| REF 41423 ZENIT ra | **ZENIT RA t-TG IgG** | A.MENARINI diagnostics |
| **INSTRUCTIONS FOR USE** | **IVD**   +8°C +2°C   Σ **50** | C E |

INTENDED USE

The *ZENIT RA t-TG IgG* test is a chemiluminescent immunoassay (CLIA) for use on the dedicated *ZENIT RA Analyzer* for quantitative determination of the specific IgG antibodies directed against tissue transglutaminase in samples of human serum or plasma (EDTA, heparin).

This assay method is employed as a supplementary diagnostic technique in evaluation of gluten enteropathy (celiac disease - CD) and dermatitis herpetiformis (Durhing's disease).

CAUTION: Medical decisions cannot be based solely on the results of this test but must take into account all the available clinical and laboratory data.

CLINICAL SIGNIFICANCE

Celiac disease (CD) or gluten intolerance is an autoimmune disorder that occurs in genetically predisposed subjects and is triggered by a diet rich in cereals such as wheat, barley, and rye.[1]

Genetic predisposition to CD is linked principally to several HLA system genes; in particular to genotypes DQ2 and DQ8, which are present in 95-98% of celiac patients and occur in 20-30% of the general population.[2,3] The prevalence of CD in the Caucasian population is 1:100 ca; that is, one person in 30 who carries the alleles coded by the HLA DQ2/DQ8 genes develops CD.[4,5]

Gliadin is a gluten protein that can trigger the autoimmune process; contact between the gliadin peptides and the cells of the immune system of the intestinal submucosa can occur following alteration of intestinal permeability induced by zonulin secreted by enterocytes. Gliadin is an excellent substrate for the tissue transglutaminase (t-TG) enzyme; deamidation and transamidation of the gliadin peptides by t-TG modify the overall charge of the molecule, permitting it to bind with the HLA DQ2-DQ8 antigens expressed by the cells presenting the antigen to form a HLA DQ2-DQ8 / deamidated peptide / t-TG complex. This complex is recognized by the CD4[6] T lymphocytes, which trigger the immune process with activation of the effector T cells, production of cytokines, proliferation of B lymphocytes, and synthesis of anti-t-TG antibodies[7] and antibodies against gliadin peptides.[8,9] The net result is an inflammatory process presenting various histologies that can escalate to (reversible) lesions of the intestinal mucosa such as villous atrophy.

Serological testing plays a crucial role in diagnosis of CD and in monitoring compliance to treatment characterized by a gluten-free dietary regime. As recommended by international guidelines, the first-line

approach to diagnosis of CD is testing for anti-t-TG IgA autoantibodies associated with the test for total IgA; this approach is suggested because the risk that subjects with an absolute IgA deficiency (IgA ≤ 5 mg/dl)[10] will develop CD is 10 times greater than in the normal population.[11]

The high sensitivity and specificity of the anti-transglutaminase IgA autoantibody assays, respectively 96-98% and 93-95%,[12] associated with the objectivity and full automation offered by the test, have meant that in recent years anti-t-TG IgA testing has superseded older serological tests for CD.[13] Testing for anti-endomysial IgA antibodies (EMA) nevertheless plays an important role in confirming positivity in all sera that test anti-t-TG IgA positive, due to the high specificity (99-100%) of the EMAs, even though significant interpretative issues remain. In selective IgA deficiencies, the anti-t-TG IgG test must be associated with testing for IgG antibodies against deamidated gliadin peptides (a-DGP).

It has recently been demonstrated that celiac subjects synthesize specific antibodies against certain deamidated gliadin peptides. The anti-DGP antibodies are highly specific markers for identifying gluten-intolerant subjects, differently from the anti-gliadin antibodies in toto, which are also found in healthy subjects or in subjects affected by other enteric disorders and are therefore less specific.[14]

The anti-DGP IgA antibody tests show a sensitivity of 86-95% and a specificity of 91-95%, while the IgG antibody tests show 84-98% sensitivity and 95-98% specificity; these performance figures suggest use in pediatric subjects,[15] in whom synthesis of the latter antibodies would seem to precede synthesis of anti-transglutaminase IgA antibodies. Use of the test for anti-DGP IgA and IgG antibodies is also recommended in all subjects, regardless of age, showing symptoms suggestive of CD and in whom the t-TG or EMA autoantibodies are absent or present only at low titers.[16]

Celiac patients on gluten-free diets show a progressive reduction of anti-t-TG and anti-gliadin antibodies. The IgG antibody titer decreases more slowly than does the IgA antibody count.

PRINCIPLE OF THE METHOD

The *ZENIT RA t-TG IgG* kit for determination of the specific anti-t-TG IgG antibodies employs an indirect, two-step immunoassay method based on the principle of chemiluminescence.

The specific antigen is used to coat magnetic particles (solid phase) and a human anti-IgG antibody is labeled with an acridine ester derivative (conjugate).

During the first incubation, the specific antibodies present in the sample, in the calibrators, or in the controls bind with the solid phase.

During the second incubation, the conjugate reacts with the anti-t-TG IgG antibodies captured on the solid phase.

After each incubation, the material that has not bonded with the solid phase is removed by suction and repeated washing.

The quantity of marked conjugate bonded to the solid phase is evaluated by chemiluminescent reaction and measurement of the light signal. The generated signal, measured in RLU (Relative Light Units), is indicative of the concentration of the specific antibodies present in the sample, in the calibrators, and in the controls.

AUTOMATION

The *ZENIT RA Analyzer* automatically performs all the operations called for by the assay protocol: addition of the samples, calibrators, controls, magnetic particles, conjugate, and chemiluminescence activator solutions to the reaction vessel; magnetic separation and washing of the particles; measurement of the emitted light.

The system calculates the assay results for the samples and the controls using the stored calibration curves and prints a report containing all the assay- and patient-related information.

MATERIALS AND REAGENTS

Materials and Reagents Provided

| REAG | 1 | MP | | 2.5 ml |

Magnetic particles coated with t-TG antigen in phosphate buffer containing stabilizing proteins, detergent, and Pro-Clin 300 and sodium azide (< 0.1%) as preservatives.

| REAG | 2 | CONJ | | 15 ml |

Mouse anti-human IgG monoclonal antibodies labeled with an acridine ester derivative (conjugate), in phosphate buffer containing stabilizer proteins and sodium azide (< 0.1%) as preservative.

| REAG | 3 | DIL | | 25 ml |

Sample Dilution Solution: phosphate buffer containing bovine serum albumin, detergent, inert blue dye, and Pro-Clin 300 and Gentamicin SO_4 as preservatives.

| REAG | 4 | CAL A | | 1.6 ml |

Human serum with low concentration of anti-t-TG IgG antibodies in phosphate buffer containing bovine serum albumin, detergent, inert blue dye, and Pro-Clin 300 and Gentamicin SO_4 as preservatives.

| REAG | 5 | CAL B | | 1.6 ml |

Human serum with high concentration of anti-t-TG IgG antibodies in phosphate buffer containing bovine serum albumin, detergent, inert blue dye, and Pro-Clin 300 and Gentamicin SO_4 as preservatives.

All the reagents are ready to use.

Reagents 1, 2, and 3 are assembled into a single reagents cartridge unit.

The concentrations of the calibrators are expressed in AU/ml (Arbitrary Unit) and calibrated against an internal reference standard. The concentration values are specific by product lot and are recorded on the DATA DISK included in the kit.

DATA DISK

A mini-DVD containing information about all the ZENIT RA products (Reagents, Calibrators, Control Sera, Auxiliary Reagents) updated to the latest production lot and excluding products expired at the date of writing of each new DATA DISK.

The only Data Disk needed to ensure that the information needed for correct system operation is always updated is that with the highest lot number.

Materials and Reagents Required but not Provided in the Kit

- ZENIT RA Analyzer   Code No. 41400

- ZENIT RA Cuvettes Cube   Code No. 41402
  Box of 960 cuvettes.

- ZENIT RA System Liquid   Code No. 41409
  1 – 0.5-liter bottle of 10x solution.

- ZENIT RA Wash Solution   Code No. 41407
  1 – 0.5-liter bottle of 20x solution.

- ZENIT RA Trigger Set   Code No. 41403
  1 – 250-ml vial of Trigger A (pre-activation solution)
  1 – 250-ml vial of Trigger B (activation solution)

- ZENIT RA D-SORB Solution   Code No. 41436
  Box containing 2 – 1-liter bottles of ready-to-use solution.

- ZENIT RA Cartridge Checking System   Code No. 41401

- ZENIT RA Top Cap Set   Code No. 41566
  300 top caps for capping the calibrator containers after first use.

Other Recommended Reagents

ZENIT RA t-TG IgG CONTROL SET   Code No. 41455

3 – 1.5 ml vials of human serum negative for anti-t-TG antibodies and 3 – 1.5 ml vials of human serum negative for anti-t-TG antibodies.

WARNINGS AND PRECAUTIONS

The reagents provided in the *ZENIT RA t-TG IgG* kit are intended for *in vitro* diagnostic use only and not for *in vivo* use in humans or animals.

This product must be used by professional users only and in strict accordance with the instructions set out in this document.

Menarini may not be held responsible for any loss or damage in any way resulting from or related to use of the product in manners not compliant with the instructions provided.

Safety Precautions

This product contains material of animal origin and therefore must be handled as though it contained infectious agents.

This product contains components of human origin. All the serum or plasma units used in the manufacture of the reagents in this kit have been tested by FDA-approved methods and have been found to be non-reactive for HBsAg and anti-HCV, anti-HIV1, and anti-HIV2 antibodies.

Nevertheless, since no analysis method can offer complete assurance of the total absence of pathogenic agents, all material of human origin should be considered potentially infected/infectious and handled as such.

If the packaging is damaged in such a way as to cause leakage of the reagents, decontaminate the affected area with a dilute sodium hypochlorite (bleach) solution while wearing appropriate personal protective equipment (lab coat, gloves, goggles).

Dispose of used cleaning materials and the packaging materials affected by the leakage in accordance with national-level regulations for disposal of potentially infected/infectious waste.

Some of the reagents contain sodium azide as preservative. Since sodium azide may react with lead, copper, or brass in plumbing to form explosive azide compounds, do not flush reagents or waste to sewer. Dispose of such waste in accordance with national-level regulations for disposal of potentially hazardous substances.

Operating Precautions

In order to obtain reliable results, follow these instructions for use and the instructions provided in the analyzer operator's manual carefully.

The reagents supplied in the kit are intended for use only with the *ZENIT RA Analyzer* system.

The reagents cartridge components cannot be removed from the cartridge and reassembled.

Do not use the kit after the expiry date.

REAGENT PREPARATION

The reagents supplied in the kit are ready to use.

REAGENT STORAGE AND STABILITY

Store the reagents supplied in the kit in an upright position, at 2-8 °C, in a dark place.

In these conditions, the reagents cartridges and the unopened calibrators reagents are stable until the expiry date.

After opening, the reagents cartridges may be used for 60 days if stored refrigerated at 2-8 °C or onboard the machine.

After opening, the calibrators may be used for 60 days if stored refrigerated at 2-8 °C or if the on-board use time does not exceed 6 hours per session.

Do not freeze the reagents and/or the calibrators.

SAMPLE PREPARATION AND STORAGE

The assay must be performed on samples of human serum or plasma (EDTA - heparin).

Use of lipemic, hemolyzed, or turbid samples is not recommended.

If the assay is performed more than 8 hours after the blood sample is drawn, after separating the serum from the coagulate or the plasma from the red blood cells transfer the surnatant from the gel separation tubes to secondary tubes with no additives, for storage.

Prior to analysis, the samples may be stored refrigerated at 2-8 °C for a maximum of 7 days.

If the samples must be stored for more than 7 days before testing, store frozen at < -20 °C.

Avoid repeated freezing and thawing.

ASSAY PROCEDURE

In order to obtain reliable analysis results, follow the instructions provided in the analyzer operator's manual carefully.

Loading the Reagents

All the reagents supplied in the kit are ready to use.

Before installing the reagents cartridge on the system, agitate the magnetic particles container by rotating horizontally, in order to ensure correct particle suspension. Do not allow the suspension to foam during agitation.

Position the reagents cartridge in the reagents area of the analyzer, using the guide for that purpose, and allow to agitate for at least 30 minutes prior to use.

The identification bar code is automatically read when the reagents cartridge is positioned on the analyzer. If the cartridge label is damaged or if for any other reason reading is not performed, the cartridge identification data may be entered manually.

The analyzer automatically performs continual agitation of the magnetic particles.

If the reagents cartridge is removed from the analyzer, store in an upright position, at 2-8 °C, in a dark place.

Loading the Calibrators and Controls

The ZENIT RA calibrators and controls are ready to use. Allow the calibrators and controls to stand at room temperature for 10 minutes before use. Agitate the contents gently, by hand or vortex; do not allow to foam. Do not upend the container and do not remove the seal cap with perforator (yellow cap for calibrators; green or blue caps for controls).

When using a calibrator or control for the first time, press the perforator cap down until it stops. This operation perforates the container seal membrane to permit accessing the liquid contents. If the perforator cap is used correctly, red strip at the top of the label will be covered (See Fig. 1 – Sealed Container and Perforated Container).

Previously-used calibrator and/or control containers will be capped with a top cap (white cap) and the red label strip will be covered.

Load only perforated containers from which the top cap (white cap) has been removed and on which the red strip is covered (Fig. 1 – Perforated Container) onto the analyzer.

Read the barcode and insert the calibrators or controls into the samples area of the analyzer. The barcode data may be entered manually if the label is damaged or if for any other reason reading is not performed.

The concentration values of the anti-t-TG IgG antibodies contained in the calibrators and the controls are stored on the DATA DISK and are automatically transferred to the analyzer. The data may be entered manually If for any reason data transfer is not successful.

At the end of each session, reseal the calibrator and control containers with the appropriate top caps (white caps) and transfer to storage at 2-8 °C until next use (See Fig. 1 – Capped Container).

The calibrators are each sufficient for four assays.

Figure 1: Container Layout

Sealed Container	Perforated Container	Capped Container
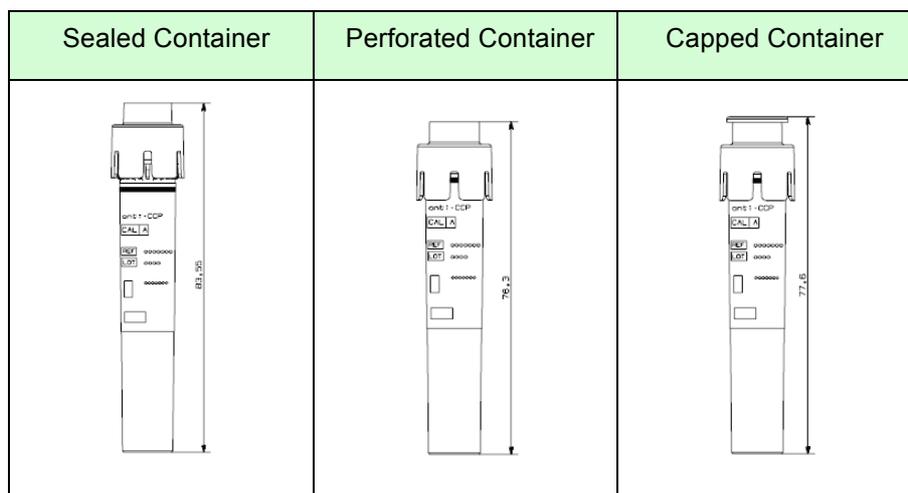
Loading the Samples

Identify the samples via the barcode reader and insert them in the appropriate container on the analyzer. If a sample barcode is missing or illegible or for any other reason not read, the sample identification data may be entered manually.

Select the analysis parameters for each sample.

Calibration

The *ZENIT RA Analyzer* uses a calibration curve (master curve) that is generated by the manufacturer for each lot of reagents cartridges.

The master curve parameters, as well as the calibrator concentration values, are stored on the DATA DISK and transferred to the analyzer database.

Calibrators A and B are used for recalibrating the master curve for the instrument used and for the reagents installed onboard.

To recalibrate, analyze three replicates of the two calibrators (A and B) and one replicate of each control. The concentration values obtained with the controls permit validating the new calibration.

Once a master curve recalibration has been accepted and stored in memory, all the successive sample can be analyzed with no further calibration being required, exception made for the cases listed below:

- when a reagents cartridge with a new lot number is installed on the analyzer;
- when the control values do not fall within the acceptability interval;
- after analyzer maintenance;
- after expiry of the period of validity of the recalibrated master curve.

A master curve recalibrated for the *ZENIT RA t-TG IgG* kit has a period of validity of 15 days.

Recalibration management is handled automatically by the analyzer.

Assay

 Press the start button.

1. The system draws 100 µl of sample dilution solution, 20 µl of magnetic particles; 100 µl of sample dilution solution, 6 µl of sample or control; for the calibrators, the positive serum is supplied prediluted with the sample dilution solution and the volume drawn is 106 µl. The solutions and suspension are dispensed into the reaction cuvette.

2. The reaction cuvette is incubated on the rotor at 37°C for 10 minutes.

3. At the end of this incubation phase, the magnetic particles are separated and washed.

4. 200 µl of conjugate are dispensed into the cuvette.

5. The reaction cuvette is incubated on the rotor at 37°C for 10 minutes.

6. At the end of this last incubation phase, the magnetic particles are separated and washed and the cuvette is transferred to the reading chamber.

7. The quantity of conjugate bound to the solid phase, expressed in RLU (Relative Light Units), is directly proportional to the concentration of anti-t-TG IgG in the sample.

8. The results are interpolated on the calibration curve and expressed in concentrations.

If the concentration value of a sample exceeds the upper limit of the measurable interval, the sample may be diluted and retested. The new value thus obtained is multiplied by the dilution factor used to obtain the final result.

QUALITY CONTROL

In order to ensure the validity of the assay, control sera at different concentrations (at least one negative serum and one positive serum) must be tested every day on which samples are assayed.

If individual laboratory practice so dictates, more frequent or more numerous controls may be performed for verification of assay results. Follow local quality control procedures.

If the ZENIT RA control sera are used, the expected mean values and the acceptability limits are those reported in the DATA DISK supplied with the controls.

Should different control sera be used, the expected values must be defined with the ZENIT RA reagents and analysis system before the products are used.

Should the values obtained with the controls not fall within the specified acceptability range, the relative assay results cannot be considered valid and it will be necessary to retest the respective samples.

In this case, recalibrate before repeating the assay/s in question.

CALCULATION AND INTERPRETATION OF RESULTS

Calculation of Results

The system automatically calculates the concentration of the anti-t-TG IgG antibodies in the tested sample. The values may be displayed on video or may be printed.

The concentrations are expressed in AU/ml.

Calculation of the analyte concentration in a sample proceeds by reading of the result obtained for each sample on a calibration curve in accordance with a 4-parameter logistic fitting model (4PL, weighted Y), which is corrected periodically on the basis of the calibrator assay results.

For detailed information on how the system calculates the results, refer to the system operator's manual.

Interpretation of Results

The measurability range for the *ZENIT RA t-TG IgG* assay is: 0.0 – 200 AU/ml.

Values less than 0.0 AU/ml are extrapolated values and may be reported as "equal to 0.0 AU/ml."

Values in excess of 200 AU/ml may be reported as "greater than 200 AU/ml" or the sample may be retested following appropriate dilution.

The results for a sample may be interpreted as set forth below:

(AU/ml)	Interpretation
< 10	The sample should be considered Negative for the presence of anti-t-TG IgG
≥ 10	The sample should be considered Positive for the presence of anti-t-TG IgG

The values reported above are suggested values only. Each laboratory will establish its own reference intervals.

LIMITS TO THE ASSAY METHOD

For diagnostic purposes, the results obtained with the *ZENIT RA t-TG IgG* kit and the *ZENIT RA Analyzer* system should always be used in conjunction with the other clinical and laboratory data available to the case physician.

Bacterial contamination of the samples and inactivation by heat may influence the results of the assay.

The heterophilic antibodies present in the human serum samples may react with immunoglobulin-based reagents, causing interference with in vitro immunoassays. Such samples may yield anomalous values when analyzed with the *ZENIT RA t-TG IgG* kit.

EXPECTED VALUES

Samples from 100 randomly-selected donors were analyzed to check for the presence of anti-t-TG IgG antibodies.

Of these samples, 4 tested positive and 96 negative; the mean value was 2.3 AU/ml and the standard deviation was 1.90 AU/ml.

The results thus obtained were used to calculate the "Limit of Blank" (LoB = the highest value that may be expected in a series of samples that do not contain the analyte). The Limit of Blank corresponding to the 95th percentile of the negative population was 6.4 AU/ml with reagents lot no. 1.

CLINICAL SENSITIVITY AND SPECIFICITY

A total of 62 samples from newly-diagnosed histologically-confirmed celiac patients and 104 non-celiac samples (60 donors, 30 patients affected by inflammatory and functional intestinal disorders and 14 patients with IgA deficiency) were tested using the *ZENIT RA t-TG IgG*. All the celiac samples and the samples from the 60 donors and the 30 patients affected by inflammatory and functional intestinal disorders showed total IgA concentrations within the normal range when tested by immunonephelometry. The samples from patients with IgA deficiency showed total IgA concentrations of less than 5 mg/dl when tested by immunonephelometry.

In the presumably negative (non-celiac) study population, 4 samples (3 from the intestinal disorders group and 1 from the IgA deficiency group) tested positive and 100 samples tested negative:

- **Diagnostic Specificity: 96.2%.**

In the presumably positive (celiac) study population, 25 17 samples gave negative results and 37 samples gave positive results:

- **Diagnostic Sensitivity: 59.7%**

Based on the diagnostic specificity and sensitivity results, **diagnostic concordance is 82.5%**.

Additionally, 8 samples from newly-diagnosed celiac patients with IgA deficiency all tested positive for anti-gliadin IgG antibodies.

PERFORMANCE

Caution: The data presented are not representative of kit operating specifications but constitute experimental evidence of how kit performance is aligned with the manufacturer's stated specifications.

Precision and Reproducibility

The precision and reproducibility of the *ZENIT RA t-TG IgG* kit assays were assessed using a protocol based on the guidelines provided by Clinical and Laboratory Standards Institute (CLSI) document EP5-A2.

Precision was calculated by analyzing the results for 20 replicates of five sera (one negative and four positive at different anti-t-TG IgG concentrations) run with two different reagent lots during the same experimental session.

The anti-t-TG IgG concentration found in the negative serum (LOB2) fell in the intervals from 1.5 to 3.5 AU/ml and 2.6 to 4.1 AU/ml when tested with reagents lots no. 1 and no. 2, respectively.

The results obtained with the 4 positive sera are reported in the table below.

Sample	Reagents Lot No.	Average Concentration (AU/ml)	SD	CV %
P1	1	10.0	0.60	6.0
	2	10.5	0.53	5.0
P2	1	15.2	0.91	6.0
	2	15.8	0.86	5.4
P3	1	33.5	2.17	6.5
	2	32.5	2.36	7.3
P4	1	46.0	3.03	6.6
	2	43.9	3.64	8.3

Reproducibility was calculated by analyzing the results for five sera (one negative and four positive at different anti-t-TG IgG concentrations) assayed in single replicates, with two different reagent lots, in 15 different sessions.

The anti-t-TG IgG concentration found in the negative serum fell in the intervals from 0.7 to 1.3 AU/ml and 0.1 to 0.7 AU/ml when tested with reagents lots no. 1 and no. 2, respectively.

The results obtained with the 4 positive sera are reported in the table below.

Sample	Reagents Lot No.	Average Concentration (AU/ml)	SD	CV %
P1	1	11.8	0.48	4.1
	2	10.7	0.52	4.9
P2	1	17.0	0.63	3.7
	2	16.4	0.84	5.1
P3	1	34.4	1.95	5.7
	2	34.1	1.88	5.5
P4	1	46.6	2.80	6.0
	2	46.9	2.59	5.5

Linearity of Dilution

The assays performed with the *ZENIT RA t-TG IgG* kit may underestimate the anti-t-TG IgG antibody content in the case of samples containing high concentrations of anti-t-TG IgA due to competition on the solid phase. In order to obtain accurate results for ant-t-TG IgG concentrations in these samples, the samples must be appropriately diluted before testing. The linearity *ZENIT RA t-TG IgG* kit dilutions is satisfactory when the sample contains a prevalence of specific IgGs (e.g., samples from patients with IgA deficiency).

It must in any case be noted that not all sera, when measured at different dilutions, can give results within the measurability interval, since the result is dependent not only on concentration but also on the affinity of the antibodies in the sample.

Analytical Sensitivity

The analytical sensitivity of the *ZENIT RA t-TG IgG* kit, expressed as the **Limit of Detection *(LoD:*** the smallest quantity of analyte that can be measured by the method), was assessed using a protocol based on the guidelines provided by Clinical and Laboratory Standards Institute (CLSI) document EP17-A and the formula: $LoD = LoB + C_\beta \, SD_s$ (where LoB is the value of the Limit of Blank, SD_s is the estimated standard deviation of the low-concentration sample distribution, and C_β is derived from the 95th percentile of the standard normal [Gaussian] distribution).

Four (4) samples at low analyte concentration were assayed in single replicates, using two different reagents lots, in 15 different experiments.

The resulting Limit of Detection of the *ZENIT RA t-TG IgG* kit was 8.3 AU/ml.

The Limit of Detection values, clinical considerations, and the results of comparison with reference methods contributed to definition of the cutoff value.

Analytical Specificity: Interferences

A study based on the guidelines provided by CLSI document EP7-A2 demonstrated that assay performance is not influenced by inclusion in the sample of the potentially interfering substances listed below, at concentrations up to those tested.

Potentially Interfering Substances	Maximum Concentration Tested
Free Bilirubin	20 mg/dl
Conjugated Bilirubin	28 mg/dl
Hemoglobin	1000 mg/dl
Fatty acids	3000 mg/dl

Use of lipemic, hemolyzed, or turbid samples is not recommended.

Analytical Specificity: Cross-reactivity

A study of 21 samples, all with high levels of other autoantibodies and negative for anti-t-TG IgG, was conducted to evaluate potential cross-reactions with the antigen used for sensitizing the magnetic particles.

The samples were so divided: SS-A (2), SS-B (3), U1-snRNP (1), Jo-1 (2), Scl-70 (3), dsDNA (3), Cenp B (1), histone (2), nucleolar (1), MPO (1), PR3 (1), and CCP (1).

The study revealed no significant cross-reactions between the solid-phase antigen and the other autoantibodies.

High-dose Hook Effect

Some methods for immunoassay of samples containing extremely high concentrations of analyte may provide apparent analyte levels that underestimate actual content (high-dose saturation or hook effect).

The dual-incubation method employed by the *ZENIT RA t-TG IgG*, kit is not influenced by this effect.

A sample containing an extremely high concentration (above the top limit of the measurement interval) of anti-t-TG IgG has confirmed the absence of the hook effect up to a concentration of 2200 AU/ml.

Relative Sensitivity and Specificity

method and a commercially-available ELISA method.

The samples for analysis were taken from newly-diagnosed celiac patients, celiac patients on a dietary regime, celiac and non-celiac patients with IgA deficiency, and normal patients.

The ZENIT RA assay and the commercially-available assay gave discordant results for 18 samples.

Relative concordance was therefore 87.5% (126/144).

Relative sensitivity was shown to be 78.8% (26/33).

Of the 7 discordant sensitivity samples, 5 were from the newly-diagnosed celiac patients group (all with high concentrations of anti-t-TG IgA), 1 (one) was from the group of celiac patients on dietary regime and 1 (one) was from the group of non-celiac patients with IgA deficiency.

Relative specificity was shown to be 90.1% (100/111).

Of the 11 discordant samples, 4 were from the newly-diagnosed celiac patients group, 6 from the group of celiac patients on dietary regime, and 1 (one) from the group of non-celiac patients with IgA deficiency.

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