VIDAS® TOXO IgG II (TXG)

IVD

VIDAS® TOXO IgG II (TXG) assay is an automated quantitative test for use on the instruments of the VIDAS family for the measurement of anti-toxoplasma IgG in human serum using the ELFA technique (Enzyme Linked Fluorescence Assay). It is intended for use as an aid in determination of immune status. This test is not intended for use in screening blood donors.

SUMMARY AND EXPLANATION OF THE TEST

Toxoplasma gondii, an obligate intracellular protozoan parasite, is a significant pathogen among humans. The parasite, whose primary host is the Felidae, is scattered in nature and invades all orders of mammals.

This infection is usually benign or asymptomatic, but can have severe consequences if it occurs in immunodeficient subjects or fetuses. Women who are seropositive before they become pregnant are essentially protected from transmitting the infection to their unborn child. Women who are seronegative are at risk of becoming infected during gestation. The transmission to their fetus occurs during the acute stage of the infection in the mother. The frequency with which *Toxoplasma* crosses the placental barrier to cause congenital infection will depend on a number of factors including the virulence of infecting strain of the parasite, size of the original inoculum, the immune response of the patient and when during gestation the mother became infected (6).

The diagnosis of Toxoplasma infection is most commonly made using serological tests, ie. specific immunoglobulin detection (IgM and IgG).

The diagnosis of the acute acquired infection during pregnancy is established by demonstration of a seroconversion (a significant rise in antibody titer) in sequential sera assayed concomitantly (6,7).

PRINCIPLE

The assay principle combines a two step enzyme immunoassay sandwich method with a final fluorescence detection (ELFA).

The Solid Phase Receptacle (SPR®), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument.

After a sample dilution step, the sample is cycled in and out of the SPR for a specified length of time. Anti-*T gondii* IgG antibodies present in the specimen will bind to the *T. gondii* antigen coating the interior of the SPR. Unbound components are eliminated during the washing steps. Mouse monoclonal anti-human IgG conjugated with alkaline phosphatase is cycled through the SPR and will attach to any human IgG bound to the SPR wall.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antibody present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

KIT COMPOSITION (60 TESTS) :

60 TXG strips	STR	Ready-to-use.
60 TXG SPRs 2 x 30	SPR	Ready-to-use. SPRs coated with membrane and cytoplasmic Toxoplasma antigen (RH Sabin strain) grown in mice (10).
Positive TXG control 1 x 2 ml (liquid)	C1	Human serum* containing anti-Toxoplasma IgG + protein stabilizer + 1 g/l of sodium azide. MLE data indicate the titer in IU/mL ("Control C1 (+) Dose Value Range").
Negative TXG control 1 x 3 ml (liquid)	C2	Human serum* negative for anti-Toxoplasma IgG + protein stabilizer + 1 g/l of sodium azide.
TXG calibrator 1 x 1 ml (liquid)	S1	Human serum* containing anti-Toxoplasma IgG and calibrated against the WHO standard + protein stabilizer + 1 g/l of sodium azide. MLE data indicate the calibrator titer in IU/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range").
Specifications for the factory master data required to calibrate the test: ● MLE data (Master Lot Entry) provided in the kit, or ● MLE bar codes printed on the box label		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR®

The interior of the TXG SPR is coated during production with membrane and cytoplasmic Toxoplasma antigen (RH Sabin strain). Each SPR is identified by the “TXG“ code. Only remove the required number of SPRs from the pouch. Make sure the pouch is carefully closed after opening.

The Reagent Strip

The strip consists of 10 wells covered with a labeled, foil seal. The label contains a bar code which indicates the type of test performed, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the TOXO IgG II (TXG) Reagent Strip

Wells	Reagents
1	Sample well
2	Serum diluent : TRIS buffer (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (600 µl).
3	Pre-washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (600 µl).
4 - 5 - 7 - 8	Washing buffer : TRIS (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (600 µl).
6	Conjugate : Alkaline phosphatase labeled monoclonal anti-human IgG antibodies (mouse) + 1 g/l of sodium azide (400 µl).
9	Serum diluent : TRIS buffer (50 mmol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l of sodium azide (400 µl).
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (DEA*) (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 : Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Safety Data Sheet.

MATERIALS REQUIRED BUT NOT PROVIDED

– Pipette with disposable tip calibrated to dispense 100 µl.
– Powderless, disposable gloves.
- For other specific materials, please refer to the Instrument Operator’s Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analytical method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use reagents after their expiration date.
- Do not mix reagents or disposables from different lots.
- **When the analysis is completed, remove the used SPRs and strips, and dispose of them appropriately (i.e autoclaving). All other contaminated material such as disposable gloves and pipette tips should** be disposed of in a similar manner**.**
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The reading cuvette with Substrate (well 10) contains an irritant agent (diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.

- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5 % sodium hypochlorite. See the instrument Operator's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the instrument Operator's Manual).

STORAGE

- Store the VIDAS® TOXO IgG II (TXG) kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR® pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SAMPLE COLLECTION AND STORAGE

The VIDAS TOXO IgG II (TXG) test can be performed on serum samples only. Samples that are obviously lipemic, icteric, or hemolyzed should not be used. Samples containing particulate matter must be centrifuged before analysis.
According to NCCLS Standard H18-A2, pg 10, separated serum should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2-10°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -25 ± 6°C. Avoid repeated freezing and thawing. Sera inactivated at 56°C for 30 minutes can be tested with VIDAS TOXO IgG II (TXG).

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.
Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified as S1, must be tested in **duplicate** (see Operator's Manual). The calibration value must be within the set RFV (Relative Fluorescence Value) range. If this is not the case, recalibrate.

Assay procedure

1. Remove necessary components from the kit and allow them to come to room temperature for at least 30 minutes.
2. Use one "TXG" strip and one "TXG" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "TXG" code on the instrument. The standard must be identified by "S1", and tested in duplicate. If the positive control is to be tested, it should be identified by "C1". If the negative control needs to be tested, it should be identified by "C2".
4. If needed label "TXG" strips with appropriate sample identification numbers.
5. Mix the standard, controls and samples using a vortex-type mixer (for serum separated from the pellet).
6. **For this test, the calibrator, control, and sample test portion is 100 µl**
7. Insert the "TXG" SPRs and "TXG" strips into the appropriate positions.
8. Initiate the analysis as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to 2–8°C after pipetting.
10. The assay will be completed in approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
11. Dispose of the used SPRs and strips into an appropriate recipient.

QUALITY CONTROL

A positive control (C1) and a negative control (C2) are included in each VIDAS TOXO IgG II (TXG) kit. These controls must be tested immediately after opening a new kit to ensure that reagent performance has not been altered. Each recalibration must also be checked using these controls. If the control values deviate from the expected values, do not report patient results. Test controls with each instrument run or as specified by your laboratory's regulatory guidelines.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the instrument. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the cuvette and substrate. The second reading is taken after the substrate has been incubated in the SPR®. The RFV is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The RFV is interpreted by the instrument. Results are expressed in IU/ml. The TOXO IgG II thresholds were established via a study involving approximately 500 samples. Each sample was tested using Dye Test, a High Sensitivity agglutination procedure, and VIDAS TOXO IgG II (TXG). Evaluation of the results using a number of statistical techniques yielded the thresholds shown below, which were confirmed with clinical trial data.

Thresholds and interpretation of results

Titer : IU/ml	Interpretation
< 4	Negative
from ≥ 4 to < 8	Equivocal
≥ 8	Positive

Equivocal samples should be retested. If the interpretation remains equivocal, then a new sample must be collected.
A negative result does not always exclude the possibility of Toxoplasma infection. Patients with negative results in suspected early disease cases should be retested in 4-6 weeks.
Samples with a VIDAS TOXO IgG II (TXG) concentration greater than 300 IU/ml should be reassayed after diluting 1/4 (1 volume of sample and 3 volumes of negative control).

If the dilution factor has not been entered when the analysis has been requested (see Operator's Manual), multiply the result by the dilution factor to obtain the TOXO sample concentration.
Samples with results greater than 300 IU/ml will be reported as "greater than 300 IU/ml". Samples with high anti-*T. gondii* IgM titers may affect dilution test results.

PERFORMANCE

The figures presented in the following tables are taken from tests which have been performed as indicated in this package insert.

1. Sensitivity - Specificity

1893 samples were evaluated at 3 sites in comparison with a legally marketed EIA.
Consolidated results for the 3 sites are as follow:

		EIA Technique			
		Pos	Equiv	Neg	Total
VIDAS	Pos	536	8	3	547
	Equiv	5	11	12	28
	Neg	9	10	1299	1318
Total		550	29	1314	1893

Overall Agreement: 97.52%
(Confidence interval at 95%: 96.70% - 98.18%)

Relative* Sensitivity: 98.35 %
(Confidence interval at 95%: 96.87% - 99.24%)

Relative* Specificity: 99.77%
(Confidence interval at 95%: 99.33% - 99.95%)

*The word "relative" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.

Discrepant samples were tested using the Dye Test. Upon resolution with Dye Test, 6 of the 9 VIDAS® false negatives resolved as true negatives, 2 remained false negatives, and 1 was unresolved. Two of the 3 VIDAS false positives resolved as true positives and 1 remained false positive.

2. Precision

Precision was tested with a negative sample, a low positive sample and a high positive sample.
Each sample was tested in duplicate two times per day for twenty days.
Within-run reproducibility (intra-assay precision) and between-run reproducibility (total precision) were calculated according to the recommendations of NCCLS Document EP5-T2, Volume 12 Number 4

a. Within-run reproducibility

Sample	N	Mean IU/ml	Standard deviation	CV %
Negative	80	1.21	0.25	NA*
Low Positive	80	17.18	0.88	5.13
High Positive	80	168.90	12.17	7.21

b. Total precision

Sample	N	Mean IU/ml	Standard deviation	CV %
Negative	80	1.21	0.43	NA*
Low Positive	80	17.18	1.15	6.70
High Positive	80	168.90	18.38	10.88

* Not applicable due to low values.

3. Cross-reactivity:

A total of 49 samples positive for RF, ANA, or EBV were obtained for evaluation of cross-reactivity and interference in the VIDAS TOXO IgG II (TXG) Assay. When tested with a commercially available TOXOIgG EIA, half of the samples in each group were positive for anti-*T. gondii* and half were negative. No cross-reactivity or interference in the VIDAS TOXO IgG II (TXG) assay was seen with any of the samples tested.

4. Linearity

A total of five samples were used to evaluate the linearity of the VIDAS TOXO IgG II (TXG) assay. Four of the samples were sera with T. gondii -specific IgG titers between 250 and 300 IU/ml. The final sample was the WHO standard diluted in negative serum.
For each of the five samples, dilutions were made in negative sera. For each dilution factor, 2 separate dilutions were prepared from the pure serum. Each pure serum and each dilution were then tested in duplicate using the VIDAS TOXO IgG II (TXG) assay. The results were evaluated using linear regression analysis.

The table below shows the equations and correlation coefficients for each of the samples.

	Linear regression	Correlation coef.
WHO Standard	y= 1.00x + 19.46	0.972
Sample 1	y= 1.00x – 12.43	0.991
Sample 2	y= 1.02x -17.37	0.987
Sample 3	y= 1.03x – 5.82	0.985
Sample 4	y= 0.99x – 0.33	0.996

LIMITATIONS

1. A negative result does not always exclude the possibility of Toxoplasma infection. Sera collected very early in the acute stage of disease may have IgG levels < 4 IU/ml. Patients with negative results who are suspected of having early disease should be retested in 4-6 weeks.
2. Positive test results from cord blood should be interpreted with caution. The presence of total or IgG anti-*T. gondii* antibodies in cord blood is usually the result of passive transfer from the mother to the fetus. A negative test, however, may be useful in excluding current infection.
3. Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.
4. VIDAS® TOXO IgG II (TXG) results should be used in conjunction with clinical symptoms and other laboratory test results, such as anti-*T. gondii* IgM results.
5. Test results from immunosuppressed patients may be difficult to interpret, due to diminished immune response.
6. The VIDAS TOXO IgG II (TXG) assay may only be used with serum samples. The use of plasma, amniotic fluid or other body fluids has not been established.
7 The concentrations of anti-Toxoplasma gondii IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity

EXPECTED VALUES

The prevalence of toxoplasmosis varies depending upon geographical location, age and gender of the population studied, specimen collection and handling, and other factors. In Europe, the prevalence rate ranges from 20% to 85%. In the United States, the prevalence is lower: 12% to 41%. Prevalence in other countries can vary from 18% to 65%.

A group of 100 random sera from healthy blood donors in the Northeast US was tested in the VIDAS TOXO IgG II (TXG) assay. Of the 100 samples, 87 were from men and 13 from women. Overall, there were 13 positive samples (12 male and 1 female) The prevalence at this site was within the range stated in the literature.

CDC TOXOPLASMA 1998 HUMAN SERUM PANEL

The following information is from a serum panel obtained from the CDC (Centers for Disease Control and Prevention) and tested by bioMerieux. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply endorsement of the assay by the CDC. The panel consists of 70% positive and 30% negative samples. The VIDAS TOXO IgG II (TXG) assay demonstrated 100% total agreement with the CDC results. Of the results obtained by bioMerieux, Inc. there was 100% agreement with the positive specimens and 100% agreement with the negative specimens.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	13687C	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	KIT COMPOSITION (60 tests) WARNINGS AND PRECAUTIONS
2015/06	13687D	Technical	KIT COMPOSITION (60 tests) INSTRUCTIONS FOR USE

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