# ACANTHOCHEILONEMA VITEAE

**Enzyme immunoassay for the diagnosis of human filariosis**

96 assays on individual wells for in vitro use

Instructions for use for article N° **9400**
EC reg. N°: H-CH/CA01/IVD/01755

CE

**Intended use:**

Screening of suspects clinical cases and routine serology (IgG) of human filarial infections including lymphatique and african filariasis (Bancroftian and Malayan filariosis, Loaosis, Onchocercosis and Mansonellosis).

**Principle and presentation:**

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Acanthocheilonema viteae*** somatic antigens. The presence of parasite specific serum antibodies is detected with a Proteine A - alkaline phosphatase conjugate. Sensitized wells are provided as breakable strips for the economical assay of small series of samples.

**Material contained in the kit (96 assays):**

| | | | | |
|---|---|---|---|---|
| WELL | 9400-01 | Breakable ELISA strips sensitized with ***Acanthocheilonema viteae*** somatic antigens | 96 | wells |
| DILB | 9400-02 | Dilution buffer (10 x) concentrate | 50 | ml |
| WASH | 9400-03 | Washing solution (10 x) concentrate | 50 | ml |
| ENZB | 9400-04 | Enzyme buffer | 50 | ml |
| STOP | 9400-05 | Stopping solution ($K_3PO_4$) | 25 | ml |
| CONTROL - | 9400-06 | Negative control serum | 200 | µl |
| CONTROL -/+ | 9400-07 | Weak positive serum (cut off) | 200 | µl |
| CONTROL + | 9400-08 | Positive control serum | 200 | µl |
| CONJ | 9400-09 | Protein A - alkaline phosphatase conjugate | 300 | µl |
| SUBS | 9400-10 | Phosphatase substrate | 20 | tablets |
| | | Multipipette reservoir, 25 ml | 1 | piece |
| | | Frame for ELISA 8-well holder | 1 | piece |

**Shelf life and storage:**

Store kit at 2° to 8° C (transport at ambient temperature). The expiry date and the lot number of the kit are printed on the side of the box.

**Equipment needed but not provided with the kit:**

Pipettes (ml and µl). Flasks. Tubes for the dilution of sera. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37° C. ELISA reader set at 405 nm.

**Preparation of reagents before use:**

**ELISA wells**: open side of aluminium bag 9400-01 and remove number of wells needed. Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with dessicant pad.

**Dilution buffer**: dilute dilution buffer (10 x) concentrate 9400-02, 1/10 in distilled water.

**Washing solution**: dilute washing solution (10 x) concentrate 9400-03, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase.

Negative, weak positive (cut off) and positive **control sera**: dilute 10 µl control sera 9400-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20).

**Sera to be tested**: dilute 10 µl serum in 2.0 ml dilution buffer solution (final dilution 1/201).

Protein A - alkaline phosphatase **conjugate**: dilute conjugate 9400-09 in dilution buffer solution (final dilution 1/51).

**Substrate solution**: prewarm enzyme buffer 9400-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate 9400-10 in undiluted buffer 9400-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s).

**Stopping solution**: use reagent 9400-05 undiluted.

**Warnings and precautions:** Solutions 9400-02, 9400-03, 9400-04 and 9400-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide ($N_aN_3$). Solution 9400-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution 9400-05 (0.5 M $K_3PO_4$) is irritant.

The negative, weak positive, and positive control sera (9400-06 to -08) are from rabbits.

**Volumes to be prepared:**

| | | | Total number of wells to be used | | | |
|---|---|---|---|---|---|---|
| | | | **3-4** | **5-6** | **7-8** | **9-10** |
| **Dilution buffer (10 x)** | 9400-02 + $H_2O$ | ml + ml | 1 + 9 | 2 + 18 | 3 + 27 | 4 + 36 |
| **Washing solution (10 x)** | 9400-03 + $H_2O$ | ml + ml | 1 + 9 | 2 + 18 | 3 + 27 | 4 + 36 |
| **Conjugate** | 9400-09 + dilution buffer | µl + µl | 10 + 500 | 15 + 750 | 20 + 1000 | 25 + 1250 |
| **Control sera** | 9400-06 to -08 + dilution buffer | µl + µl | 10 + 190 | 10 + 190 | 10 + 190 | 10 + 190 |
| **Sera to be tested** | Serum + dilution buffer | µl + µl | 10 + 2000 | 10 + 2000 | 10 + 2000 | 10 + 2000 |
| **Substrate solution** | 9400-10 + 9400-04 | tabl. + ml | 1 + 2.5 | 1 + 2.5 | 1 + 2.5 | 1 + 2.5 |

**Procedure:**

**Step 1: Blocking:**

| |
|---|
| Fill completely wells with dilution buffer solution.<br><br>Incubate for 5 to 15 minutes at ambient temperature (blocking).<br><br>Remove dilution buffer by aspiration or by shaking the strips over the sink. |

**Step 2: Incubation with serum samples:**

| |
|---|
| Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).<br><br>Fill the subsequent three wells with 100 µl diluted negative, weak positive (cut off) and positive control sera respectively (100 µl each).<br><br>Fill remaining wells with the diluted sera to be tested (100 µl each).<br><br>Cover wells with adhesive tape and incubate for 30 minutes at 37° C.<br><br>Remove sera and wash 4 x with washing solution. |

**Step 3: Incubation with conjugate:**

| |
|---|
| Distribute 100 µl diluted proteine A - alkaline phosphatase conjugate in each well.<br><br>Cover wells with adhesive tape and incubate for 30 minutes at 37° C.<br><br>Remove conjugate and wash 4 x with washing solution. |

**Step 4: Incubation with substrate:**

| |
|---|
| Distribute 100 µl substrate solution per well.<br><br>Cover wells with adhesive tape and incubate for 30 minutes at 37° C.<br><br>Stop the reaction by the addition of 100 µl stopping solution to each well. |

**Step 5: Measurement of absorbances:**

| |
|---|
| Wipe bottom of wells, eliminate bubbles and measure absorbances at 405 nm. |

## Interpretation:

Subtract value of the no-serum blank from all measured values. The test is valid if the following criteria are met: absorbance (A) of positive control > 1.200, A of negative control < 12% of A of positive control, A of blank against air < 0.350.

The titer of the weak positive (cut off) serum 9400-07 has been set to discriminate optimally between sera of clinically documented cases of filarioses and healthy human sera.

The result is **negative** when the absorbance of the analyzed sample is lower than the absorbance of the weak positive serum 9400-07. In this case, the IgG antibody concentration against ***Acanthocheilonema viteae*** somatic antigens is clinically non-significant. If filariasis is strongly suspected, patients sould be examined for microfilariae. Negative serology may be observed in patients with microfilaremia.

The result is **positive** when the absorbance of the analyzed sample is higher than the absorbance of the weak positive control 9400-07. In this case, the IgG antibody concentration against ***Acanthocheilonema viteae*** somatic antigens is is considered as clinically significant. This result should be considered with regards to the endemic situation and clinical symptoms.

## Sensitivity and specificity of the assay:

The sensitivity and the specificity of the test for human filarioses are respectively of 95% and 98 %. This test does not differentiate between the different filarial infections. It is used as a first screening method. Cross-reactivity often occur with antibodies to other parasites such as *Ascaris*, *Trichinella*, *Ancylostoma*, *Fasciola hepatica* et *Echinococcus granulosus*. Positive results have to be interpreted in relation to the endemic and clinical background.
The kit has been evaluated by an independent laboratory : Out of 22 sera from positive patients for filariasis (patients with microfilaremia and/or with positive serology with other techniques and an epidemiological and clinical background of filarasis) 21 sera were positifs by this test. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.
Reproducibility were assessed by testing the 2 human serum samples on 10 differents assays.

| | **Repeatability** | | **Reproducibility** | |
|---|---|---|---|---|
| | **Sample 1** | **Sample 2** | **Sample 1** | **Sample 2** |
| **Average (absorbance)** | 0.525 | 1.535 | 0.764 | 1.905 |
| **Standard deviation (absorbance)** | 0.026 | 0.070 | 0.068 | 0.112 |
| **Variation coefficient (%)** | 5.0 | 4.5 | 8.9 | 5.9 |



## References:

**Gueglio, B., Bordier, C. et Marjolet, M**. (1995) Mise au point d'un test ELISA pour le diagnostic des filarioses humaines. Bulletin de la société Française de parasitologie. **13 :** 67-72.

**Laverbratt, C., Ljungström, I., Guzman, G., Thors, C., Eriksson, T. et Akuffo, H. O.** (1997) Evaluation of serological assays for diagnosis of onchocercosis. Scand. J. Infect. Dis. **29 :** 65 -70.

**BORDIER AFFINITY PRODUCTS SA**
Biokema building, Chatanerie 2, CH-1023 Crissier, Switzerland.
Phone: + 41 21 633 31 67, Fax : + 41 21 633 31 78, www.bordier.ch