VIDAS® TOXO IgG Avidity (TXGA)

IVD

The VIDAS® TOXO IgG Avidity assay is an automated qualitative test for the determination of anti-toxoplasma IgG avidity in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay). The VIDAS® TOXO IgG Avidity assay is intended for use in conjunction with results from the VIDAS TOXO IgG II and must have a positive titer (≥ 8 IU/mL); other laboratory findings and clinical information to aid in the presumptive exclusion of a recently acquired (≤ 4 months) *Toxoplasma gondii* infection in pregnant women and patients with lymphadenopathy.

VIDAS TOXO IgG Avidity assay performance has not been established for prenatal screening, for newborn testing, for use in immunocompromised patients and in cases of endogenous or exogenous reinfection by *Toxoplasma gondii*. This assay has not been cleared or approved by the FDA for blood/plasma donor screening.

SUMMARY AND EXPLANATION

Toxoplasma gondii is a protozoan parasite that infects most species of warm-blooded animals, including humans. Members of the cat family (*Felidae*) are the only known definitive hosts for the sexual stages of *T. gondii* and thus are the main reservoirs of infection. A *T. gondii* infection during pregnancy may be transmitted to the fetus and result in congential toxoplasmosis. *T. gondii* infection is acquired primarily by ingestion of cysts in infected, undercooked meat or exposure to oocysts that may contaminate soil, water, and food. Transmission to the fetus occurs almost solely in women who acquire their primary infection during gestation and can result in visual and hearing loss, mental and psychomotor retardation, seizures, hematological abnormalities, hepatosplenomegaly, or death. In rare cases, congenital transmission has occurred in women with latent infection whose infection was reactivated because of their immunocompromised state (e.g., from AIDS or treatment with corticosteroids for their underlying disease). Most pregnant women with acute acquired infection do not experience obvious symptoms or signs. A minority may experience malaise, low-grade fever, and lymphadenopathy. Rarely, pregnant women will present with visible changes due to toxoplasmic chorioretinitis as a result of recently acquired infection or reactivation of a chronic infection. The frequency of transmission to the fetus increases with the gestational age. In contrast, severe clinical signs in the infected infant are more commonly observed in offspring of women whose infection was acquired early in gestation.

Laboratory tests are used to diagnose toxoplasmosis in pregnant women. Because infection may occur in the absence of known toxoplasmosis exposure, the decision to perform *T. gondii* serological tests during pregnancy should not be based solely on clinical (e.g. presence or absence of symptoms) or epidemiological (i.e., history of exposure to *T. gondii*) grounds. (1)

Avidity index determination (2, 3) is a test for use as a complement to detection of anti-toxoplasma IgG and IgM. After initial detection of anti-toxoplasma IgG and IgM, the avidity index may aid in the determination that the infection occurred at least 4 months previously. (4, 5) One of the elements used to establish a diagnosis of acute toxoplasmosis infection in immunocompetent patients is the detection of specific IgM; however, if seroconversion is not strictly demonstrated, other approaches may be needed, especially during pregnancy, to assess the date of infection. (6)

The VIDAS TOXO IgG Avidity assay enables weak avidity antibodies to be differentiated from high avidity antibodies. Since IgG avidity rises progressively during the course of infection, detection of high avidity antibodies is a strong indication of a primary infection dating back more than 4 months (chronic infection) and can be used to exclude recent infections of less than or equal to 4 months (acute infection) (7, 8, 9, 10). Low or equivocal (borderline) IgG avidity antibody results should not be interpreted as diagnostic of recently acquired infection; low or equivocal (borderline) avidity antibodies can persist for > 1 year are therefore not reliable for the diagnosis of recently acquired infection (11). Interpretation of avidity test results should be made in conjunction with the patient history, and the results of any other tests performed.

PRINCIPLE

The addition of a dissociating agent (such as urea) which disrupts the Ag-Ab link during an ELISA test has little effect on the high avidity Ag-Ab link, but great effect on that of weak avidity Ab. Comparison of results obtained with and without a dissociating agent corresponds to one measure of avidity.

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent 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 predispensed in the sealed reagent strips.

VIDAS TOXO IgG Avidity uses a dual strip comprising one reference strip and one test strip. The sample to be tested, after dilution, is dispensed into both sample wells of the dual strip: reference and test.

Anti-toxoplasma IgG present in the sample forms complexes with antigen coated to the solid phase. In the reference strip, non-specific antibodies are eliminated by washing, whereas specific antibodies remain coated to the solid phase. In the test strip, washing with the dissociating agent changes antigen-antibody links: high avidity antibodies remain bound to the solid phase, whereas low avidity antibodies are eliminated.

Alkaline phosphatase labeled with human anti-IgG antibody (conjugate) is then cycled in and out of the SPR, and binds with any human IgG coated on the interior of the SPR. Unbound conjugate is removed by washing.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. 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. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each dual reagent strip. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate.

The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. The RFV for the two assays (reference and test) appear on the result sheet. The intensity of the fluorescence is proportional to the concentration of antibodies present in the sample. At the end of the assay, results are automatically calculated by the instrument and then printed out.

The ratio between the quantity of high avidity antibodies (test strip) and the quantity of total antibodies (reference strip) provides an index that indicates antibody avidity in the tested sample.

CONTENT OF THE KIT (30 TESTS):

30 dual TXGA strips	STR	Ready-to-use.
60 TXGA SPRs 2 x 30	SPR®	Ready-to-use. SPRs coated with toxoplasma antigen, RH Sabin strain grown in mice (5).
TXGA High avidity control 1 x 2 mL (liquid)	C1	Human serum* containing anti-toxoplasma IgG + protein stabilizer + 1 g/L sodium azide. MLE data indicate the confidence interval in "Relative Fluorescence Value ("C1 Ref RFV Range").
TXGA Low avidity control 1 x 1.6 mL (liquid)	C2	Human serum* containing anti-toxoplasma IgG + protein stabilizer + 1 g/L sodium azide. MLE data indicate the index: confidence interval ("Control C2 (-) Test Value Range").
Sample diluent 2 x 6.5 mL (liquid)	R1	Human serum* containing protein stabilizer + 1 g/L sodium azide
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 SPR® is coated during production with membrane and cytoplasmic toxoplasma antigen (RH Sabin strain). Each SPR is identified by the TXGA code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The Dual Reagent Strip

Each of the reagent strips consists of 10 wells covered with a labeled, foil seal. The label contains a bar code that mainly indicates the assay code, 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 strips contain the various reagents required for the assay.

Description of the Reference Strip (left)

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L sodium azide (600 µL).
3 – 4 – 5 – 7 – 8	Wash buffer: TRIS (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L sodium azide (600 µL).
6	Conjugate: Alkaline phosphatase labeled human anti-IgG antibodies (mouse) + 0.9 g/L sodium azide (400 µL).
9	Sample diluent: TRIS buffer (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L 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).

Description of the Test Strip (right)

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L sodium azide (600 µL).
3 – 7 – 8	Wash buffer: TRIS (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L sodium azide (600 µL).
4 – 5	Wash buffer: TRIS (50 mmol/L, pH 7.4) + dissociating agent (urease solution) + protein and chemical stabilizers + 1 g/L sodium azide (600 µL).
6	Conjugate: Alkaline phosphatase labeled human anti-IgG antibodies (mouse) + 0.9 g/L sodium azide (400 µL).
9	Sample diluent: TRIS buffer (50 mmol/L, pH 7.4) + protein and chemical stabilizers + 1 g/L 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 10 µL, 50 µL, 100 µL, 1000 µL.
- Powderless, disposable latex gloves.
- For other specific materials, refer to the Instrument Operator Manual.
- VIDAS or miniVIDAS instrument.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis 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 the 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 the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- 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 optical cuvette with substrate (well 10) contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5% sodium hypochlorite. See the VIDAS Operator Manual for cleaning spills on or in the VIDAS instruments. Do not autoclave solutions containing bleach.
- The instruments should be regularly cleaned and decontaminated (see the VIDAS Operator Manual).

STORAGE CONDITIONS

- Store the VIDAS TOXO IgG Avidity 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.

SPECIMENS

Specimen type and collection:

The test should only be used with serum from immunocompetent populations.

Plain and serum separator tubes were used for testing. Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used. It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.
It is recommended that clearly hemolyzed, lipemic or icteric samples not be used and, if possible, to collect a new sample.
Sera inactivated at 56°C for 30 minutes can be tested with VIDAS TOXO IgG Avidity.

Specimen stability:

Samples can be stored at 2-8°C in capped tubes for up to 5 days; if longer storage is required, freeze the sera at -25 ± 6°C.

A maximum of three freeze/thaw cycles is recommended.

Samples containing impurities (i.e. suspended fibrin particles or erythrocyte stroma) must be centrifuged before analysis.

INSTRUCTIONS FOR USE

For complete instructions, see the User 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 Manual).

Procedure

All samples to be assayed by VIDAS TOXO IgG Avidity must have been previously tested using VIDAS TOXO IgG II and must have a positive titer (≥ 8 IU/mL**).**

Samples found to be higher than 300 IU/mL will have been previously diluted, according to the VIDAS TOXO IgG II package insert, in order to obtain a working titer.
Only an IgG titer determined according to the VIDAS TOXO IgG II can be used to calculate the dilution factor to be used to bring the titer to 15 IU/mL.

1. **Important – Calculation of the dilution factor: to test a sample with VIDAS TOXO IgG Avidity, bring its titer back to 15 IU/mL by dilution. The dilution factor is calculated as follows:**

$$d = \frac{\text{titer in IU/mL VIDAS TOXO IgG II}}{15}$$

Example: VIDAS TOXO IgG II titer = 150 IU/mL

$$d = \frac{150}{15} = 10$$

In this example, the sample will be diluted 1/10 in the diluent for the kit, and then tested using VIDAS TOXO IgG Avidity.

Positive samples ≤ 15 IU/mL **are tested as is, with no dilution required.**

2. **Remove only required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
3. Use one dual "TXGA" strip and two "TXGA" SPRs for each sample or control to be tested. Make sure the storage pouch has been resealed after the required SPRs have been removed.
4. The test is identified by the "TXGA" code on the instrument. If the high avidity control is to be tested, it should be identified by "C1". If the low avidity control needs to be tested, it should be identified by "C2".
5. Mix the controls and samples using a Vortex-type mixer (for serum separated from the pellet).

6. Control and sample test portion is 100 µL for each sample well of the dual strip.

7. Insert the VIDAS SPRs and strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the dual Reagent Strips match.
8. Initiate the assay as directed in the Operator 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 within 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.

INTERPRETATION OF RESULTS

The instrument automatically calculates the avidity index as follows:

$$\text{Index} = \frac{\text{test RFV (washing with dissociating agent)}}{\text{reference RFV (washing without dissociating agent)}}$$

Interpretation of the avidity index is as follows:

Avidity	Results	Interpretation
index < 0.200	Low IgG avidity (Acute infection not excluded)	Unable to differentiate between past or recent infection. Infection ≤ 4 months not excluded, re-test with other method(s)
0.200 ≤ index < 0.300	Equivocal IgG avidity (Borderline)	
index ≥ 0.300	High IgG avidity (Chronic)	Strong indication of a primary infection excluded, i.e., primary infection occurred > 4 months previously.

An avidity index greater than or equal to 0.300 is a strong indication of a primary infection dating back more than 4 months. This result must be confirmed using a second sample collected 2 to 3 weeks later. Alternatively, the result may be confirmed on the original sample using another confirmatory test method(s). Refer to the Limitations section for a description of the confirmatory test methods.

An index lower than 0.300 does not enable recent infection to be differentiated from infection > 4 months prior. These samples should be retested using other markers or techniques.

- Samples with Low Avidity or Equivocal (borderline) results should be retested using other Toxoplasma testing methods to determine whether serological test results are more likely consistent with infection acquired in the recent or more distant past. These tests include the Sabin-Feldman Dye Test (DT), Differential Agglutination (AC/HS) test and Toxoplasma IgM ELISA. A Toxoplasma IgA ELISA assay and Toxoplasma IgE ELISA assay may also be used as supportive information. Laboratories not performing these tests should submit the sample to a reference laboratory for evaluation.

The instrument will display an error message if the reference assay RFV deviates from the expected values.

- If the RFV are lower than the limits, the sample dilution is too high. Retest with the appropriate dilution. If the sample has been tested *without* dilution, the result is uninterpretable.
- If the RFV are higher than the limits, the sample dilution is insufficient. Retest using an appropriate dilution.

Interpretation of test results should be made in conjunction with the patient history, and the results of any other tests performed.

QUALITY CONTROL

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

Two controls are included in each VIDAS TOXO IgG Avidity kit.
These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. The user must check that the relative fluorescence value (RFV) of the high avidity control C1 (reference strip) is included in the expected values. The instrument will automatically check the avidity index of the controls if they are identified as C1 and C2.
Results cannot be validated if one of the values deviates from the expected values.

LIMITATIONS

- This test should not be used as an initial pregnancy screening test for pregnant women, nor as a substitute for tests confirming the presence of IgG and IgM.
- All samples to be assayed by VIDAS TOXO IgG Avidity must have been previously tested using VIDAS TOXO IgG II and must have a positive titer (≥ 8 IU/mL).
- Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted in conjunction with the patient history, and any other tests performed.
- This test has not been validated:
  - for newborn infants,
  - for immunocompromised patients,
  - in cases of endogenous or exogenous reinfection by *Toxoplasma gondii*.
- This test was not established for use with patients who have received blood transfusions or other blood products within the past few months.
- Test interpretation may be erroneous if the patient has previously received anti-parasitic treatment, since this may affect IgG maturation kinetics during infection (9).
- This test is to be used with the VIDAS or miniVIDAS instrument.
- It is recommended that clearly hemolyzed, lipemic or icteric samples not be used and, if possible, to collect a new sample.

EXPECTED VALUES

The TOXO IgG Avidity assay was tested with 148 prospectively collected specimens (fresh and frozen) representing subjects for whom the VIDAS TOXO IgG II result was ≥ 8 IU/mL. Of the 148 serum samples, 142 corresponded to pregnant women, and 6 to patients with lymphadenopathy. The percentage of high avidity results in these populations as determined by the VIDAS TOXO IgG Avidity assay was 85.2% for the pregnant women population and 66.7% for the lymphadenopathy population.

CLINICAL PERFORMANCE

The clinical performance of the VIDAS TOXO IgG Avidity assay was studied in a single study of samples from 386 subjects, enrolled prospectively or from samples previously collected for toxoplasmosis testing ('retrospective').

Study Design Population

A total of 386 subjects provided samples; all patients were required to have a VIDAS TOXO IgG II assay positive result (i.e. result ≥ 8 IU/mL), and either be pregnant or have acute lymphadenopathy due to suspected or confirmed toxoplasmosis infection. Samples were obtained from four clinical sites: Palo Alto, CA, Grenoble and Marseille, France and Cali, Colombia. Testing was performed at 3 US sites: Palo Alto, CA, Hackensack, NJ and Albuquerque, NM.

Of the 386 subjects, 104 subjects had initially equivocal results on composite reference testing. Thirty-one subjects had an additional sample available for repeat composite reference testing and are included in the primary analysis; results for the remaining 73 subjects without an evaluable response on the composite reference standard were excluded. Of the 313 total subjects analyzed (283 frozen samples and 30 fresh samples), 271 samples were from pregnant women, 42 from patients with lymphadenopathy (both males or females); 14 of the 271 pregnant women also had acute lymphadenopathy. Among the 313 subjects included in the final analysis, 31 had an initial equivocal result but a follow-up sample was available.

Of the 271 samples from pregnant women, 142 were from subjects enrolled prospectively and 129 subject samples were retrospectively tested samples; of the 56 patients with lymphadenopathy (including 14 pregnant women), 7 were from prospectively enrolled subjects and 49 were from retrospective samples.

All specimens were tested by both the VIDAS TOXO IgG Avidity assay and a composite reference method; the composite reference method was used for determining whether infection occurred recently or was more distant, and included the Sabin-Feldman Dye Test (DT), the VIDAS TOXO IgM ELISA assay, and the Differential Agglutination (AC/HS) test. Toxoplasmosis IgA and IgE ELISA assays were also performed and used as supportive information. All reference testing was performed at a Palo Alto, CA laboratory. 'Diagnostic truth' was assigned by results from the composite reference method and assigned as whether infection occurred ≤ 4 months or > 4 months previous to sample collection.

VIDAS TOXO IgG Avidity assay and composite referencing testing were performed on an initial specimen from each subject; if the initial specimen was equivocal by the composite reference method, a second follow up sample was tested in parallel with the initial specimen by the composite reference method to assist in determining a final diagnostic classification. Patients for which a follow up sample was required but was unavailable were not included in the results below.

Study Results

The comparison between the VIDAS TOXO IgG Avidity assay and the Composite Reference Method was analyzed as follows: all samples (tables 1 and 2), frozen samples (tables 3 and 4), pregnant women (tables 5 and 6) and lymphadenopathy patients excluding pregnant women (tables 7 and 8). The results and interpretation are shown below.

Table 1: All samples – Results

		Composite Reference Method			
		≤ 4 months [Acute]	Equivocal	> 4 months [Chronic]	Total
VIDAS TXGA	Low	129	2	11	142
	Equivocal	6	1	10	17
	High	3	2	149	154
	Total	138	5*	170	313

* Results for five (1.6%) patients remained equivocal by the Composite Reference Method after follow-up sampling testing.

Table 2. All samples – Interpretation and Performance

		Composite Reference Method Interpretation		
		≤ 4 months not excluded [Acute & Equivocal] *	> 4 months [Chronic]	Total
VIDAS TXGA Interpretation	≤ 4 months not excluded [Low & Equivocal] *	138	21	159
	> 4 months [High]	5	149	154
	Total	143	170	313

Performance	%	95% Confidence Interval
Sensitivity	96.5	92.0 – 98.9
Specificity	87.6	81.7 – 92.2

* Samples equivocal by either the VIDAS TOXO IgG Avidity assay or the Composite Reference Method were analyzed as ≤ 4 months not excluded for their respective category.

Additional analyses: Of the 31 subjects included in the analysis with initial results that were equivocal on composite reference testing, results for testing additional samples available for these patients by the composite reference method were as follows: 13 (41.9%) were interpreted as consistent with acute disease, 5 patients (16.1%) again had equivocal results, and 13 (41.9%) were interpreted as consistent with chronic disease. All of the 13 acute samples by composite reference testing (100%) had low avidity on the VIDAS TOXO IgG Avidity assay; however, of the 13 samples consistent with chronic infection, on composite reference testing, 7 (53.8%) had low avidity, 1 (7.7%) had equivocal results, and 5 (38.5%) had high avidity on the VIDAS TOXO IgG Avidity assay. An exploratory analysis where the results from these 31 samples were extrapolated to the 73 samples with equivocal composite reference testing (and not included in the analyses above) and combined with the 313 analyzed samples to approximate results for the original 386 subjects yielded a similar overall sensitivity of 94.6% (95% CI 90.3 – 97.4) but a noticeable drop in specificity to 80.5% (95% CI 74.3 – 85.8). These latter estimates may more closely approximate the results that would have been observed had repeat testing been possible on all initially equivocal composite reference samples.

Table 3: Frozen samples – Results

		Composite Reference Method			
		≤ 4 months [Acute]	Equivocal	> 4 months [Chronic]	Total
VIDAS TXGA	Low	129	2	10	141
	Equivocal	6	1	9	16
	High	3	2	121	126
	Total	138	5	140	283

Table 4: Frozen samples – Interpretation and Performance

		Composite Reference Method Interpretation		
		≤ 4 months not excluded [Acute & Equivocal]	> 4 months [Chronic]	Total
VIDAS TXGA Interpretation	≤ 4 months not excluded [Low & Equivocal]	138	19	157
	> 4 months [High]	5	121	126
	Total	143	140	283

Performance	%	95% Confidence Interval
Sensitivity	96.5	92.0 – 98.9
Specificity	86.4	79.6 – 91.6

For frozen samples from a combined pregnant women and lymphadenopathy population, the composite reference method results established that an infection occurring ≤ 4 months could not be excluded in 143 patients. In 138 (96.5%) of these 143 patients, VIDAS TXGA test results were Low or Equivocal at < 0.300. Thus the sensitivity of the VIDAS TXGA for the diagnosis that an acute infection (≤ 4 months) cannot be ruled out was 96.5%.

Table 5: Pregnant women – Results

		Composite Reference Method			
		≤ 4 months [Acute]	Equivocal	> 4 months [Chronic]	Total
VIDAS TXGA	Low	97 (89)*	2 (0)	9 (6)	108 (95)
	Equivocal	6 (5)	1 (1)	10 (3)	17 (9)
	High	3 (3)	1 (0)	142 (22)	146 (25)
	Total	106 (97)	4 (1)	161 (31)	271 (129)

* In each cell, the number not in parentheses represents the results from both prospective and retrospective samples. The number in parentheses represents the results from only the retrospective samples.

Table 6: Pregnant women – Interpretation and Performance

		Composite Reference Method Interpretation		
		≤ 4 months not excluded [Acute & Equivocal]	> 4 months [Chronic]	Total
VIDAS TXGA Interpretation	≤ 4 months not excluded [Low & Equivocal]	106 (95)*	19 (9)	125 (104)
	> 4 months [High]	4 (3)	142 (22)	146 (25)
	Total	110 (98)	161 (31)	271 (129)

* In each cell, the number not in parentheses represents the results from both prospective and retrospective samples. The number in parentheses represents the results from only the retrospective samples.

Performance	%	95% Confidence Interval
Overall Sensitivity	96.4	91.0 – 99.0
Overall Specificity	88.2	82.2 – 92.7
Retrospective Sensitivity	96.9	91.3 – 99.4
Retrospective Specificity	71.0	52.0 – 85.8
Prospective Sensitivity	91.7	61.5 – 99.8
Prospective Specificity	92.3	86.3 – 96.2

In the overall pregnant women population, the composite reference method results established that an infection occurring ≤ 4 months could not be excluded in 110 patients. In 106 (96.4%) of these 110 patients, VIDAS TXGA test results were Low or Equivocal at < 0.300. Thus the sensitivity of VIDAS TXGA for the diagnosis that an acute infection (≤ 4 months) cannot be ruled out was 96.4%.

Table 7: Lymphadenopathy patients (excluding pregnant women) – Results

		Composite Reference Method			
		≤ 4 months [Acute]	Equivocal	> 4 months [Chronic]	Total
VIDAS TXGA	Low	32 (30)*	0 (0)	2 (2)	34 (32)
	Equivocal	0 (0)	0 (0)	0 (0)	0 (0)
	High	0 (0)	1 (1)	7 (3)	8 (4)
	Total	32 (30)	1 (1)	9 (5)	42 (36)

* In each cell, the number not in parentheses represents the results from both prospective and retrospective samples. The number in parentheses represents the results from only the retrospective samples.

Table 8: Lymphadenopathy patients (excluding pregnant women) – Interpretation and Performance

		Composite Reference Method Interpretation		
		≤ 4 months not excluded [Acute & Equivocal]	> 4 months [Chronic]	Total
VIDAS TXGA Interpretation	≤ 4 months not excluded [Low & Equivocal]	32 (30)*	2 (2)	34 (32)
	> 4 months [High]	1 (1)	7 (3)	8 (4)
	Total	33 (31)	9 (5)	42 (36)

* In each cell, the number not in parentheses represents the results from both prospective and retrospective samples. The number in parentheses represents the results from only the retrospective samples.

Performance	%	95% Confidence Interval
Overall Sensitivity	97.0	84.2 – 99.9
Overall Specificity	77.8	40.0 – 97.2
Retrospective Sensitivity	96.8	83.3 – 99.9
Retrospective Specificity	60.0	14.7 – 94.7
Prospective Sensitivity	100.0	15.8 – 100.0
Prospective Specificity	100.0	39.8 – 100.0

In the overall lymphadenopathy population, the composite reference method results established that an infection occurring ≤ 4 months could not be excluded in 33 patients. In 32 (97.0%) of these 33 patients, VIDAS TXGA test results were Low or Equivocal at < 0.300. Thus the sensitivity of the VIDAS TXGA for the diagnosis that an acute infection (≤ 4 months) cannot be ruled out was 97.0%.

ANALYTICAL PERFORMANCE

Precision and Reproducibility Study

Five serum samples, including low avidity (sample 1), equivocal avidity (sample 2), and high avidity (samples 3, 4 and 5), were tested in duplicate in 20 different runs (2 runs per day over 10 days) with 2 reagent lots at three sites (N = 240). Repeatability (within-run precision), between-run, between-day, between-lot, between-system and total precision were calculated based on the recommendations of CLSI EP5-A2:

Sample		1	2	3	4	5
Mean index		(0.1196)	(0.2620)	(0.3209)	(0.5352)	(0.6843)
Source of Variation	N	CV (%)	CV (%)	CV (%)	CV (%)	CV (%)
Repeatability	240	7.9	7.8	5.7	6.1	7.1
Between-run	240	<0.1*	<0.1*	4.3	3.1	2.1
Between-day	240	2.0	2.9	<0.1*	<0.1*	<0.1*
Between-lot	240	<0.1*	5.0	1.8	1.5	0.6
Between-System	240	2.0	<0.1*	<0.1*	<0.1*	0.1
Total	240	8.4	9.7	7.4	7.0	7.4

* For the precision study, the components in which the %CVs were determined to be negligible are reported as <0.1.

Analytical Specificity

Cross-reactivity

Cross-reactivity of the VIDAS TOXO IgG Avidity assay was evaluated according to the recommendations of CLSI EP7-A2. Cross-reactivity was evaluated at two TOXO IgG Avidity Index levels. Testing included at least 5 samples with a low (or borderline) avidity index containing high titers of antibodies to a potentially interfering disease state, and five samples with a high avidity index containing high titers of antibodies to a potentially interfering disease state. Clinically significant interference was defined as either a > 20% change in index values or a result that altered assay interpretation, i.e., a low or equivocal avidity result changing to a high avidity result or a high avidity result changing to a borderline or low avidity result.

Results moving from low to borderline (and conversely) were not considered clinically relevant.

Samples	Samples with clinically significant interference
ANA (Anti-nuclear antibodies)	0/12
CMV (Cytomegalovirus)	0/10
EBV (Epstein Barr Virus)	0/10
HAMA (Human anti-mouse antibodies)	0/10
HAV (Hepatitis A Virus)	0/10
HBV (Hepatitis B Virus)	0/10
HSV-1 (Herpes Simplex Virus type 1)	1/12
HSV-2 (Herpes Simplex Virus type 2)	0/10
RF (Rheumatoid factor)	0/12
Rubella Virus	0/12
VZV (Varicella-Zoster Virus)	0/10

No clinically significant interference was observed with ten disease conditions tested: ANA, CMV, EBV, HAMA, HAV, HBV, HSV-2, RF, Rubella, and VZV. A clinically significant interference was observed with one sample of the twelve tested for HSV-1.

Interferences

The following substances were tested by adding the identified substances in known concentrations to serum samples at three TOXO IgG Avidity Index levels (Low, Equivocal and High), per recommendations of CLSI EP7-A2. None of the substances below were found to interfere with test results at the concentrations indicated.

Substance	Tested Concentration
Clindamycin	89.1 µmol/L (45 µg/mL)
Pyrimethamine	60 µg/mL
Spiramycin	15.0 µg/mL
Sulfamethoxazole	1.58 mmol/L (400 µg/mL)
Sulfapyridine	1.20 mmol/L (300 µg/mL)
Sulfasalazine	754 umol/L (300 µg/mL)
Trimethoprim	138 µmol/L (40 µg/mL)
Trimethoprim/Sulfamethoxazole	1.58 mmol/L (400 µg/mL) and 138 µmol/L (40 µg/mL), respectively
Bilirubin	0 to 510 µmol/L
Hemoglobin	0 to 300 µmol/L (monomer)
Human serum albumin	0 to 5 g/dL
Lipids	0 to 30 mg/mL equivalent in triglycerides

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.

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INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	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
2015/02	14704D	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE TEST (30 tests) WARNINGS AND PRECAUTIONS
2015/06	14704E	Technical	CONTENT OF THE TEST (30 tests) INSTRUCTIONS FOR USE
2017/03	14704F	Technical	CONTENT OF THE TEST (30 tests)

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