VIR-ELISA TOXO-IgG AVIDITY

GB

1. INTENDED USE

The VIR-ELISA TOXO-IgG AVIDITY is a supplementary reagent kit for the determination of Toxoplasma gondii-specific IgG avidity in human serum and plasma. It has to be used in combination with the VIR-ELISA ANTI-TOXO-IgG [REF] EG 127.

[REF] EAv 127 S 48 [IVD]

2. PRINCIPLE OF THE ASSAY

The detection of antibodies is based on the principle of an enzyme-linked immunosorbent assay (ELISA).The purified, homogeneous antigen is fixed to each well of the microtiterstrips. Any specific antibodies present in the patient's sample (set up in duplicate) are bound during the first incubation.
After removing unbound material by washing, avidity reagent [UREA] is added to one well of the duplicate and wash solution (ready to use) to the other well of the duplicate. Using the special reagent for avidity the binding capacity of the immune complex becomes dissolved but only for antibodies with low affinity whereas antibodies with high affinity remain bound to the antigen.
After removing unbound material by a further wash cycle, the presence of specific antibodies which are still bound to the antigen are detected using Anti-human IgG during the third incubation.
Excess peroxidase conjugate is then removed and TMB substrate is added, resulting in the development of a blue colour. The enzyme reaction is terminated by the addition of a stop solution. The intensity of the yellow colour thus developed is proportional to the concentration of antibodies in the sample.

3. DIAGNOSTIC RELEVANCE

Toxoplasmosis is an infection caused by a single-celled parasite called Toxoplasma gondii which has been found worldwide. The infection is most commonly acquired from contact with cats and their feces or with raw or undercooked meat.
In most adults the initial infection with Toxoplasma gondii does not cause serious illness and confers lifelong immunity on immunocompetent persons. However, primary infection during pregnancy can cause blindness and mental retardation in congenitally infected children or can lead to abort or stillbirth. Toxoplasmosis can also result in complications in immunocompromised individuals.
The prevalence of Toxoplasma infection in women of child-bearing age is about 26 to 54%. Considerable importance is attached to detection of early primary infections in pregnant women.
With the parallel determination of pathogen specific IgG and IgM antibodies a clear differentiation between acute and past infections is not always possible in case of Toxoplasma. Diagnosis of an infection can be simplified by determining the avidity of the IgG antibodies. The IgG antibodies have a maturation cycle in which early-phase antibodies show low avidity and the antibodies from a completed infection cycle show high avidity. The measurement of Toxoplasma gondii-specific IgG avidity is therefore a valuable method in addition to serological routine diagnosis of Toxoplasmosis.

4. COMPONENTS OF THE TEST KIT

The test kit includes:

AVIDITY REAGENT [UREA]
One vial containing 5,0 ml of ready-to-use avidity reagent. The avidity reagent contains 0,095% sodium azide as preservative.

POSITIVE CONTROL / LOW AVIDITY [CONTROL|LA],
One vial containing 1,2 ml human plasma (low avide) with 0,095% sodium azide as preservative. Ready to use.

The safety data sheet (MSDS) is available upon request.

5. STORAGE AND STABILITY

Store all reagents at 2-8°C. Protect them from intense light and do not freeze. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date.

6. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- VIR-ELISA ANTI-TOXO-IgG [REF] EG 127
- Test tubes for sample dilution
- Timer
- Micropipettes, multipipettes 10-1000 µl
- One-litre graduated cylinder, distilled water
- ELISA washer or multichannel pipette
- Spectrophotometer for micro-plates (450-nm/ reference wavelength 630/620 nm)
- Paper towels, pipette tips
- Incubator for t 37°C with high relative humidity

7. WARNINGS OR PRECAUTIONS SAFETY PRECAUTIONS

The ELISA test is for [IVD] use only.

1. Only qualified and well-trained employees should carry out the assay procedure.
2. The instruction for use describes the applicable test method. In case of modification or applications others than the intended use, or the use of automatic processors, the user has to validate the procedure and take the responsibility for it.
3. Seal all bottles properly after use in order to avoid bacterial contamination. All samples and kit components should be considered potentially infectious. All control samples have been tested for Hepatitis Bs antigen, anti-HIV I and II, anti-HCV (CE/FDA) and found to be negative.
4. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact and if necessary consult a doctor.
5. Reagents containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents. Hazard warning: Harmful if swallowd. Seek medical advice.
6. For disposal the legal regulations have to be followed.

8. SPECIMEN COLLECTION AND STORAGE

1. Microbially contaminated specimen may cause interference.
2. Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
3. Suitable specimens are serum or plasma (heparinized, EDTA) samples obtained by standard laboratory techniques.
4. The samples should not be heat-inactivated since non-specific results may occur.
5. Patient samples should be stored at t 2-8°C.
   For long term storage t -20°C or lower is recommended.
   Avoid repeated freeze-thaw cycles.
6. **Note:** Diluted patient samples must be used on the same day.

9. GENERAL INFORMATION Please note: important!

Impact of Antibody Concentration on the Determination of Avidity
Due to the competition of specific antibodies with low and high affinity to the same binding site of the corresponding antigen (solid phase on microtiter plate), the concentration of antigen-specific antibodies will influence the level of the avidity index (AI). In order to keep the ratio between antigen and antibody constant, the Toxo IgG antibody concentration of the sample has to be between the upper value of the cut-off range and 100 IU. The avidity indices of samples with antibody concentrations within this range can be calculated right away without further dilution of the samples. But samples with an antibody concentration <= 20 IU might cause a false classification of the avidity index and should therefore be retested with a new serum sample.

For the correct determination of avidity, samples higher than 100 IU must be prediluted 1:10 prior to the test.
If the prediluted samples are found again above 100 I.U., the samples must be diluted further (e.g. 1:20, 1:40) and if they are lower than the cut-off value, the samples must be diluted less than 1:10 (e.g. 1:5).

10. REAGENT PREPARATION

For the determination of Toxo-specific IgG avidity the VIR-ELISA TOXO-IgG AVIDITY test [REF] EAv 127 has to be used in combination with VIR-ELISA ANTI-TOXO-IgG [REF] EG 127.

Bring all reagents to room temprature (21°C-25°C) prior to use!

Please note!
Crystals may form in the **Avidity Reagent** during cold storage at 2°C-8°C. They have to be dissolved by warming (max. 37°C) and/or stirring at room temperature. **Use only completely dissolved Avidity Reagent for the test!**

[WASHBUF]: Dilute the [WASHBUF|25x] 1:25 with distilled water
e.g. add 40ml of [WASHBUF|25x] to 960 ml distilled water and mix well.

Dilution of samples IgG: Dilute patient samples **1:101** with [SPE|DIL] e.g. 10µl sample + 1ml [SPE|DIL] or resp. 10µl prediluted sample (e.g. 1:10) with 1ml [SPE|DIL]; mix thoroughly.

[CONTROL|-] / Calibrators are ready to use.

Take the required [MTS] out of the foil packets and place them in the holder. Possibly remaining wells of a [MTS] have to be stored at 2-8°C tightly sealed in the plastic bag provided, with the desiccant inside.

11. PIPETTING AND INCUBATION STEPS

A. Pipette 100 µl of sample diluent into well A1 (Blank). Pipette 100µl of the negative control, of calibrator 1 to 4 and of each diluted patient sample into the wells. The [CAL|4], the [CONTROL|LA] and the patient samples must be set up in duplicates.

B. Cover the [MTS] with an adhesive seal and incubate the wells for 60 minutes at 37°C (± 1°C) in an incubator with relative high humidity or in a humid chamber.

C. Wash the wells four times as described in section N. WASHING PROCEDURE

D. Add 100µl of ready to use avidity reagent [UREA] to one well of the duplicate, add 100µl of the diluted wash solution to the other well of the duplicate and to all other wells (Blank, negative control and calibrator 1-3).

E. Cover the [MTS] with an adhesive seal and incubate the wells at 37°C (± 1°C) in an incubator with relative high humidity or in a humid chamber for exactly 15 minutes.

F. Repeat washing as in section C above.

G. Add 100µl of ready-to-use peroxidase conjugate to each well.

H. Cover the [MTS] with an adhesive seal and incubate the wells for 30 minutes at 37°C (± 1°C) in an incubator with relative high humidity or in a humid chamber.

I. Repeat washing as described in section C above.

J. Add 100µl of ready-to-use TMB substrate to each well.

K. Incubate the wells at room temperature (21...25°C), in the dark for exactly 15 minutes.

L. Add 100µl of stop solution to each well. Tap gently to ensure a homogenous color distribution and read within 10 minutes.

M. To read the plate, make sure that the bottom is free from moisture and that no air bubbles are in the wells. Read the absorbance of the well contents at 450nm on a suitable plate reader. On readers equipped with a dual wavelength facility, set the reference filter to 620/630 nm.

Attention: The absorbance (OD) of the Blank must be always substracted from the OD values of the controls and samples.

PROCEDURAL NOTES
Do not allow the wells to dry out between incubations.
Comply with the given incubation temperatures and times.

N. WASHING PROCEDURE
The washing procedure can be done manually with a multichannel pipette or on an automatic plate washer. Empty the wells, invert and tap dry on paper towel. Wash four times with a soaking time of approx. 30 seconds (300 µl).

12. CALCULATION OF RESULTS

For each sample, for [CAL|4] and for the [CONTROL|LA], calculate the percent ratio between the O.D. of the well treated with Avidity Reagent and the O.D. of the well treated with Wash Solution and multiply with the factor 100. This ratio will be the sample/control percent Avidity.

$$\frac{\text{O.D. value (with avidity reagent)}}{\text{O.D. value (with wash solution)}} \times 100 = \text{Avidity Index (AI \%)}$$

Interpretation of sample results:

RESULT	DEFINITION
low avidity IgG	avidity index **< 35%**
equivocal range	avidity index **35% - 40%**
high avidity IgG	avidity index **> 40%**

Determination of Toxo-specific IgG antibody avidity could be used as a diagnostic marker to distinguish between primary infection and prior Toxoplasma infection. With an avidity index less than 35% a primary infection, acquired within the past 3 – 4 months, can be assumed. The range between 35% and 40% is defined as equivoval. Equivocal results should be retested. If the equivocal result is confirmed it is not possible to determine the time of infection. The presence of high avidity IgG, that means an avidity index >40%, almost excludes the possibility that the infection occurred within the previous 3 months.
Note! For the interpretation of the avidity test results, other results of Toxoplasma gondii diagnostic and the patient's clinical background have to be taken into account. Particular attention should be paid to the effect that the production of IgG antibodies and maturation of IgG avidity can be delayed under the influence of medication.

13. VALIDITY OF THE ASSAY

All calibrators and controls have to be used in every test run.

The test must comply with the following validation criteria:

- Avidity index of the [CONTROL|LA] should be <35%.
- Avidity index of the [CAL|4] should be >50%.

See also the validation criteria of the VIR-ELISA ANTI-TOXO-IgG [REF] EG 127.

If calibrators, the negative control and the [CONTROL|LA] give invalid levels then results from the test samples are invalid too and a retesting is required.

14. PERFORMANCE CHARACTERISTICS

14.1 Avidity testing on 70 *T. gondii*-IgG-positive blood donors

Among the 70 blood donor samples tested, 66 showed a Toxo IgG high avidity result (AI% >40%). 2 samples had a low-avidity index. Both samples had Toxo-IgG antibody concentrations <= 20 IU that might cause a false classification of the avidity index (see also **9.** on page 1). 2 samples had confirmed avidity indices within the equivocal range. These results show that immune maturation might be delayed or inhibited in individual persons.

14.2 Avidity testing on 39 samples with defined avidity by Instand e.V.

Based on the results of **39 samples** with well-defined avidity it could be demonstrated that the VIR-ELISA TOXO-IgG AVIDITY can differentiate between high and low avidity-antibodies with a high reliability.
29 samples were classified as "high-avid" resp. as "latent infection" during the last few years by Instand e.V. All samples showed high avidity (>40%) with the VIR-ELISA TOXO-IgG AVIDITY test and correlated completely with the specifications for all samples (100%).
10 samples were classified as "low-avid" resp. as "acute/active infection" during the last few years by Instand e.V. All samples showed low avidity (<35%) with the VIR-ELISA TOXO-IgG AVIDITY test and correlated completely with the specifications for all samples (100%).

14.3 Avidity testing on samples from pregnant women (n=44)

A serum panel with 20 samples from pregnant women with toxoplasmosis, where the time of infection was more than 4 month ago, was tested. 19 out of these 20 patients had high-avidity antibodies with an avidity index (AI %) > 40%. 1 patient had still low avidity antibodies. This result shows that the immune maturation of the IgG response varies considerably between individuals.
It is also reported that treatment may influence the maturation of T. *gondii*-specific IgG (95%).

In addition a serum panel of 24 samples from pregnant women, with evidence of acute primary toxoplasmosis within the last 3 months, was tested. All of these 24 samples had low-avidity antibodies with an avidity index (AI) less than 35% (100%).

14.4 Avidity testing external study (n=38)

38 samples from 24 patients were tested in comparison to a reference method (ELISA).

11 patients (16 samples) with a latent or subacute *Toxoplasma gondii* infection (6-12 months post infection) were found with a high avidity index (AI >40%). The concordance with the results of the reference method was 100 %.

13 patients (22 samples) with an acute *Toxoplasma gondii* infection (less than 4 months post infection) were found with a low avidity index (AI <35%). The concordance with the results of the reference method was 100 %.

All results refer only to the groups of samples investigated.

14.5 Precision and reproducibility

Intra-assay reproducibility was determined with samples of different avidity by testing each sample for at least 22 times in one test run. The calculated coefficients of variation (CV) of the samples were < 10%.

Inter-assay reproducibility was determined by testing samples of different avidity in 10 independent test runs. The calculated CV's of the samples were < 10%.

15. [Bib]

1. Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. Jamshaid Iqbal and Nabila Khalid. Journal of Medical Microbiology (2007), 56, 1495-1499
2. Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity. Maija LAPPALAINEN and Klaus HEDMAN. Ann 1st Super Sanità 2004;40(1):81-88.
3. Performance of the Immunoglobulin G Avidity and Enzyme Immunoassay IgG/IgM Screening Tests for Differentiation of the Clinical Spectrum of Toxoplasmosis. Mehmet Tanyuksel, Cakir Guney, Engin Araz, M.Ali Saracli and Levent Doganci. The Journal of Microbiology, September 2004, p 211-215, Vol. 42, No. 3.
4. VIDAS Test for Avidity of Toxoplasma-Specific Immunoglobulin G for Confirmatory Testing of Pregnant Women. Jose G. Montoya, Oliver Liesenfeld, Sandra Kinney, Cynthia Press and Jack S. Remington. Journal of Clinical Microbiology, July 2002, p. 2504-2508, Vol. 40 No. 7.

Symbole nach IVD/ symbols used with IVD devices/ Symbole / Símbolos/ Simboli/ Simbolos/ Symboly/ Címkékén/ Συμβολα IVD

[SPE|DIL]	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição/ Ředidlo na Vzorek/ Mintahígító/ Αραιωτικό Δείγματος
[WASHBUF|25x]	Waschlösung/ wash solution/ solution de lavage/ solución limpiadora/ soluzione lavaggio/ solução de lavagem Konzentrat/ concentrate / Concentré / concentrado/ concentrato/ concentrado 25x/ Promývací Roztok 25x/ Mosópufferkoncentrátum 25x/ Διάλυμα Πλύσης 25x
[UREA]	Aviditätsreagenz / avidity reagent / réactif d'avidité / reactivo de avidina / reagente di aviditá / reagente de avidez / reagencie pro testování avidity / άντιδράστήριο δϋνάμης σϋνδέσης
[CONTROL|LA]	Positive Kontrolle/Niedrig-avide / positive control/low aviditá contrôle positif/faible avidité / control positivo/bajo grado de avidez / controllo positivo/bassa aviditá / controle positivo/baixa avidez / pozitivní kontrola/nízká avidita / θετικός μάρτυρας/χάμηλή δϋναμη σϋνδεσης
[Bib]	Literatur/ Literature/ Littérature/ Bibliografia/ Bibliografía/ Literatura/ Irodalom/ Βιβλιογραθια
[LOT]	Charge/ lot/ Lot/ lote/ carcia/ lote/ Číslo Šarže/ Lot Szám/ Αριθμός παρτίδας
[IVD]	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro/ diagnóstico in-vitro/ In-vitro diagnostic/ diagnóstico In-vitro/ In vitro Diagnostický Zdravotnický Prostředek/In Vitro Diagnosztikum/ Διαγνωστική ιατρική συσκευή In vitro
[REF]	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda/ Katalogové Číslo/ Katalógusban Szereplő Kód/ Κωδικός Καταλόγου
S 48	48 Bestimmungen/ tests/ testś/ determinazioni/ testes / Počet Testů/ Vizsgálatok Száma/ Αριθμός εξετάσεων
I	Gebrauchsanweisung beachten/ consult instructions for use/ consulter le mode d'emploi/consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruçõesde uso/ Přečtěte si Návod k Použití/ Olvassa el a Használati Utasítást/ Δείτε Οδηγίες Χρήσεως
t	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura/ Teplotní Omezení/ Hőmérsékleti Korlátozások/ Θερμοκρασιακά όρια
e	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade/ Datum Expirace/ Lejárati Idő/ Ημερομηνία λήξης (Χρήση έως ...)
M	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por/ Výrobce/ Gyártó/ Κατασκευάζεται από...

M : | **0123**

VIRO-IMMUN Labor-Diagnostika GmbH, In der Au 29, D-61440 Oberursel, Germany -Tel.: +49-6171-6281-00 - Fax: +49-6171-6281-12
email: info@viro-immun.de