NovaTec Immundiagnostica GmbH

NovaLisa®

Echinococcus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: ECHG0130 (96 Determinations)

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ENGLISH

1. INTRODUCTION

Echinococci are microscopic cestodes (tapeworms) of 1-6 mm which are dependent on their genus found either in dogs or other canids (E. granulosus) or in foxes, coyotes and wolves (E. multilocularis). In their larval stage they are the causative agent of human echinococcosis (Hydatiosis, or hydatic disease). The adult tapeworms reside in the small bowel of the definitive hosts, and gravid proglottids release eggs that are passed in the feces. After ingestion of a suitable intermediate host, the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and through the circulatory system into various organs, especially the liver and lungs, where it develops into a cyst. Echinococcus infections remain silent for years before the enlarging cysts cause symptoms in the affected organs (liver, lung, and less commonly other organs as brain, bone, heart). E. granulosus occurs practically worldwide, and more frequently in rural, grazing areas where dogs ingest organs from infected animals. E. multilocularis occurs in the northern hemisphere, including central Europe and the northern parts of Europe, Asia, and North America. Although human cases are rare, infection in humans causes parasitic tumors to form in the liver, the lungs, and less commonly, the brain, and other organs. If left untreated, infection can be fatal.

Species	Disease	Symptoms (e.g.)	Transmission route
E. granulosus	Cystic chinococcosis (Cystic Hydatid Disease, CHD)	(Depends on localization size, and number of cysts) Liver: Upper abdominal pain, hepatomegaly, cholestasis, jaundice, etc.	"hand-to-mouth" transmission. Infection by oral uptake of eggs. e.g.: contaminated wild berries.
E. multilocularis	Alveolar Echinococcosis (Alveolar Hydatid Disease, AHD)	Lungs: Thoracic pains, cough, expectoration, dyspnea, etc. CNS: Neurological symptoms	

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g by ELISA

2. INTENDED USE

The Echinococcus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Echinococcus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Echinococcus antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap;
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Echinococcus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.479	8.00
#2	24	0.863	3.43
#3	24	0.657	3.33
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	17.88	3.87
#2	12	13.28	4.74
#3	12	6.02	6.61

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 98.82% (95% confidence interval: 95.81% - 99.86%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 97.22% (95% confidence interval: 85.47% - 99.93%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: ECHG0130 Echinococcus IgG ELISA (96 Determinations)

Anti-Echinococcus EUROLINE WB (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DY 2321-1601-1 G	Echinococcus antigen extract (whole antigen) plus Em18, Em95 and EgAgB	IgG	Antigen coated membrane strips	16 x 01 (16)

Indication: The EUROLINE-WB test kit provides a qualitative in vitro assay for human antibodies of the immunoglobulin class IgG against Echinococcus granulosus and Echinococcus multilocularis antigens in serum or plasma for the diagnosis of echinococcosis.

Application: The Anti-Echinococcus EUROLINE-WB (IgG) is excellently suited for result confirmation of serological screening tests for the detection of Echinococcus-specific antibodies (IHA, IIFT, ELISA). The use of an Echinococcus whole antigen extract and biochemically produced single antigens of Echinococcus granulosus and Echinococcus multilocularis in the Anti-Echinococcus EUROLINE-WB (IgG) also enables differentiation between cystic and alveolar echinococcosis, caused by Echinococcus granulosus and multilocularis, respectively.

Test principle: The test kit contains blot strips with electrophoretically separated antigen extract of Echinococcus. Additionally, each blot has a membrane chip coated with the biochemically produced antigens Em18, Em95 and EgAgB. In the first reaction step the blot strips are incubated with diluted patient samples. In the case of positive samples, specific antibodies of class IgG (and IgA, IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format	Symbol
1. Test strips Single strips with electrophoretically separated Echinococcus antigen extract plus Em18, Em95 and EgAgB	16 x 1	STRIPS
2. Evaluation matrix with control strip Test strip incubated with a positive control serum	1 pattern	---
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 50 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	TRAY
7. Test instruction	1 booklet	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Undiluted patient sera and incubated blot strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers. Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels (black)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under: ZD 2321-0101-1 Evaluation protocol visual Anti-Echinococcus EUROLINE-WB.

Preparation and stability of the reagents

Note: The bag containing the blot strips is printed with a number in addition to the test kit lot number. This number refers to the strip batch and is also printed on the corresponding evaluation template. These two numbers must match to ensure correct evaluation of test results.
All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, if earlier, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the packing with the test strips only when the strips have reached room temperature to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2°C to +8°C. To ensure correct evaluation of results, the lot number on the bag must match the lot number on the strips as well as on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with ready for use diluted universal buffer. For 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml ready for use diluted universal buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the ready for use universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 1.5 ml buffer concentrate should be diluted with 13.5 ml deionised or distilled water. The ready for use diluted universal buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light ✳.

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the serum or plasma samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** in the ready for use diluted universal buffer. For example, add 30 µl of sample to 1.5 ml ready for use diluted universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

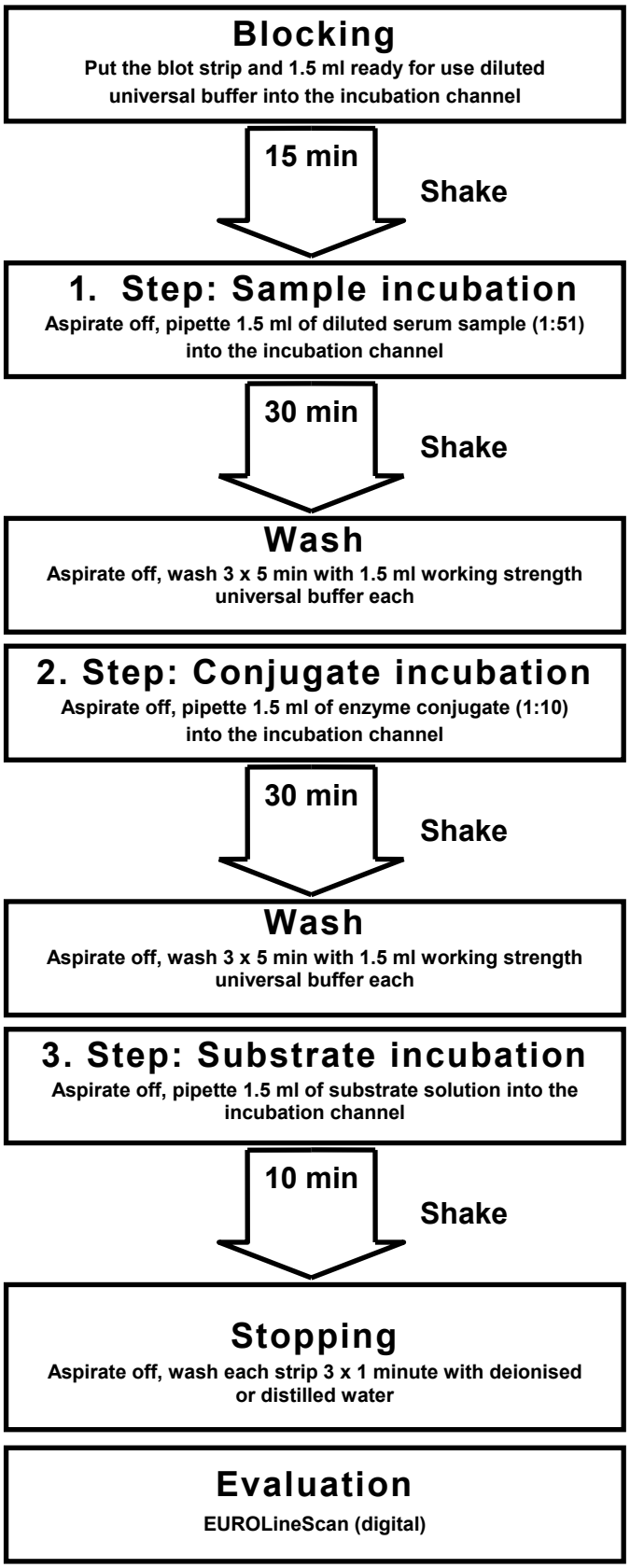
Blocking:	According to the number of serum samples to be tested fill each channel of the incubation tray with 1.5 ml ready for use diluted universal buffer and a blot strip. Remove the required amount of blot strips from the packing using a pair of tweezers. The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples and incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml ready for use diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgG) into each channel and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

For automated incubation with the EUROBlotMaster select the program **Euro02 Inf WB 30**.

For automated incubation with the EUROBlotOne select the program **Euro 01/02**.

Anti-Echinococcus EUROLINE-WB (IgG)

Incubation protocol



Evaluation and Interpretation of the results of the Anti-Echinococcus EUROLINE-WB (IgG)

Handling: For evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned with a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **Test** in EUROLineScan is **Echino_EL-WB_IgG**.

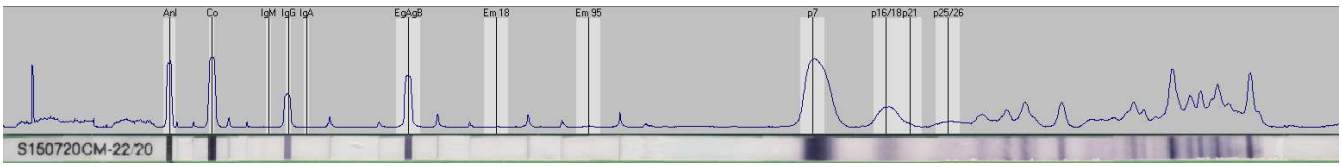
If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must match the lot number on the blot strips.** Clearly recognisable bands on the blot strips which concur with the labelled bands on the evaluation matrix are noted in the evaluation protocol.

Antigens: The antigen source for the **EUROIMMUN Anti-Echinococcus EUROLINE-WB** is provided by a particularly suitable Echinococcus antigen preparation. This antigen has been solubilised using sodium dodecyl sulphate followed by a separation of the solubilised protein using discontinuous polyacrylamide gel electrophoresis according to molecular mass and transfer of the separated proteins to nitrocellulose. Additional membrane strips containing the antigens Em 95, Em 18 and EgAgB are applied to each nitrocellulose membrane.

Control blot strips were taken from each nitrocellulose membrane and incubated with a reference serum. One of these stained strips is included in the kit.

Specificity of the antigens on the test strips:

Band	Antigen	Specificity
24-26 kDa	p25/26	Unspecific
21 kDa	p21	Specific for Echinococcus and other parasites
16-18 kDa	p16/18	Specific for Echinococcus
7 kDa	p7	Specific for Echinococcus
Em 95	Em 95	Specific for Echinococcus multilocularis
Em 18	Em 18	Specific for Echinococcus multilocularis
EgAgB	EgAgB	Specific for Echinococcus



In the lower part of the test strip there is a conjugate control membrane chip (IgA, IgG and IgM). Below the conjugate control, there is a membrane chip with a control band (Control).

Attention: A correctly performed determination of antibodies of class IgG against the antigens described above is indicated by a positive reaction of the control band and a positive reaction of the IgG band.

If one of these bands only shows a very weak reaction or none at all, the result is not valid.

IgG class antibodies against Echinococcus

In order to evaluate the signals, the band position and the intensity of the staining must be taken into account, as negative sera can also in some cases produce weak signals at individual band positions.

The defined antigens on the Anti-Echinococcus EUROLINE-WB can be divided into 5 categories:

Category	Antigens
1	antigen: p25/26
2	genus-specific Echinococcus antigen: EgAgB
3	Echinococcus antigen: p21
4	Echinococcus antigens: p7 and p16/18
5	Echinococcus multilocularis antigens: Em18 and Em95

Result interpretation: The results obtained with the Anti-Echinococcus EUROLINE-WB can be classified into negative, borderline and positive.

Result	Characteristics
Negative	No bands, or one positive antigen band of category 1, or one borderline antigen band of category 2.
Borderline	Positive antigen band of category 2, or at least one borderline antigen band of category 3, 4 or 5. It is recommended that a fresh sample be taken and the test be repeated after a few weeks.
Positive	At least one positive antigen band of category 3, 4 or 5. If one antigen band of category 3 is positive, either alone or in combination with an antigen band of category 1, cross reactivity may have occurred due to an Ascaris or Anisakis infection.

The Anti-Echinococcus EUROLINE-WB allows a serological differentiation between an infection with Echinococcus granulosus and Echinococcus multilocularis in many cases. The differentiation is automatically made during evaluation with EUROLineScan.

For differentiation the following applies:

Result	Characteristics
Echinococcus granulosus	Positive antigen band of category 2 and additionally at least one positive antigen band of category 3 or 4.
Echinococcus multilocularis	At least one positive antigen band of category 5. Further positive antigen bands from the other categories may also occur.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The EUROLINE-WB is a qualitative method. No measurement range is provided.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE-WB displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera showed no effect on the analytical results.

Cross reactions: The cross reactivity of the EUROLINE-WB was investigated using patient samples with the following parasite infections:

Species	Number of samples	Number of positive results
Ascaris lumbricoides	10	3
Anisakis simplex	16	1
Filarioidea	5	0
Strongyloides stercoralis	10	0
Schistosoma ssp.	9	0
Multi-Helminth infection	2	0
Plasmodium ssp.	7	0
Toxocara canis	10	0
Taenia solium	7	0
Trichinella spiralis	13	0
Fasciola hepatica	17	0
Entamoeba histolytica	11	0
Leishmania ssp.	5	0

Specificity and sensitivity: A panel of 107 defined patient samples with a positive Echinococcus result and a control panel with serum samples from 50 healthy blood donors and 50 tumour patients were analysed using the Anti-Echinococcus EUROLINE-WB (IgG). The samples had been provided by the Institute of Parasitology of the University of Bern, Switzerland.

n = 207		Characterisation: Institute of Parasitology, University of Bern	
		positive	negative
Anti-Echinococcus EUROLINE-WB (IgG)	positive	99	0
	negative	8	100

The Anti-Echinococcus EUROLINE-WB (IgG) has a specificity of 100% and a sensitivity of 93%.

Of the 99 Echinococcus samples that were positive in the Anti-Echinococcus EUROLINE-WB (IgG) 80 samples could be differentiated further:

Echinococcus IgG positive: n = 99	Number of samples
Echinococcus granulosus	47
Echinococcus multilocularis	33
No differentiation possible	19

The rate of differentiation between Echinococcus granulosus and Echinococcus multilocularis is 81%.

Clinical significance

Echinococcosis is an infectious disease caused by parasites of the genus *Echinococcus*. In Europe, the dog tapeworm (*E. granulosus*), causing cystic echinococcosis (CE), and the fox tapeworm (*E. multilocularis*), causing alveolar echinococcosis (AE), are most important from the medical point of view.
The development of all *Echinococcus* species includes an obligatory host change: the definitive hosts are carnivores, the intermediate hosts mostly herbivores. The mature worms of the fox tapeworm, *E. multilocularis*, live in the intestine of their definitive hosts (in Europe, mainly red foxes, seldom dogs and cats) who release the mature cestode eggs with their droppings. These eggs are very robust and may be infectious for several months if the conditions are good. The intermediate hosts (small mammals like field mice or European water voles) take the eggs in with their food. The larvae of the worms then develop in their inner organs (mostly in the liver).
Humans can be infected with *E. granulosus* by smear infection, through dealing with contaminated soil or consuming contaminated foods. Also here, the eggs are dispersed with the definitive hosts' droppings (mostly dogs, at times cats) and stay infectious for months. The larvae develop in liquid-filled blisters (hyatides) which are found in liver, lung, other organs and also in the skeletal system of the intermediate host (e.g. hoofed animals such as cows or sheep).
Humans are incidental hosts for fox and dog tapeworms. Human infection usually takes place by intake of cestodes after contact with infected hosts (e.g. smear infections or via the animal's fur).
The clinical image of both echinococcoses (CE and AE) differs in the different developmental behaviours of both parasites in the human body. The clinical appearance of AE corresponds to that of a malignoma. After haematogenous transport of the cestodes into the liver, infection of the liver takes place (usually unnoticed for a long time) and an alveolar tumour develops.
In infections with *E. granulosus*, larvae are released in the human intestine. They are transported haematogenously via the portal vein first into the liver and then into other organs, e.g. the lungs. The clinical course may vary significantly and is characterised by the slowly growing cysts and their different localisation.
In humans, both diseases remain asymptomatic for many years, until after 10 to 15 years they show symptoms like cholestatic icterus, epigastric pains, fatigue, weight loss and hepatomegaly. By invasion and destruction of the healthy liver tissue, an untreated echinococcosis may lead to the patient's death. Differential diagnosis from cysts, malignant and benign tumours, abscesses and the distinction between AE and CE are important for a diagnosis.

Imaging techniques, such as sonography, CT and MRT are used for diagnosis. The use of serological test systems for the detection of parasite-specific antibodies in serum or plasma helps to confirm results from imaging techniques. If whole antigens of *Echinococcus* are used, an echinococcosis can be detected with good sensitivity by ELISA or IFT tests.
The use of species-specific antigens in Westernblot and ELISA tests enables in many cases the serological differentiation of *E. granulosus* and *E. multilocularis.*

A negative result in this serological investigation does not exclude an infection. In liver CE, serum antibodies can be detected in 80 to 94% of cases. In lung echinococcosis, the prevalence amounts to only 65 to 70%.
In practice, molecular detection methods (PCR for the detection of *Echinococcus* DNA and RNA) have not proven to deliver.

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