA, ELISA)
- *E.granulosus* and *E.multilocularis* serological differentiation.

Introduction

Echinococcosis are zoonosis caused by larvae of tape-worms living as intestinal parasites of wild or domesticated carnivores and belonging to the genus *Echinococcus*. Four species are of medical importance in that they may inflict severe disease in humans. *E.granulosus*, one of the world’s major zoonosis, affects both man and his domestic animals. It occurs world-wide, but social behaviour and low hygienic conditions favour the prevalence of the infection [4, 10, 16]. *E.multilocularis*, the most lethal, is found in the northern hemisphere (Europe, Russia, Siberia, China, Japan, Canada, U.S.A., Turkey...). *E.vogeli* and *E.oligarthus* are restricted to the northern half of South America, but represent no longer a medical curiosity [5]. Simultaneous infections of humans with *E.granulosus* and *E.multilocularis* occur in Central Europe, Russia, and China [4, 11].

Human infections occur by ingestion of eggs excreted with the faeces of carnivores. A digestive process in the host gut results in release of a larva which penetrates intestinal villi and migrates to other anatomical sites. In some cases, disease does not appear and lesions become calcified. In these cases, died-out larvae are able to maintain a high IgG antibody response for several years [2, 8].
However, the infection may cause dramatic damage in a large proportion of individuals. The clinical pattern of the disease is determined by the development of larvae which is quite different from a species to another [16]. *E.granulosus* grows as an hydatid cyst by internal budding of brood capsules, mainly in the liver and the lungs. However, no organ of the body is exempt. With time (several months or years), a fully mature and large larva is formed, constituted by a fluid-filled, usually unilocular bladder, surrounded by a thick fibrotic wall formed by the host response [17]. The pathology of the disease is mainly due to the physical pressure developing cyst exerts on the viscera. Anaphylaxis and peritoneal seeding may also occur when a cyst ruptures and releases cyst fluid and parasitic cells, respectively, into the body cavity [10, 17].

In contrast, *E.multilocularis* larvae establish almost exclusively in the liver of humans and develop by external budding to form an infiltrative growing tumour. They are surrounded by an intense inflammatory process which leads to scattered liver fibrosis, and not to a limiting host-tissue barrier. Larval cells can thus give rise to metastatic foci through distribution via lymph or blood [17].

By the time the disease becomes clinically manifest, alveolar echinococcosis is often misdiagnosed as liver cancer and the lesions have often reached an extension too large for complete surgical resection [2]. *E.vogeli* and *E.oligarthus* develop mainly by internal budding. Larvae are termed polycystic since they are characterized by the internal division of fluid-filled cysts to form multichambered growths [5, 17]. Liver localizations are more frequent than those of mesentery, lungs, muscles or orbit. Due to the extensive nature of lesions by the time of diagnosis, prognosis may be similar to that of alveolar echinococcosis [5, 6].

There is rarely any parasitological evidence of infection. Furthermore, the puncture of *E.granulosus* cysts is discouraged because of risks of anaphylactic shock [10, 17]. The diagnosis is mainly suggested by imaging techniques (ultrasound scanning and computerized tomography) and sometimes by non specific biological abnormalities. Imaging techniques must be combined with serological assays.

Since each of these parasitic infections may be lethal without treatment, and since the efficiency of antiparasitic drugs depends upon the cyst size [17], early serological diagnosis and subsequent treatment (chemotherapy and/or surgery) may reduce mortality [2, 7]. Among all available serological assay methods used for the screening of human echinococcosis IHA or ELISA are satisfactory [17]. Antibody detection in human sera is usually based upon the use of either crude *E.granulosus* or crude *E.multilocularis*, both of which lack specificity. Indeed, sera from patients with other helminthic parasites exhibit cross-reactivity [2, 7]. The use of highly purified antigens improves the specificity of serological assays but leads to a loss of sensitivity. Indeed, some patients with echinococcosis do not produce any antibodies against these antigens [2, 3, 7, 14].
The remarkable sensitivity and specificity of the immunoblot technique led to the use of it as a powerful confirmatory test and a serological tool for the differentiation between both infections of major relevance: cystic and alveolar echinococcosis [1, 9, 11, 12, 13].

Principle of the test:

1/ The strips:
Echinococcus multilocularis antigens from a crude larval extract have been resolved by electrophoresis into bands and transferred by electro blotting onto a nitrocellulose membrane (Western Blot procedure). The antigen bearing membrane has been cut into ready to use numbered strips.

2/ The test:
Each serum to be tested is incubated separately with a membrane strip. Echinococcus-specific IgG, if present in the serum, will bind to the antigens on the strips. The first wash step removes any unbound serum components. The strip is then incubated with an alkaline phosphatase-anti human IgG conjugate. Following the second wash step, the whole complex is then detected by addition of substrate (NBT/BCIP) which precipitates as a dark blue-purple colour.

The colour development is stopped by rinsing the strips with distilled water. Echinococcus-specific IgG, if present in the serum sample will appear on the strips as violet coloured bands. The strips are dried and their coloration remains stable for years once stored away from direct light.

3/ Reading (see page 8 § Interpretation for details):
The presence on the strip of the 7 and/or the 26-28 kDa band(s) is indicative of the presence of Echinococcus-specific IgG in the serum sample. Specific intermediate bands (between 7 and 26-28 kDa) allow thereafter the differentiation between E.granulosus and E.multilocularis infections in more than 2/3 of cases.

Kit components

Nota: The numbers (in italic) are the volumes and quantities corresponding to the 12 tests kit #ECH-WB12G. The numbers (in bold) are the volumes and quantities corresponding to the 96 tests kit #ECH-WB96G.

R1 One (1) folder containing 24 (12 or 4x24) numbered TEST STRIPS
These are nitrocellulose membrane strips bearing electrophoresis
separated *E. multilocularis* larval antigens. **Ready to use.**

**R2** One (1) vial containing 30 (16 or 120) ml of SAMPLE BUFFER: buffer + surfactant + NaN3 (<0.1%) + red dye. **Pink solution - Ready to use.**

**R3** One (1) vial containing 60 (30 or 240) ml of WASH CONCENTRATE 10X concentrated buffer + surfactant + NaN3 (<0.1%) **colourless solution - to be diluted 1/10 in distilled water.**

**R4** One (1) vial containing 30 (16 or 120) ml of ANTI IgG CONJUGATE buffer + polyclonal goat anti-human IgG - Alkaline Phosphatase conjugate + stabilisers + NaN3 (<0.1%) + blue dye. **Blue solution - Ready to use.**

**R5** One (1) vial containing 200 (200 or 400) µl of POSITIVE CONTROL buffer + human serum positive in *Echinococcus multilocularis* serology + stabilisers + NaN3 (<0.1%). **red cap vial - Ready to use.**

**R6** One (1) vial containing 30 (16 or 120) ml of SUBSTRATE buffer + NBT + BCIP + stabilisers **Brown vial - Ready to use.**

- One (1) prestained Molecular Weights (MW) Standards Strip (recombinant proteins), visible on the right side of the nitrocellulose membrane (R1 folder) for estimating the MW of the revealed bands (kDa): Blue = 250, Blue = 150, Blue = 100, Pink = 75, Blue = 50, Green = 37, Pink = 25, Blue = 20, Blue = 15, Yellow (very pale) = 10.
- The kit also contains pages 18 et 19, as example, a Reference Sheet: the scanner copy of ECHINOCOCCUS immunoblots.

**Additional Materials Required**
- Latex powderless gloves and tweezers for handling the strips
- Multi-channel polypropylene incubation tray for mini-blots (LDBIO # WBPP-8 or equivalent)
- Rocking platform
- Filtering flask connected to vacuum system (a water aspirator is sufficient)
- Cutter or scalpel
- Flat transparent ruler
- Absorbent paper (Whatman or equivalent)
- Aluminium foil
- High quality distilled or deionised water
- Automatic pipettes, micropipettes and disposable tips for dispensing volumes of 25µl, 1.2ml and 2ml
- Graduated cylinders and flasks
- Timer
- good quality transparent adhesive tape
- Serum collection equipment

Storage and stability
All reagents are stable until the expiry date (indicated on the outer box and bottle labels) when stored between 2-8°C.
Wash buffer solution is stable for 2 months after dilution when stored at 2-8°C

Precautions
1/ Safety
- All reagents derived from human origin have been tested and found negative for antibodies to HIV, hepatitis B surface antigen and hepatitis C antibodies. However, since no test can provide complete assurance of the absence of virus, treat all controls as potentially infectious.
- For in vitro use only.
- Do not mouth pipette reagents and avoid contact with skin.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the test. Wash hands thoroughly when finished.
- Never eat, drink or smoke at the laboratory workbench.
- Most of the reagents contain sodium azide as preservative. NaN3 may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagents should be flushed away with large volumes of water to prevent azide build up.
- Both samples and kit reagents should be handled with caution, following Good Laboratory Practice. Use care to prevent aerosols and decontaminate any sample spills.
- Substrate contains NBT and BCIP which may be toxic if inhaled or after contact with skin or mucous membranes. Any substrate that comes into contact with the skin should be rinsed off with water.
- Contact a licensed professional waste disposal service to dispose of all biological material (reagents, washing buffers, tips, vials, human sera…), follow the GMP’s and observe all state and local environmental regulations.

2/ Procedural
- Use powderless gloves and tweezers when handling western blots.
- Do not use reagents after their expiry date.
- Do not mix reagents from different lot numbers.
- Do not use the kit if liquid reagents become cloudy or develop precipitate.
- Do not freeze reagents.
- Deviation from the standard protocol provided may cause erroneous results.
- Mix well the reagents before using.
- Avoid leaving reagents in direct sunlight and/or above 4-8°C for extended periods.
- Return unused components to 2-8°C.
- Unused strips should be returned into their original plastic bag and resealed.
- If specimens cannot be tested on the day of collection, they should be stored at 2-8°C for up to 3 days. If longer storage is required, samples should be frozen at -20°C ± 5. Avoid repeated freeze-thaw cycles. On thawing, mix thoroughly.
- Do not contaminate samples or components and always use fresh pipette tips for each sample and component.
- High quality distilled or deionised water is required for the wash solution. The use of poor quality or contaminated water may lead to background colour in the assay.
- Mix the wash concentrate well, before dilution.
- Always use clean, preferably disposable, containers for all reagent preparation.
- Use care to prevent inter-channel contaminations.
- Use care to prevent aerosols formation.
- Establish a distribution plan before starting the test.
- Care must be taken to ensure that specimens are dispensed correctly into their designated test channel. If a specimen is inadvertently not added to a channel, the result for that channel will be negative, regardless of the actual status of the specimen.

**Specimen collection**
Serum samples should be obtained following Good Medical Practice. A minimum of 25µl of serum is required for ECHINOCCUS WB IgG testing. If specimens cannot be tested on the day of collection, they should be stored at 2-8°C for up to 3 days. If longer storage is required, samples should be frozen at -20°C.

**Preparation of reagents**
**Wash Buffer (R3)** - For 4 ECHINOCCUS WB IgG assays, dilute 10 ml of Wash Concentrate 10X (R3) in 90 ml of distilled or deionised water. Swirl to mix. A squeeze bottle can be used to dispense the diluted Wash buffer during the washing steps. Wash buffer solution is stable for 2 months after dilution when stored at 2-8°C.
Test Procedure

1/ Establish a distribution plan for control and samples before starting the assay. Strips are in numerical order, attached by the top in the folder R1.

2/ Dispense 1.2 ml of sample buffer (R2) into the designated channels.

3/ Wear powderless gloves. Don't touch the strips with fingers, handle strips with tweezers. Using a flat transparent ruler (well cleaned and dried) and a cutter (or a scalpel), cut out as many strips R1 as necessary: hold the strips in place by pressing the ruler firmly on the strips (numbers can be seen through the ruler) and cut out the strips along the bookbinding. Ensure that you keep the numbers and the blue line on the strips.

4/ Using tweezers, place one membrane strip, face up, into each channel of the tray following the distribution plan. Return the remaining strips into their bag and reseal. Shake the tray gently to ensure that the strips are well covered with the buffer. Incubate for 5-10 min to thoroughly wet the strips.

5/ Dispense 25 µl of each specimen and positive control into the channels following the distribution plan. Shake the tray gently (avoid cross contamination) after each dispense for mixing. Incubate on the rocking platform (about 10 cycles/mn) for 90 min ± 5mn at 18-25°C.

6/ Wash step:
   6.1 - Aspirate the liquid from each channel with a disposable tip connected to a vacuum system. Dispense about 2 ml of diluted Wash Buffer into each channel. Shake the tray gently and aspirate the liquid from the channels.
   6.2 - Dispense another 2 ml of diluted Wash Buffer into each channel. Incubate on the rocking platform for 3-5 mn. Aspirate the liquid from the channels.
   6.3 - Repeat step 6.2 one more time.

7/ Put 1.2 ml of anti IgG conjugate (R4) into each channel of the tray. Shake the tray gently to ensure that the strips are well covered with the buffer, face up. Incubate on the rocking platform for 60 min ± 5mn at 18-25°C.

8/ Wash step: Repeat step No 6.

9/ Dispense 1.2 ml of NBT/BCIP substrate (R6) into the channels. Shake the tray gently to ensure that the strips are well covered with the substrate, face up.
Incubate on the rocking platform at 18-25°C. Cover the trays with a piece of aluminium foil.
Check the colour regularly (every 5 mn). The usual range of time for the colour development is 20-60mn but there are no fixed rules regarding this time. Allow the colour to develop until the bands appear very well contrasted in comparison with the pink-grey background colour of the strips.
Stop the colour development by aspirating the liquid from the channel and dispensing 2 ml of distilled water. Repeat this step one more time.

Remarks: - The sera from some patients could have low level of *Echinococcus* antibodies. Be careful to allow sufficient time (i.e. 90 mn) for correct band colour development (check the strip's background colour to ensure pink-grey colouring).
- The quality of the washing steps is essential for getting a good contrast by reducing speed of the background coloration.

10/ Take the strips at the top, using the tweezers and transfer the strips face up onto a sheet of filter paper and let air dry. Leave them to dry about 15mn : the colour of the strips becomes much lighter after drying.

11/ Keeping them in place with a flat ruler, stick the top of the strips to the paper with a transparent adhesive tape.
Warning : strips are more fragile when dried.

Interpretation

◊ Molecular Weight (MW): *identification tools*

- The MW of unknown bands can be estimated by comparing their positions with those of bands on the prestained MW Standards Strip (R1 folder). Before using, cut out the Standards Strip from the folder R1 with the help of a flat ruler and a cutter.
- The strip obtained from the R5 positive control, when tested with an adjoining membrane strip at the same time as the serum samples, is the most precise positive standard of the kit. It will supply an exact model for the definite identification of the specific bands.
- The kit also contains one (1) Reference Sheet (page 18 et 19): a printed scanner copy of typical *E.granulosus* and *E.multilocularis* immunoblots. It can only be used as example.
◊ **Interpretation I :** serodiagnosis of the genus *Echinococcus*

With the help of the MW identification tools, search for the presence of the 7 and 26-28 kDa bands for each of the samples. These bands are usually easy to locate.

*Remark:* the 26-28 kDa band can present various aspects. It can be a narrow band at 26 or 28 kDa, it can be a double narrow band at 26 and 28 kDa, it can also be a single broad band between 26 and 28 kDa.

The presence on the strip of the 7 and/or the 26-28 kDa band(s) is indicative of the presence of *Echinococcus*-specific IgG in the serum sample.

◊ **Interpretation II :** differential diagnosis between *E.granulosus* and *E.multilocularis*

It is made by reading intermediate specific bands between 7 and 26 kDa.

- Bands which may be observed from 7 to 26 kDa:
  - Bands shared by both species: 12, 15, 20, 24, kDa
  - **Narrow** bands shared by *E.multilocularis* only: 16, 17, 18 kDa
  - Band shared by *E.granulosus* only: a **large fuzzy** band at 17 kDa.

- Five typical patterns (P1 to P5) are found. These 5 patterns are reproduced on the Reference Sheet of the kit (pages 18 and 19).

- P1, P2 and P3 (found in 2/3 of cases) are species specific and differentiate *E.granulosus* from *E.multilocularis*.

- P4 and P5, (found in 1/3 of cases) can not distinguish between the 2 species.
The five typical patterns (Cf: Reference Sheets page 18 and 19)

Remark 1: The isolated presence of intermediate bands (12, 15, 16, 17, 18, 20, 24 kDa) is not specific and cannot be used for the diagnosis. These bands are always associated with the band at 7 and/or 26-28 kDa during the *Echinococcus* infection.

Remark 2: A lot of other bands of a generally higher MW (more rarely lower MW) are often present on the strips. Some of them are specific of *Echinococcus*. Some of them are not. Because of the difficulty in distinguishing between the two, these other bands are not used for ECHINOCOCCUS WB IgG interpretation.

<table>
<thead>
<tr>
<th>Pattern (P1)</th>
<th>Description</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1:</td>
<td>Band 7 kDa only.</td>
<td>= <em>E. granulosus</em></td>
</tr>
<tr>
<td>P2:</td>
<td>Band 7 kDa + large fuzzy band 17 kDa. (NB: the 26-28 kDa band is usually also present)</td>
<td>= <em>E. granulosus</em></td>
</tr>
<tr>
<td>P3:</td>
<td>Band 26-28 kDa + both narrow bands 16 and/or 18 kDa. (NB: most of the other bands (7, 12, 15, 17, 20, 24 kDa) can also be present)</td>
<td>= <em>E. multilocularis</em></td>
</tr>
<tr>
<td>P4:</td>
<td>Band 26-28 kDa only. WITHOUT any additional intermediate band</td>
<td>= <em>E. multilocularis</em></td>
</tr>
<tr>
<td>P5:</td>
<td>Association of bands 7 + 26-28 kDa WITHOUT any additional intermediate band</td>
<td>or <em>E. granulosus</em></td>
</tr>
</tbody>
</table>
Remark 3: very rarely, the band 16 kDa has been seen more large than usually with a sample from *E.multilocularis* infected patient. Be care not to confuse this band with the large 17KDa *E.granulosus* specific band.

**Limitations of use:**

1/ Results of ECHINOCOCCUS WB IgG should be considered in the context of all available clinical, epidemiological and imaging (ultrasound scanning and computerized tomography) findings.

2/ Bands may have variable appearances: thin, thick, more or less coloured...
It is recommended therefore to start using ECHINOCOCCUS WB IgG assay by running some known sera for training.

3/ A negative ECHINOCOCCUS WB IgG result does not exclude the possibility of an infection.

4/ The test can not differentiate a co-infection with *E.multilocularis* and *E. granulosus* from an *E.multilocularis* infection.

**Trouble Shooting**

"Bands are pale, without contrast" :
The concentration of specific antibodies in the serum could be very low.

"Bands become rapidly large and dark so that they can't be clearly interpreted".
The concentration of specific antibodies in the serum could be very high. Monitor colour development (last incubation) to avoid over-development.

"The strip seems partly coloured with fuzzy, wave-like stains" :
The strip was not totally covered with reagent during one of the incubation steps. Artifact stains may be present on the strip at the dispensing place when the sample has not been mixed after dispensing.

"The strip presents a high background staining" :
Washing steps were insufficient or the last incubation step was too long.
Check the technical procedure, especially the washing steps and the quality of the deionised water. Monitor colour development to avoid over-development. Exceptionally, the sample can be the cause of the phenomenon (non-specific binding). The WB is not interpretable. Non-specific binding may concern only one part of the strip. The WB is then not interpretable on this part only.
"A dark pourple precipitate appears in the buffer during the last incubation (colour development step)"

The substrate can partially precipitate in the buffer during the last incubation. This will not influence the normal colour development of the bands on the strips. Solid particles of precipitate will be washed out during the last washing step. Don't mind and continue the incubation following the test procedure.

Quality control

A positive control (pattern P3) is included in the kit. It is necessary for:
- the validation of the technical procedure (the bands must appear clearly on the strip),
- the precise identification of the significant bands between 7 and 26-28kDA.

Performance characteristics

A multicentric study [9] has been carried out by 2 independent reference laboratories.

259 serums have been tested with the kit ECHINOCOCCUS WB IgG in order to determine its performance characteristics.

- **Sensitivity** :
  112 patient serums from 51 cystic echinococcosis and from 61 alveolar echinococcosis have been included in the study.

<table>
<thead>
<tr>
<th>Echinococcus WB IgG : patterns</th>
<th>Neg.</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic echinococcosis (n=50)</td>
<td>1</td>
<td>12</td>
<td>22</td>
<td>0</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Alveolar echinococcosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=61)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Total (n=111)</td>
<td>3</td>
<td>12</td>
<td>22</td>
<td>41</td>
<td>8</td>
<td>25</td>
</tr>
</tbody>
</table>

*Fig.1: sensitivity of the test - patterns frequency*

Nota : 1 serum from a cystic echinococcosis can not be interpreted (non-specific binding)

Sensitivity of the test  
= 97.3% for the genus *Echinococcus*  
= 98% for the species *E.granulosus*  
= 96.7% for the species *E. multilocularis*
• Discrimination between *E. granulosus* and *E. multilocularis*
  
  Calculated from the precedent data (*Fig. 1*), the differentiation between both species has been possible in **69.4%** of the cases (patterns *P1*, *P2*, and *P3*).

• Specificity

147 serum samples from various origin have been tested including the following infections or diseases: *T. solium* neurocysticercosis (42), *Schistosoma* (42), *F. hepatica* (10), filariasis (6), *T. spiralis* (6), *T. canis* (6), *S. stercoralis* (4), *E. histolytica* (4), *L. infantum* (4), *P. falciparum* (3), autoimmune diseases: FR+ (8), ANA+ (12).

139 sera are negative: on this population, the test specificity reaches 95%.

The 11 false positive results have been observed with the following samples:

- Cysticercosis: 5/42 samples presented a single band at 7 kDa.
- Autoimmune diseases: 1/8 patient FR+ and 2/12 patients ANA+ presented a single fine band at 28 kDa.

NB: Fasciolosis: 4/10 patients presented a very large fuzzy band at 25-30 kDa that cannot be confused with the typical 26-28 kDa specific band.

Results of ECHINOCOCCUS WB IgG should be considered in the context of all available clinical, epidemiological and imaging findings.

• Correlations:

The 112 positive samples (patients suffering from alveolar and cystic Echinococcosis) have been tested with the 4 following tests: a commercial indirect haemagglutination test (IHA), an ELISA using a crude antigen from sheep cyst fluid (*E. g ELISA*), an ELISA using a crude *E. multilocularis* larval Antigen (*E. m ELISA*) and an indirect immunofluorescence test using frozen sections of *E. granulosus* protoscolex (*E. g IFA*).

<table>
<thead>
<tr>
<th></th>
<th>IHA</th>
<th><em>E. g ELISA</em></th>
<th><em>E. m ELISA</em></th>
<th><em>E. g IFA</em></th>
<th>Immuno-blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic echinococcosis</td>
<td>82%</td>
<td>79%</td>
<td>56%</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>Alveolar echinococcosis</td>
<td>68%</td>
<td>92%</td>
<td>86%</td>
<td>ND</td>
<td>97%</td>
</tr>
</tbody>
</table>

*Fig. 2: Comparative sensitivity between five serological tests*
• Interferences
even no cross-reaction has been observed with lipidic, haemolysed or icteric sera, any result obtained from such samples must be interpreted cautiously.

• Reproducibility

- The inter-lot reproducibility of the assay has been checked by testing 10 positive sera (5 alveolar and 5 cystic echinococcosis) with 2 kits from different lot numbers (Echinococcus antigen from 2 different lot numbers). The same WB patterns were respectively observed with the 2 lot numbers.

- The inter-assay reproducibility has been checked by testing 8 positive sera (4 alveolar and 4 cystic echinococcosis) on 4 different occasions. The same WB patterns were respectively observed on the 4 occasions.
References


Recent communications:


Summary of Assay Procedure:
This summary is for quick reference only.
Read the full instructions for use before starting an assay.

1/ Establish a distribution plan

2/ Dispense 1.2ml of R2 (sample buffer - pink) into each channel.

3/ Place one strip R1, face up, into each channel.
   Shake gently.

4/ Dispense 25µl of samples and R5 (positive control).
   Shake gently the tray after each dispense.
   Incubate 90mn on rocking platform.

5/ Wash 3 times with 1/10 diluted R3.

6/ Dispense 1.2ml of R4 (conjugate - blue), shake gently.
   Incubate 60mn on rocking platform.

7/ Wash 3 times with 1/10 diluted R3.

8/ Dispense 1.2ml of R6 (substrate), shake gently.
   Incubate 20 - 60mn on rocking platform.

9/ Stop the reaction by rinsing 2 times with distilled water.

10/ Transfer strips on a filter paper – Let air dry 15mn.

11/ Compare each specimen strip to R5 positive control strip.
1/ Diagnosis of the Genus *Echinococcus*:
- Presence on the strip of a band at 7 and/or 26-28 kDa

2/ Differentiation between both Species:
- Pattern **P1** or **P2**: *Echinococcus granulosus* (*E.g*)
- Pattern **P3**: *Echinococcus multilocularis* (*E.m*)
- Pattern **P4** or **P5**: *E.multilocularis* or *E.granulosus*

Remark: The first strip on the left (*E.m*, P3 profile) has been done by using the positive control (**R5**) included in the kit.
Additional examples of WB positive samples from patients infected by *E.multilocularis* and *E.granulosus*.

These samples were especially chosen to be weak positive: most of *E.m* profiles are not complete (strip 13 excepted).

It is interesting to note the usual opposition of the profiles between both species:

*E.multilocularis*: The band 26-28 is often a double band and the strongest.

*E.granulosus*: inversely the strongest is the band 7 kDa.

But this rule is not absolute (ex. the *E.m* strip No 24)
## Index

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended use</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1-3</td>
</tr>
<tr>
<td>Principle of the test</td>
<td>3</td>
</tr>
<tr>
<td>Kit components</td>
<td>3-4</td>
</tr>
<tr>
<td>Additional materials required</td>
<td>4-5</td>
</tr>
<tr>
<td>Storage and stability</td>
<td>5</td>
</tr>
<tr>
<td>Precautions</td>
<td>5</td>
</tr>
<tr>
<td>Safety</td>
<td>5</td>
</tr>
<tr>
<td>Procedural</td>
<td>5-6</td>
</tr>
<tr>
<td>Specimen collection</td>
<td>6</td>
</tr>
<tr>
<td>Preparation of reagents</td>
<td>6</td>
</tr>
<tr>
<td>Test procedure</td>
<td>7-8</td>
</tr>
<tr>
<td>Interpretation</td>
<td>8-10</td>
</tr>
<tr>
<td>Limitations of use</td>
<td>11</td>
</tr>
<tr>
<td>Trouble shooting</td>
<td>11-12</td>
</tr>
<tr>
<td>Quality control</td>
<td>12</td>
</tr>
<tr>
<td>Performance characteristics</td>
<td>12-14</td>
</tr>
<tr>
<td>References</td>
<td>15-16</td>
</tr>
<tr>
<td>Summary of assay procedure</td>
<td>17</td>
</tr>
<tr>
<td>Reference sheet (ex. of positive and negative result)</td>
<td>18 - 19</td>
</tr>
</tbody>
</table>