REF 43735	ZENIT RA GBM	Distributed by
INSTRUCTIONS FOR USE	IVD +2°C +8°C Σ 50	CE

INTENDED USE

The *ZENIT RA GBM* test is a chemiluminescent immunoassay (CLIA) for use on the dedicated *ZENIT RA Analyzer* for quantitative determination of the specific IgG antibodies directed against the Glomerular Basement Membrane (GBM) in samples of human serum or plasma (EDTA, sodium citrate).
This assay method is employed as a supplementary diagnostic technique in evaluation of Goodpasture's syndrome and for differential diagnosis of vasculitides.
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

The anti-glomerular basement membrane (anti-GBM) antibodies are the serologic markers for a rare autoimmune disease presenting clinically with rapidly progressive glomerulonephritis and, histologically, with extra-capillary necrotizing glomerulonephritis with a linear immunofluorescence profile (anti-GBM antibody-induced glomerulonephritis). When the lungs are also involved (hemorrhagic alveolitis), the disease takes the name Goodpasture's syndrome (GP).[1]
The pathogenic role of the antibodies has been determined with certainty; the tissue damage is mediated by bonding of the anti-GBM antibodies to the glomerular (and alveolar) basement membrane.[2]
The target auto-antigen has been identified in the non-collagenous globular domain (NC1) on the α3 chain of Type IV collagen, which is present only in the basement membranes of the kidneys, lungs, cochlea, and eye.[3]
Goodpasture's syndrome is a very severe disease which, if not quickly and adequately treated, can often take a very rapid course.[4] Despite progress in treatment, the survival of the patient and the organ still depend heavily on the degree of kidney damage with which the patient presents; for this reason, early diagnosis is essential for patient survival and recovery of renal function.
Diagnosis of anti-GBM antibody disease or GP is based on detection, via direct immunofluorescence assay of biopsied kidney tissue, of linear deposits of immunoglobulins on the glomerular basement membrane. Since in many cases a kidney biopsy cannot be performed or must be postponed, serologic diagnosis assumes fundamental importance. Circulating anti-GBMs can be detected in primate kidneys by indirect immunofluorescence assay, although the method presents a high specificity but inadequate sensitivity.[5]
Quantitative, antigen-specific immunometric methods, based on ELISA, fluoroimmunoenzymatic (FLEA), and chemilumenescence (CLIA) assay methods, which make use of whole solubilized GBM, the α3(IV) collagen

chain, and, more recently, the GP antigen in human recombinant form, are now available.[6] The diagnostic sensitivity of the antigen-specific tests is very high, between 94.7% and 100%, and the specificity toward pathological controls varies between 90.9% and 100%.[6] Very recent data confirm the fact that despite the excellent diagnostic performance of these methods, circulating antibodies are not detectable in 5% ca. of patients affected with anti-GBM antibody induced diseases / Goodpasture's syndrome.[7]

Given its good clinical significance and high predictive values, anti-GBM antibody assay is indicated for diagnosis of patients whose clinical profiles reveal kidney failure of unknown origin with microscopic hematuria, especially in rapidly progressive cases.

The titer of circulating anti-GBM antibodies is of utility in determining the prognosis.[8]

Anti-GBM antibodies can be detected in about one-third of patients with a kidney-lung syndrome.

The anti-GBM antibodies are directly responsible for organ damage; monitoring these antibodies is therefore considered very useful for guiding treatment in general and plasmapheresis in particular. Persisting negativity for anti-GBM antibodies is a indispensible in prospective kidney transplant patients, since the absence of anti-GBM antibodies reduces the risk that the disease may re-present in the implanted organ.

Since ANCA-associated systemic vasculitides can present with a clinical picture of rapidly progressive glomerulonephritis, it is worthwhile running ANCA assays at the same time as anti-GBM assays. It must be remembered that a significant percentage of patients showing anti-GBMs (10-38%) also show ANCAs, generally with specificity for myeloperoxidases (ANCA-MPO); the clinical significance of this association is not yet clear.[6,9,10] A GNRP presentation may sometimes be secondary to systemic connective tissue disease or infections.

Regarding the diagnostic utility of the laboratory datum, it is worthwhile noting that the positive and negative predictive values (PPV, NPV) depend, besides on the sensitivity and the specificity of the test, on the prevalence of the disease in the study population. An appropriate requirement (high pre-testing probability) permits obtaining a result with real clinical utility and significantly reduces the incidence of false positive results.

PRINCIPLE OF THE METHOD

The *ZENIT RA GBM* kit for quantitative determination of the specific anti-glomerular basement membrane (anti-GBM) IgG antibodies employs an indirect, two-step immunoassay method based on the principle of chemiluminescence.

The highly-purified NC1α3(IV) 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-GBM 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 the highly-purified NC1α3(IV) 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 monoclonal anti-human IgG antibody labeled with an acridine ester derivative (conjugate), in phosphate buffer containing stabilizing proteins and sodium azide (< 0.1%) as a preservative.

REAG	3	DIL	25 ml

Sample Dilution Solution: phosphate buffer containing bovine serum albumin, detergent, blue dye, and sodium azide (<0.1%) as a preservative.

REAG	4	CAL A	1.6 ml

Human serum with a low concentration of anti-GBM 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 a high concentration of anti-GBM 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 Units) 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), 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 Cuvette Cube * Code No. 41402
  Pack of 960 cuvettes.

- ZENIT RA System Liquid * Code No. 41409
  1 bottle containing 5 litres of ready-to-use solution.
- ZENIT RA Wash Solution * Code No. 41407
  1 bottle containing 10 litres of ready-to-use solution.

- ZENIT RA Trigger Set * Code No. 41403
  1 250 mL-bottle of Trigger A (pre-trigger solution)
  1 250 mL-bottle of Trigger B (trigger solution)

- ZENIT RA D-SORB Solution Code No. 41436
  Pack of 2 bottles containing 1 litre of ready-to-use solution.

- ZENIT RA Cartridge Checking System * Code No. 41401

- ZENIT RA Top Cap Set Code No. 41566
  300 red top caps to close the calibrator containers after first use.

(*) The ZENIT RA Analyzer and the accessories tagged with an asterisk are manufactured by Immunodiagnostic Systems S.A., Rue E. Solvay, 101, B-4000 Liège, Belgium, and distributed by A. Menarini Diagnostics Srl.

Other Recommended Reagents

ZENIT RA ANCA/GBM CONTROL SET Code No. 41449

3 – 1.5 ml vials of human serum negative for anti-GBM antibodies and 3 – 1.5 ml vials of human serum positive for anti-GBM antibodies.

WARNINGS AND PRECAUTIONS

The reagents provided in the *ZENIT RA GBM* 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, 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 a 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, take care to follow these instructions for use, and the instructions provided in the analyzer operator's manual, to the letter.

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 unopened reagents cartridge and the calibrators are stable until the expiry date.
After opening, the reagents cartridge 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 – sodium citrate).
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 supernatant from the gel separation tubes to secondary tubes.
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, take care to follow the instructions provided in the analyzer operator's manual to the letter.

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 60 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

The ZENIT RA calibrators 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.

When using the calibrators for the first time, eliminate the seal and the white cap before inserting the calibrators in the analyzer.

Previously-used calibrator containers will be capped with a top cap (red cap) and the container will no longer have the seal. Remove the red top caps before inserting the calibrators in the analyzer.

Load the calibrators in the samples area of the analyzer; consult the analyzer use manual for identifying the calibrator placement positions on the instrument. 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-GBM IgG antibodies contained in the calibrators are stored on the DATA DISK and are automatically transferred to the analyzer.

At the end of each session, reseal the calibrator containers with the appropriate top caps (red caps) and transfer to storage at 2-8 °C until next use.

The calibrators are sufficient for up to four uses.

Loading the Controls

Load the controls in the samples area of the analyzer; consult the analyzer use manual for identifying the control placement positions on the instrument. The barcode data may be entered manually if the label is damaged or if for any other reason reading is not performed. If Zenit RA controls are used, consult the relative instructions for use. The concentration values of the anti-GBM IgG antibodies contained in the Zenit RA controls are stored on the DATA DISK and are automatically transferred to the analyzer. Select the analysis parameters for each control.

Loading the Samples

Insert the sample into the samples area of the analyzer; consult the analyzer use manual for identifying the sample placement positions on the instrument. 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 on the basis of the instrument used and 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 samples 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 GBM* kit has a period of validity of 21 days.

Recalibration management is handled automatically by the analyzer.

Assay

Press the start button.

1. The system draws 80 µl of sample dilution solution, 40 µl of magnetic particles, 100 µl of sample dilution solution, and 10 µl of sample or control, in that order (for the calibrators, the positive serum is supplied prediluted with the sample dilution solution and the volume drawn is 110 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-GBM 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 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 control tests 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 anti-GBM IgG antibodies in the tested samples. 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 calculated 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 GBM* assays is 0.0 – 1000 AU/ml.

Values less than 0.0 AU/ml are extrapolated values: the message/s "OMR-" and/or ORA is displayed and the values are reported as "equal to 0.0 AU/ml".

Values in excess of 1000 AU/ml are accompanied by the message "OMR+" and/or ORA; the sample may be retested following appropriate dilution.

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

(AU/ml)	Interpretation
< 40	The sample should be considered Negative for the presence of anti-GBM IgG
≥ 40	The sample should be considered Positive for the presence of anti-GBM 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 GBM* 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.

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 GBM* kit.
In some cases, samples from patients affected with chronic liver disorders, chronic infections, collagen disorders, and myeloma with hypergammaglobulinemia (IgG concentration greater than 1800 mg/dl) may give positive values for anti-GBM IgGs.

EXPECTED VALUES

Samples from 100 healthy patients were analyzed to check for the presence of anti-GBM IgG antibodies.
The results for all the samples analyzed were negative, with a mean value of 1.1 AU/ml and a standard deviation of 3.2 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 6.3 AU/ml with reagents lot no. 2.

CLINICAL SENSITIVITY AND SPECIFICITY

A total of 156 samples were tested with the *ZENIT RA GBM* kit. Of these, 30 samples were from patients affected with Goodpasture's syndrome (GP), 2 were from patients affected with rapidly progressive glomerulonephritis (GNRP), 95 were from patients affected with different pathologies (18 with systemic connective tissue disorders, 12 with ANCA-associated vasculitides, 15 with rheumatoid arthritis, 4 celiac patients, 38 with infectious diseases, 8 with various conditions), and 29 samples were from normal subjects.

None of the samples from the presumably negative study population (18 with systemic connective tissue disorders, 12 with ANCA-associated vasculitides, 15 with rheumatoid arthritis, 4 celiac patients, 38 with infectious diseases, 8 with various conditions, and the 29 samples from normal subjects) tested positive.

- **Diagnostic Specificity: 100 %** (confidence interval at 95%: 96.3 - 99.9%); on 124 samples, 124 samples tested negative.

All of the samples from the presumably positive study population (30 samples from patients affected with Goodpasture's syndrome (GP) and 2 samples from patients affected with rapidly progressive glomerulonephritis (GNRP)) tested positive.

- **Diagnostic Sensitivity: 100 %** (confidence interval at 95%: 86.7 - 99.7%); (32/32 samples).

Based on the diagnostic specificity and sensitivity results, **diagnostic concordance is 100 %** (confidence interval at 95%: 96.7 - 99.9%); (156/156 samples).

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

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

The concentration found for the anti-GBM IgG-negative serum (N4) fell in the interval from 0.0 to 0.4 AU/ml when tested with reagent lot no. 2 and to be 0.0 AU/ml when tested with reagent lot no. 3.

The results obtained with the 3 positive sera are reported in the table below.

Sample	Reagents Lot No.	Average Concentration (AU/ml)	SD (AU/ml)	CV %
P1	2	81.4	2.52	3.1
	3	64.7	2.07	3.2
P2	2	243.5	18.82	7.7
	3	270.9	11.88	4.4
P3	2	471.5	13.57	2.9
	3	371.9	12.06	3.2

Reproducibility was calculated by analyzing the results for six sera (one negative and five positive, at different anti-GBM IgG concentrations) assayed in single replicates in 14 different sessions, with a single reagent lot.

The concentration of the anti-GBM IgG-negative serum (GBM-N3) fell in the interval from 0.5 to 2.2 AU/ml.

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

Sample	Average Concentration (AU/ml)	SD (AU/ml)	CV %
GBM-P1	51.2	4.62	9.0
GBM-P2	205.0	15.06	7.3
GBM-P3	296.3	28.84	9.7
GBM-P4	91.6	7.16	7.8
GBM-P5	222.8	18.68	8.4

Linearity of Dilution

The linearity of the *ZENIT RA GBM* kit dilutions was evaluated by assaying scaled dilutions of 3 sera containing high concentrations of anti-GBM IgGs, diluted with the system liquid.

The results of this study are summarized in the table below.

Sample	Dilution Factor	Measured Concentration (AU/ml)	Expected Concentration (AU/ml)	Recovery %
1	1	338.0	--	(100)
	2	169.0	169.0	100.0
	4	83.2	84.5	98.5
	8	42.1	42.3	99.5
2	1	353.3	-	(100)
	2	173.0	176.7	97.9
	4	93.0	88.3	105.3
	8	42.7	44.2	96.6
3	1	223.6	-	(100)
	2	124.0	111.8	110.9
	4	67.4	55.9	121.0
	8	33.9	28.0	121.1

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.

Analytic Sensitivity

The analytic sensitivity of the *ZENIT RA GBM* kit, expressed as the ***Limit of Detection*** (***LoD:*** the smallest quantity of analyte that can be measured by the method), was calculated by the formula LoD = LoB + C_β 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).

Five (5) samples at low analyte concentration were assayed in single replicates, using one reagent lot, in 14 different experiments.

The resulting Limit of Detection of the *ZENIT RA GBM* kit was 13.5 AU/ml.

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

Analytic Specificity: Interferences

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	20 mg/dl
Hemoglobin	1000 mg/dl
Triglycerides	3000 mg/dl

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

Analytic Specificity: Cross-reactivity

A study of 49 samples, all with high levels of other autoantibodies and negative for anti-GBM IgGs, was conducted to evaluate potential cross-reactions with the antigen used for sensitizing the magnetic particles.
The samples were so subdivided: CCP and/or FR (15), Gliadin A and/or G and/or tTG-A (4), ANCA-positive (12), and ANA and/or ENA (18).
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 GBM* kit is not influenced by this effect.
A sample containing an extremely high concentration (above the top limit of the measurement interval) of anti-GBM IgG has confirmed the absence of the hook effect up to a concentration of 1552 AU/ml.

Relative Sensitivity and Specificity

The presence of anti-GBM IgG antibodies was determined, using the *ZENIT RA GBM* kit and a commercially-available ELISA method, in 156 samples: 30 samples from patients affected with Goodpasture's syndrome (GP), 2 samples from patients affected with rapidly progressive glomerulonephritis (GNRP), 95 samples from patients affected with different pathologies (18 with systemic connective tissue disorders, 12 with ANCA-associated vasculitides, 15 with rheumatoid arthritis, 4 celiac patients, 38 with infectious diseases, 8 with various conditions), and 29 samples from normal subjects.
The ZENIT RA assay and the commercially-available ELISA assay gave discordant results for 5 samples.
Relative concordance was therefore 96.8% (confidence interval at 95%: 92.3 - 98.8%) (151/156 samples).
Relative sensitivity was shown to be 90.9% (confidence interval at 95%: 74.5 - 97.6%) (30/33 samples).
Relative specificity was shown to be 98.4% (confidence interval at 95%: 93.7 - 98.7%) (121/123 samples).
The three samples that tested negative with the *ZENIT RA GBM* kit and positive with the ELISA kit were: one (1) from the infectious diseases group, 1 from the various conditions group, and 1 from the normal subjects group.
The two samples that tested positive with the *ZENIT RA GBM* kit and negative with the ELISA kit were both from the Goodpasture's syndrome sample group.

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