CE EN v21 – DL 17/02/2023

EC - Declaration of Conformity

We, LDBIO Diagnostics, 24 Av Joannes Masset, 69009 Lyon, France, hereby confirm that we have installed a quality management system according to the harmonized standard NF EN ISO 13485:2016. Our quality management system has been certified for compliance with said standard by the LNE/GMED (Certification no. 9742 rev. 11, valid until 20 July 2024).

Compliance with additional requirements of Annex IV (Directive 98/79/EC) for products classified as either Annex II list B has been certified by the notified bodies LNE/GMED 0459 (Certification 9760 rev. 10, valid until 26 May 2025 and additional document 38916).

We further confirm that the IVD products listed in Attachment 1 are designed, manufactured and controlled by us in accordance with the European Directive 98/79/EC and that they meet the essential requirements according to Annex I of said Directive. Applicable conformity assessment procedures according to Annex III or Annex IV.3, whatever applicable, of said directive have been completed for these products.

The products marketed by LDBIO Diagnostics benefit from the transition period between Directive 98/79/EC and Regulation 2017/746. The conformity assessment is based on Annex IX (Regulation 2017/746) and the company is registered under the number FR-MF-000021697 on the EUDAMED database.

Lyon, le 17/02/2023

LDBIO DIAGNOSTICS
Dr Denis LIMONNE
Président, responsable qualité

Denis LIMONNE
2023.02.17
12:55:20 +01'00'

Digitally signed by Ceaicovschi Tudor
Date: 2024.03.12 15:39:24 EET
Reason: MoldSign Signature
Location: Moldova

Attachment 1: Products List

Dénominations	**Références**	**Classification**
TOXOPLASMA WB IgG-IgM	TOP-WB12GM, TOP-WB24GM, TOP-WB96GM	Annexe II, Liste B (Directive 98/79/CE)
TOXOCARA WB IgG	TXA-WB12G, TXA-WB24G, TXA-WB96G	
LEISHMANIA WB IgG	LES-WB12G, LES-WB24G, LES-WB96G	
ECHINOCOCCUS WB IgG	ECH-WB12G, ECH-WB24G, ECH-WB96G	
CYSTICERCOSIS WB IgG	CYS-WB12G, CYS-WB24G, CYS-WB96G	
SCHISTO II WB IgG	SCH II-WB12G, SCH II-WB24G, SCH II WB96G	
TRICHINELLA ES WB IgG	TRI ES-WB12G, TRI ES-WB24G, TRI ES-WB 96G	
FASCIOLA ES WB IgG	FAS ES-WB12G, FAS ES-WB24G, FAS ES-WB96G	
LDBIO TOXO II IgG	TOXO II 12G, TOXO II 24G, TOXO II WB96G	Annexe II, Liste B (Directive 98/79/CE)
ASPERGILLUS WB IgG	ASP-WB12G, ASP-WB24G, ASP- WB96G	
CHAGAS WB IgG	CHA-WB12G, CHA-WB24G, CHA- WB96G	
LDBIO TOXO II IgM	T2M-12M, T2M-24M, T2M-96M	Annexe II, Liste B (Directive 98/79/CE)

A.FUMIGATUS WB IgE	AFU-WB12E, AFU-WB24E, AFU-WB96E	
PEO WB IgG (Bird breeder's disease)	PEO-WB12G, PEO-WB24G, PEO-WB96G	
SCHISTOSOMA ICT IgG-IgM	BILZ Ab ICT20, BILZ Ab ICT100	
TOXOPLASMA ICT IgG-IgM	TOXO Ab ICT20, TOXO Ab ICT100	Annexe II, Liste B (Directive 98/79/CE)
ASPERGILLUS ICT IgG-IgM	ASPG Ab ICT20, ASPG Ab ICT100	
PEO ICT IgG-IgM (Bird breeder's disease)	MPEO Ab ICT20, MPEO Ab ICT100	
CONJUGUE ANTI-IgG	WB-IG30, WB-IG60	Annexe II, Liste B (Directive 98/79/CE)
CONJUGUE ANTI-IgM	WB-IM30, WB-IM60	Annexe II, Liste B (Directive 98/79/CE)
CONJUGUE ANTI-IgA	WB-IA30, WB-IA60	Annexe II, Liste B (Directive 98/79/CE)
CONJUGUE ANTI-IgE	WB-IE30, WB-IE60	
SUBSTRAT NBT-BCIP	WB-SA30, WB-SA125	Annexe II, Liste B (Directive 98/79/CE)
DILUANT ECHANTILLON	WB-DE30, WB-DE125	Annexe II, Liste B (Directive 98/79/CE)
LAVAGE CONCENTRE	WB-LC60, WB-LC250	Annexe II, Liste B (Directive 98/79/CE)

TOXOCARA CE

Western Blot IgG

Test immunoblot de diagnostic *in vitro*
Technique manuelle / semi-automatisable

In vitro diagnostic Immunoblot assay
Semi-automated / manual technique

#TXA-WB24G: 24 tests
#TXA-WB12G: 12 tests
#TXA-WB96G: 96 tests

NOTICE D'UTILISATION

INSTRUCTIONS FOR USE - page 9

Retrouvez plus d'informations et les notices traduites dans votre langue
sur notre site internet **www.ldbiodiagnostics.com**

Find more information and IFU in your language on our website **www.ldbiodiagnostics.com**

Toxocara Western Blot IgG

INTENDED USE

TOXOCARA Western Blot (WB) IgG is a single use qualitative test of serological IgG diagnosis by Immunoblot Assay of toxocariasis intended for confirmatory testing of a positive or equivocal result obtained through classic screening tests. It can be performed on sera, cerebrospinal fluid (CSF) or aqueous humour.

PRINCIPLE OF THE TEST

Western Blot technique

The excretory/secretory (ES) antigens of *Toxocara canis*, once separated by electrophoresis, are bound by electroblotting to the surface of a nitrocellulose membrane (called the transfer) cut into 24 strips numbered from 1 to 24.

Conduct of the test

Each specimen to be tested is separately incubated with a strip. The specific antibodies potentially present in the sample selectively bind themselves onto the antigens. The alkaline phosphatase-anti human IgG conjugate then binds itself to the bound antibodies. Finally, the immunocomplexes react with the substrate. The antigens recognized by the specific antibodies of type IgG present in the samples are revealed as purple transversal bands.

REAGENTS SUPPLIED

Default: package of 24 tests (#TXA-WB24G)

Italic: package of *12 tests* (#*TXA-WB12G*) – **Bold**: package of **96 tests** (#**TXA-WB96G**)

ID	Qty	Description	Composition
R1	1	Folder(s) of 24 (*12*, **4x24**) STRIPS: precut + coloured Standards. (Each folder and each transfer are identified by a unique serial number).	Sensitized nitrocellulose. Coloured Molecular Weight (kDa): Blue: 250, Blue: 150, Blue: 100, Pink: 75, Blue: 50, Green: 37, Pink: 25, Blue: 20, Blue: 15.
R2	1	Vial of 30 (*30,* **125**) mL of SAMPLE BUFFER (Ready to use - pink solution).	Buffer + surfactant.
R3	1	Vial(s) of 30 (*30,* **2x60**) mL of ANTI IgG CONJUGATE (Ready to use - blue solution).	Buffer + anti-human IgG polyclonal goat sera conjugated with Alkaline Phosphatase + NaN3 (<0.1%) + stabilisers.
R5	1	Vial of 30 (*30,* **125**) mL of SUBSTRATE (Ready to use - opaque brown vial).	Buffer + NBT + BCIP + stabilisers.
R6	1	Vial of 60 (*60,* **250**) mL of WASH CONCENTRATE 10X BUFFER (To be diluted 10 times in distilled water - colourless solution).	Buffer + surfactant.
R10	1	Tube of 100 (*100,* **2x100**) µL of POSITIVE CONTROL SERUM (Ready to use - red cap).	Buffer + pool of human sera positive in *Toxocara* serology + NaN3 (<0.1%) + stabilisers.

R1: The letter before each strip number is specific to the parameter.

R2, R3, R5 and R6 are common to all kits and have a unique lot number depending only on the date of their production**. It is recommended to perform multiparameter testing (see the LDBIO immunoblot range) to limit the number of vials opened and to ensure better quality control.**
R10 is calibrated in immunoblot according to a reference lot and is only dedicated to this technique.

R3, R10 (NaN3): EUH 032 - Contact with acids liberates very toxic gas.

EUH 210 Safety data sheet available on request as well as on our website **www.ldbiodiagnostics.com**

ADDITIONAL MATERIAL REQUIRED BUT NOT PROVIDED

- One multi-channel polypropylene incubation trays for mini-blots (#WBPP-08 or equivalent).
- One rocking platform for immunoblots, one vacuum system for liquids (the #WBPP-08 tubs that we supply can be emptied by simply turning them over).
- Tubes and material for drawing the samples, graduated cylinders, adapted containers. Automatic pipettes, micropipettes and disposable tips (volumes of 10µL, 25 µL, 1.2 mL and 2 mL).
- Distilled or deionised water. Absorbent paper (e.g., Whatman filter paper), transparent adhesive tape.
- Gloves, tweezers to handle the strips, cutter or scalpel, flat transparent ruler.

Note: Our reagents can be used in an automated immunoblot processor. Care should be taken with possible chemical contaminations of our reagents if the processor is shared with reagents from another manufacturer (known example: contamination by the TWEEN 20), and bacterial contaminations. Reserve vials for the processor. After processing, do not place the remaining used reagents back into the original vials.

STORAGE AND STABILITY

Store between 2 and 8°C. The reagents from the kit are stable until the expiry date indicated on the outer box and the vial labels. Do not use contaminated or cloudy reagent. Wash buffer diluted to 1/10 is stable for 2 months at +2 to +8 °C and one week at room temperature.

PRECAUTIONS FOR USE

Safety

- For *in vitro* use only. For professional use only. Only for technically trained personnel. Handle according to Good Laboratory Practices and consider any reagent and any sample as potentially toxic and/or infectious.
- Wear a lab coat, gloves and glasses; do not drink, eat or smoke in the laboratory. Do not mouth the pipettes.
- The positive control is a serum of human origin that has been inactivated for HIV 1 and 2, hepatitis B and hepatitis C viruses. However, it must be handled like a potentially infectious product.
- The substrate contains a mixture of NBT and BCIP, toxic on contact (skin and mucous membranes) and inhalation.
- The reagents contain sodium azide which can form explosive metallic salts with lead and copper. Rinse any spill with water.
- Dispose of waste (samples, tips, tubes, wash liquid, used reagent...) according to good practices used in the industry and current regulations in the country.
- Any serious incident must be the subject of a declaration to the manufacturer and the competent authority.

Precautions

- Read and interpret the results under direct white light.
- It is preferable to use all reagents from the same batch. If different batches are used, ensure traceability.
- Use the strips in numerical order. Do not mix strips from different serial numbers; use the transfers in succession. Establish a specific distribution plan before starting the test.
- Do not touch the strips with your fingers; use tweezers.
- The reagents must be mixed well before use, particularly the concentrated wash buffer.
- Close the vials after use; do not use if a substance was accidentally introduced in the reagents. Do not use reagent from a vial that presents signs of leakage. Do not use cloudy or precipitated solution.
- Use only disposable pipette tips. Avoid any inter-channel contamination. Watch for the formation of foam or bubbles in the pipette tips (bacterial contamination of reagent vials).
- Clean incubation trays only with distilled water (never use detergent or bleach).
- The omission of a sample or the distribution of an inadequate volume may render the test result negative or positive, regardless of its actual status.

SPECIMEN COLLECTION

Aseptically collect the samples in dry tubes. A minimum of 10 µL of serum, aqueous humour or CSF is required. In cases of aqueous humour or CSF, using 25 µL will increase the sensitivity of the test.

Keep the samples at 2-8 °C until they are processed. If they need to be stored more than a week, freeze the samples at -20 ± 5 °C. Do not use a contaminated sample. Avoid freezing and thawing the samples repeatedly.

Even though no particular cross-reaction has been observed with haemolysed, icteric or lipidic sera, it is recommended to interpret the results from the use of such samples with care.

PREPARATION OF REAGENTS

Wash buffer: For 4 tests, in a clean bottle, dilute 10 mL of Wash Concentrate 10X (R6) in 90 mL of distilled or deionised water. Be careful to mix the diluted buffer well.

TEST PROCEDURE

Nota Bene: It is recommended to perform multiparameter testing (see the LDBIO immunoblot range) to limit the number of vials opened and to ensure better quality control.

1. Prepare a distribution plan for the samples and C+ positive control (R10).

Only by using this control can the test be technically validated and identification made, for a given serial number, of the specific bands developed. A C+ strip cannot be used to interpret the results of strips from a blot of a different serial number.

2. Cut the required number of strips (R1) using a scalpel and a clean and dry flat transparent ruler, keeping the blue positioning line on the strips: hold the strips firmly in place with the ruler and cut them on the side of the strain (the numbers are visible through the ruler).
3. Distribute 1.2 mL of sample buffer (R2) in each channel according to the established plan.
4. Deposit, in their numerical order, the numbered strips in the channels: Let the strips rehydrate themselves at the surface of the buffer for approximately 2 minutes, with the number visible at the top, THEN gently shake the tray to totally immerse them in the buffer.
5. Distribute the samples and positive control(s): according to the distribution plan, at a rate of 10 µL per channel (preferably 25 µL for aqueous humour or CSF). Gently shake the tray after each dispense. Place the tray on a rocking platform.
   Incubate for 90 minutes ± 5 minutes at 20-26 °C.
6. Wash step: Empty the contents of the channels with a Pasteur pipette or by turning the incubation tray over. Dispense 2 to 3 mL of diluted Wash Buffer in each channel. Incubate on the rocking platform for 3 minutes. Repeat 2 times, then empty the contents of the channels. Ensure that the strips don't turn during these steps.
7. Dispense 1.2 mL of anti IgG conjugate (R3) into each channel. Place the tray on the rocking platform. **Incubate for 60 minutes** ± 5 minutes at 20-26 °C.
8. Wash step: repeat step 6.
9. Distribute 1.2 mL of NBT/BCIP substrate (R5) into each of the channels. Place on the rocking platform and protect from direct light. **Incubate for 60 minutes** ± 5 minutes at 20-26 °C.

Regardless of the parameter, monitor the development of the colour. The development can be stopped if the background colour of the strip darkens to the point where reading is difficult (the quality of the wash steps has a fundamental influence on the background coloration). Note that the strips will lighten as they dry.

10. Stop the reaction by aspirating substrate with a Pasteur pipette or by turning the incubation tub over and dispensing 2 mL of distilled or deionised water in the channels. Repeat this last washing step one more time.
11. Drying the strips: With the channels still water-filled, take the strips by the numbered end using the tweezers and deposit them, with the number visible, onto a Whatman absorbent paper. Let air dry. The colour of the strips will naturally lighten while drying. Interpretation must only be performed after drying is complete.
12. Storage: Transfer the strips onto a sheet of paper, which will be used to archive them. Align the blue positioning lines. Keeping them in place with the flat ruler, stick the top of the strips with transparent adhesive tape.

For a good interpretation, the strips must be ordered by transfer and in their numerical order, spaced at a maximum of a few millimetres apart. It is unreliable to compare strips that are spaced far apart (e.g., no.2 with no.15). **It is dangerous** (false results) to compare strips from different kits (strips with different serial numbers).

QUALITY CONTROL AND INTERPRETATION

The serum control (R10) provided with the kit must be systematically included in any immunoblot series. It shows the typical profile and allows for technical validation of the good conduct of the test (the bands must appear very clearly on the strip) and to calibrate precisely the position and aspect of the specific bands to allow interpretation of the results of the strips from the same transfer (same serial number).

Nota Bene: The positive control (R10) profile may vary according to the lot number of the reagents used. Corresponding images are available on our website **www.ldbiodiagnostics.com** as an example.

Description of the bands

A positive sample can present numerous bands between 15 and 200 kilodaltons (kDa). Search for bands of 24-35 kDa Low Molecular Weight (LMW) for each of the tested samples using the identification tools described above. These bands, grouped and well isolated, are characteristic and generally easily found.

Two groups of bands of High Molecular Weight (HMW) may be observed in the 70-90 kDa and 100-200 kDa range. These bands are not specific to toxocariasis: possible cross reaction with another helminthiasis.

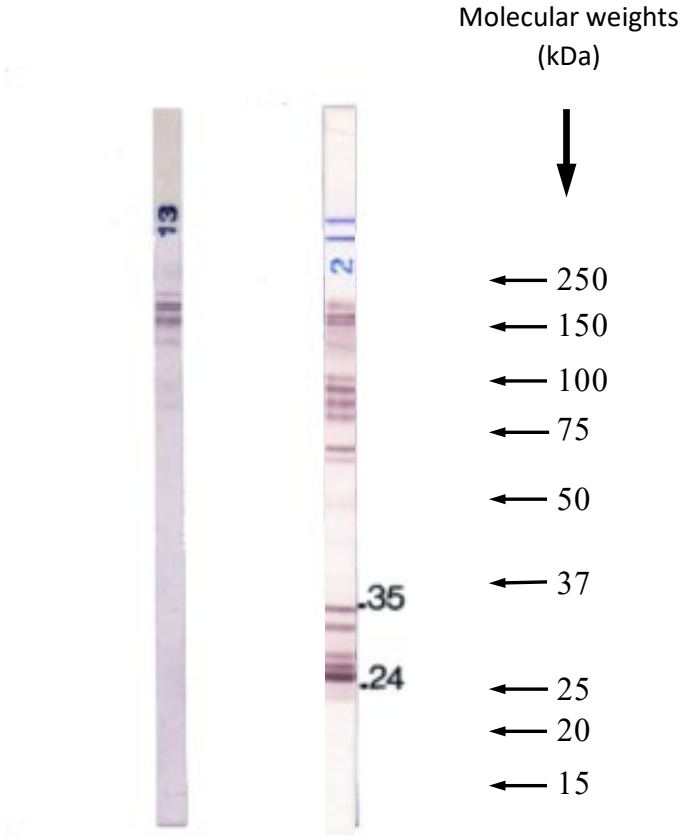

***Fig. 1**: Examples of positive and negative results*

The profiles are given as examples. **The strips are marked with the letter "B" specific to the parameter from batch "01009".**

Interpretation

The **simultaneous** presence of 2 bands between 24 and 35 kDa is indicative of the presence of anti-*Toxocara* specific antibodies.

To validate the results, always compare the profile of the immunoblot of each sample with that of the R10 positive control. The aspect of the bands is important when interpreting the test.

LIMITATION OF USE

- The diagnosis of an infectious disease cannot be established on the basis of a single test result.
- Serological results must be interpreted according to available information (e.g., epidemiology, clinical, imaging, biology…) in order to establish a diagnosis. They should not be used to make a diagnosis based on their positivity alone.

PERFORMANCES (see literature references p. 16)

The **Toxocara WB IgG** test was the subject of a comparative study with the reference immunoblot from the Toulouse CHU (University Hospital Centre). The interpretation criteria and the performance of the two tests are closely comparable.

Sensitivity (Se)

The data from literature describe excellent sensitivity of the **Toxocara WB IgG** test, often significantly higher than that of the ES ELISA screening tests, confirming immunoblot's place as a diagnostic and confirmatory technique.

Note: The numbered value of the sensitivity cannot be calculated due to the absence of a reference diagnostic method.

Specificity (Sp)

The specificity of the 24-35 bands from the ES antigen is 100%. The bands outside of this range are not considered specific.

Reproducibility

Inter-series and inter-lot reproducibility were tested. In both cases, the serum to serum correlation with respect to specific bands is excellent.

Interferences

Even though no particular cross-reaction has been observed with haemolysed, icteric or lipidic sera, it is recommended to interpret the results from the use of such samples with care.

TROUBLESHOOTING

*"**The bands are pale with little contrast**":* Certain sera with low concentrations of antibodies may give such results.

*"**Shaded areas can be seen, more or less coloured, slightly diffuse**":* The strip was not totally submerged in one of the reagents and did not incubate correctly along its entire length. Stains can also be present where the sample was deposited if the tray was not shaken after dispensing.

*"**The background noise is significant, making reading very difficult**":* The washes were insufficient or the last incubation was too long. Ensure good test performance techniques, respect wash times and ensure water quality. Reduce the time of the last incubation. Exceptionally, certain sera may react in a non-specific manner. Then, the result of the immunoblot cannot be used.
This non-specific background noise may involve only part of the strip, making the results uninterpretable for that part only.

*"**A precipitate appears in the solution during the last step of development**":* the substrate may in fact partially precipitate (black flakes) in the buffer at the end of development. This phenomenon does not alter the quality of the development which must be continued normally. The last wash with distilled water eliminates the possible solid particles present.

BIBLIOGRAPHIE/BIBLIOGRAPHY

C. N. L. Macpherson, « The epidemiology and public health importance of toxocariasis: A zoonosis of global importance », *Int. J. Parasitol.*, vol. 43, nº 12-13, p. 999-1008, nov. 2013.

J. F. Magnaval, R. Fabre, P. Maurières, J. P. Charlet, et B. de Larrard, « Application of the western blotting procedure for the immunodiagnosis of human toxocariasis », *Parasitol. Res.*, vol. 77, nº 8, p. 697-702, 1991.

J. Fillaux et J.-F. Magnaval, « Laboratory diagnosis of human toxocariasis », *Vet. Parasitol.*, vol. 193, nº 4, p. 327-336, avr. 2013.

B. Gavignet, R. Piarroux, F. Aubin, L. Millon, et P. Humbert, « Cutaneous manifestations of human toxocariasis », *J. Am. Acad. Dermatol.*, vol. 59, nº 6, p. 1031-1042, déc. 2008.

A. Nicoletti, V. Sofia, A. Mantella, G. Vitale, D. Contrafatto, V. Sorbello, R. Biondi, P.-M. Preux, H. H. Garcia, M. Zappia, et A. Bartoloni, « Epilepsy and toxocariasis: a case–control study in Italy », *Epilepsia*, vol. 49, nº 4, p. 594-599, avr. 2008.

M. Zibaei, F. Firoozeh, P. Bahrami, et S. M. Sadjjadi, « Investigation of Anti-Toxocara Antibodies in Epileptic Patients and Comparison of Two Methods: ELISA and Western Blotting », *Epilepsy Res. Treat.*, vol. 2013, p. 1-5, 2013.

E. Artinyan, H. K. Uysal, O. Akgul, S. Altiparmak, et Y. A. Oner, « Research on Toxocara canis antibodies obtained from patients with eosinophilia », *Indian J. Med. Microbiol.*, vol. 32, nº 4, p. 383-386, déc. 2014.

C. Incorvaia, Qualizza, Grande, et L. Allegra, « Seroprevalence of IgG anti-Toxocara species antibodies in a population of patients with suspected allergy », *Int. J. Gen. Med.*, p. 783, nov. 2011.

J. Logar, B. Šoba, A. Kraut, et B. Stirn-Kranjc, « Seroprevalence of Toxocara antibodies among patients suspected of ocular toxocariasis in Slovenia », *Korean J. Parasitol.*, vol. 42, nº 3, p. 137, 2004.

UPDATE NOTIFICATION – Please read carefuly

RELEASE DATE	VERSION	MODIFICATION SUMMARY
28/07/2021	Vs 14	Removal of security warning R5 - Contact email address – NaN3 EUH 032.
30/11/2022	Vs15	New address
21/12/2022	Vs16	R6 without NaN3. Strip identified by letter B. Possible use of reagents from different batches.

LDBIO Diagnostics

NF EN ISO 13485

24 Av. Joannes MASSET – 69009 LYON – FRANCE
Tel : +33(0)4 7883 3487 – Fax : +33(0)4 7883 3430
www.ldbiodiagnostics.com – info@ldbiodiag.com