Snibe Diagnostic

CE REF 130217501M:100 tests/kit
130617501M: 50 tests/kit
130717501M: 30 tests/kit

MAGLUMI® Anti-CCP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of IgG antibodies to cyclic citrullinated peptides (Anti-CCP) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of individuals with suspected or confirmed Rheumatoid Arthritis (RA).

SUMMARY

Rheumatoid arthritis (RA) is a common, autoimmune disease of unknow etiology affecting approximately 0.5-1% of the population worldwide[1-3]. It is characterized by chronic inflammation of the synovial joints, which commonly leads to progressive joint destruction and consequent disability and reduction of quality of life[3]. Until recently, treatment for RA was limited, and severe joint damage and overall debility were common[2]. It is important to early, reliable diagnosis of RA in order to control the progression of the disease and avoid irreversible joint damage[4].

RA is diagnosed primarily according to clinical disease manifestations, and serologic support is primarily restricted to the determination of rheumatoid factor (RF). Increased levels of RF can be detected in 50%-80% of RA sera but are also encountered in the sera of patients with other connective tissue diseases, patients with infectious diseases, and elderly healthy individuals[5]. A very specific antibody for RA is antiperinuclear factor (APF) showed an acceptable sensitivity and compared to rheumatoid factor (RF) a much higher specificity[6]. Another group of RA specific antibodies, the so-called antikeratin antibodies (AKA) was described[6]. It has been documented that the modification to a citrulline containing protein is essential for the autoantigenicity of filaggrin and citrullinated filaggrin is the antigen targeted by APF and AKA[7]. Based on the knowledge that mature filaggrin is the target of the APF and AKA antibodies referred to as anti-filaggrin antibodies (AFA), synthetic citrulline-containing peptides were developed and tested for their reactivity with RA sera[3]. Using a citrulline-containing peptide which was derived from filaggrin sequences, antibodies could be detected in RA sera with moderate sensitivity and high specificity[3].

Antibodies against citrullinated proteins/peptides are the second serological marker (apart from RF) to have recently been included in the 2010 ACR/EULAR classification criteria for RA, which are focused on early diagnosis and therapy[8].

TEST PRINCIPLE

Indirect chemiluminescence immunoassay.

The prediluted sample, buffer, magnetic microbeads coated with synthetic CCP antigen mixed thoroughly and incubated to form immune-complexes. After incubation, materials bound to the magnetic microbeads are held in a magnetic field while unbound materials are washed away during a wash cycle. Then adding ABEI labeled with mouse monoclonal anti-human IgG antibody, incubated to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then another wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of anti-CCP antibodies present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with synthetic CCP antigen (~2.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of anti-CCP antibodies in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of anti-CCP antibodies in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	BSA, NaN_3 (<0.1%).	13.5 mL	8.0 mL	4.8 mL
ABEI Label	ABEI labeled with anti-human IgG monoclonal antibody (mouse) (~25.0 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	23.5 mL	13.0 mL	7.8 mL
Diluent	PBS buffer, NaN_3 (<0.1%).	25.0 mL	15.0 mL	8.0 mL
Control 1	A low concentration of anti-CCP antibodies (10.0 U/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of anti-CCP antibodies (100 U/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
All reagents are provided ready-to-use.				

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 18-25°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA or sodium heparin tubes

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Samples should be free of fibrin, red blood cells, or other particulate matter. Such samples may give reliable results and must be centrifuged prior to testing. Transfer clarified samples to a sample cup or secondary tube for testing. For centrifuged samples with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 10 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 18-25°C, or 3 days at 2-8°C, or 3 months frozen at -20°C or colder. Frozen specimens subjected to up to 3 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, anti-CCP concentrations above the analytical measuring interval, can be diluted with Diluent either automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:20. The concentration of the diluted sample must be >25 U/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

Anti-CCP (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X3, MAGLUMI X6, MAGLUMI X8, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the Snibe internal reference standard.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 7 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other

published guidelines[9].
Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Anti-CCP assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.
The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.
Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Anti-CCP (CLIA) Controls (REF: 160201404MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the anti-CCP concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in U/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

In an external study using the Snibe Anti-CCP assay on samples from 181 confirmed RA patients, 78 patients with other disease and 253 apparently healthy individuals, an optimal cut-off of 17.0 U/mL was determined.

- Samples with anti-CCP antibodies concentration <17.0 U/mL should be considered negative.
- Samples with anti-CCP antibodies concentration ≥17.0 U/mL should be considered positive.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the anti-CCP results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[10, 11]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[12].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (U/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (U/mL)	%CV	SD (U/mL)	%CV	SD (U/mL)	%CV
Serum Pool 1	5.137	0.225	4.38	0.106	2.06	0.297	5.78
Serum Pool 2	21.055	0.578	2.75	0.474	2.25	1.269	6.03
Serum Pool 3	201.137	5.778	2.87	1.258	0.63	8.420	4.19
Plasma Pool 1	4.873	0.205	4.21	0.108	2.22	0.290	5.95
Plasma Pool 2	21.399	0.551	2.57	0.422	1.97	0.780	4.24
Plasma Pool 3	200.366	5.591	2.79	2.657	1.33	7.747	3.87
Control 1	9.989	0.376	3.76	0.119	1.19	0.600	6.01
Control 2	101.123	3.370	3.33	1.435	1.42	4.577	4.53

Linear Range

1.00-500 U/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.850-10000 U/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.500 U/mL.
Limit of Detection (LoD) =0.850 U/mL.
Limit of Quantitation (LoQ) =1.00 U/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	25 mg/dL	Rheumatoid factor	150 IU/mL
Hemoglobin	500 mg/dL	HAMA	40 ng/mL
Intralipid	1500 mg/dL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
anti-dsDNA IgG	800 IU/mL	anti-SS-B IgG	400 AU/mL
anti-Sm/RNP IgG	400 AU/mL	anti-Scl-70 IgG	400 AU/mL
anti-Sm IgG	400 AU/mL	anti-Jo-1 IgG	400 AU/mL
anti-SS-A /Ro IgG	400 AU/mL	anti-Centromeres IgG	400 AU/mL

Clinical Sensitivity

The clinical sensitivity was determined for 152 confirmed Rheumatoid Arthritis specimens (RA patients were classified according to the ACR Criteria). The clinical sensitivity was calculated to be 70.4%. The following results were obtained:

Specimen Category	Anti-CCP (CLIA)		
	N	Positive	%Sensitivity
Confirmed RA	152	107	70.4

Clinical Specificity

The clinical specificity was determined for 327 non-RA specimens, consisting of 115 patients with other disease (Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Sjogren's Syndrome, Systemic Sclerosis, Polymyositis/Dermatomyositis, Primary Biliary Cirrhosis, Autoimmune Thyroiditis, Osteoarthritis, Reactive Arthritis, Renal failure, Epstein-Barr Virus) and 212 apparently healthy individuals. The clinical specificity was calculated to be 98.2%. The following results were obtained:

Specimen Category	Anti-CCP (CLIA)		
	N	Negative	%Specificity
Non-RA Disease	115	110	95.7
Apparently Healthy	212	211	99.5
Total	327	321	98.2

High-Dose Hook

No high-dose hook effect was seen for anti-CCP concentrations up to 10000 U/mL.

Method Comparison

A comparison of the Anti-CCP (CLIA) assay with a commercially available immunoassay, gave the following correlations (U/mL):
Number of samples measured: 108
Passing-Bablok: y=1.0277x-0.6479, r=0.960.
The clinical specimen concentrations were between 2.04 and 485.56 U/mL.

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE	CE marking		

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