Snibe Diagnostic

CE REF 130217503M:100 tests/kit
130617503M: 50 tests/kit
130717503M: 30 tests/kit

MAGLUMI® ANA Screen (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of IgG class antinuclear antibodies (ANA) 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 multiple systemic autoimmune diseases (Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's Syndrome (SS), Systemic Sclerosis (SSc), Polymyositis/Dermatomyositis (PM/DM), Primary Biliary Cirrhosis (PBC).).

SUMMARY

Antinuclear antibodies (ANA) are a diverse group of autoantibodies that recognize multiple intracellular antigens, classically consisting of nuclear specificities such as deoxyribonucleic acid or small nuclear ribonucleoproteins[1, 2]. It is currently accepted that ANA contain two major types of antibodies, the first group includes antibodies against DNA and histones and the second group includes autoantibodies to extractable nuclear antigens (ENA) [3]. Among the most important nuclear antigens are dsDNA, Histones, Rib-P, Sm/RNP, Sm, SS-A/Ro, SS-B, Scl-70, Jo-1, Centromeres and mitochondria M2 antigens[2, 4]. These nuclear particles recognized by ANA have essential intracellular functions such as replication and transcription, and thus are structurally conserved among species[5].

ANA are key biomarkers in the diagnosis of rheumatic diseases such as Systemic Lupus Erythematosus (SLE), Sjögren's Syndrome (SS), Systemic Sclerosis (SSc), Mixed Connective Tissue Disease (MCTD), Polymyositis/Dermatomyositis (PM/DM) and Primary Biliary Cirrhosis (PBC)[1, 6]. ANA can be detected in 90-95% of patients with SLE, 50-60% of patients with SS, 85-95% of patients with SSc, 90-100% of patients with MCTD, 50-60% of patients with PM/DM and 50-80% of patients with PBC[2]. The number of different ANA specificities is large and, whereas some antibodies are highly associated with particular diseases, others are expressed more widely among patients. The association between ANA and certain disease entities suggests that these antibodies could be useful biomarkers for screening and diagnosis and could provide insights for understanding disease mechanisms[5].

TEST PRINCIPLE

Indirect chemiluminescence immunoassay.

The prediluted sample, buffer, magnetic microbeads coated with nuclear antigens (dsDNA, Histones, Rib-P, Sm/RNP, Sm, SS-A/Ro, SS-B, Scl-70, Jo-1, Centromeres, mitochondria M2 antigens together with HEp-2 cell nuclear extract) 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 antinuclear antibodies present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Lyophilized Magnetic Microbeads	Magnetic microbeads coated with nuclear antigens (dsDNA, Histones, Rib-P, Sm/RNP, Sm, SS-A/Ro, SS-B, Scl-70, Jo-1, Centromeres, mitochondria M2 antigens together with HEp-2 cell nuclear extract) (~58.8 μg/bottle) in PBS buffer, NaN_3 (<0.1%).	1 bottle	1 bottle	1 bottle
Magnetic Microbeads Buffer	PBS buffer, NaN_3 (<0.1%).	2.8 mL	2.8 mL	2.8 mL
Calibrator Low	A low concentration of antinuclear antibodies in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of antinuclear 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 antinuclear antibodies (20.0 AU/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of antinuclear antibodies (100 AU/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Magnetic Microbeads is lyophilized and must be reconstituted with Magnetic Microbeads Buffer, refer to the Preparation of Magnetic Microbeads section.				

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 Lyophilized 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 reconstitution and mixing instructions, refer to the Preparation of Magnetic Microbeads and 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.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens 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 20 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 7 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, antinuclear antibodies 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 >20 AU/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

ANA Screen (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.

Preparation of Magnetic Microbeads

- ***The Magnetic Microbeads is provided in a lyophilized form. The vial containing the lyophilized magnetic microbeads must be opened carefully and reconstituted with the Magnetic Microbeads Buffer.***
- ***Remove 2 mL Magnetic Microbeads Buffer from the magnetic microbeads tube (blue collar and serrated reagent tube on the bottom) into the vial containing lyophilized magnetic microbeads before use, cover with a rubber stopper, and gently shake. Allow the dissolved magnetic microbeads to stand for 10-15 minutes.***
- ***Swirl gently to ensure homogeneity. Avoid heavy shaking when dissolving (avoid formation of foam).***
- ***Transfer all reconstituted magnetic microbeads in vial to the magnetic microbeads tube and mix it with the remaining Magnetic Microbeads Buffer evenly, then place prepared kit onto the analyzer.***
- ***After use, the kit including the reconstituted magnetic microbeads should be stored at 2-8°C in an upright position.***

- 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.
- Each time a new kit is used.

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[7].

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 ANA Screen 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 ANA Screen (CLIA) Controls (REF: 160201405MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the antinuclear antibodies 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 AU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The optimal cut-off of the ANA Screen assay was obtained by testing 195 confirmed systemic autoimmune diseases (such as Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Sjögren's Syndrome, Systemic Sclerosis, Polymyositis/Dermatomyositis and Rheumatoid Arthritis), 77 patients with other disease and 253 apparently healthy individuals.

- Samples with antinuclear antibodies concentration <40.0 AU/mL should be considered negative.
- Samples with antinuclear antibodies concentration ≥40.0 AU/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 antinuclear antibodies 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[8, 9]. 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[10].
- 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 (AU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
Serum Pool 1	9.989	0.442	4.42	0.145	1.45	0.565	5.66
Serum Pool 2	40.987	1.362	3.32	0.883	2.15	2.696	6.58
Serum Pool 3	202.452	4.731	2.34	3.925	1.94	7.908	3.91
Plasma Pool 1	10.196	0.439	4.31	0.168	1.65	0.584	5.73
Plasma Pool 2	40.365	1.477	3.66	0.475	1.18	1.830	4.75
Plasma Pool 3	199.924	5.892	2.95	2.853	1.43	10.496	5.25
Control 1	19.883	0.817	4.11	0.486	2.44	1.106	5.56
Control 2	99.976	3.657	3.66	1.563	1.56	4.658	4.66

Linear Range

1.00-400 AU/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.792-8000 AU/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 AU/mL.

Limit of Detection (LoD) =0.792 AU/mL.

Limit of Quantitation (LoQ) =1.00 AU/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	40 mg/dL	Rheumatoid factor	500 IU/mL
Hemoglobin	1000 mg/dL	HAMA	40 ng/mL
Intralipid	2000 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-CCP	500 U/mL	IAA	175 IU/mL
TRAb	300 IU/mL	GAD65	280 IU/mL
TGAb	280 IU/mL		

Clinical Sensitivity

The clinical sensitivity was determined for a panel of 161 systemic autoimmune diseases specimens. The clinical sensitivity was calculated to be 82.6%. The following results were obtained:

Specimen Category	ANA Screen (CLIA)		
	N	Positive	%Sensitivity
Systemic Lupus Erythematosus	48	43	89.6
Mixed Connective Tissue Disease	38	36	94.7
Sjögren's Syndrome	28	22	78.6
Systemic Sclerosis	27	21	77.8
Polymyositis/Dermatomyositis	12	3	25.0
Primary Biliary Cirrhosis	8	8	100
Total	161	133	82.6

Clinical Specificity

The clinical specificity was determined for 277 non-systemic autoimmune diseases specimens, consisting of 124 patients with other diseases (Celiac disease, Type 2 diabetes, Autoimmune Thyroiditis, Renal failure) and 153 apparently healthy individuals. The clinical specificity was calculated to be 96.8%. The following results were obtained:

Specimen Category	ANA Screen (CLIA)		
	N	Negative	%Specificity
Celiac disease	25	25	100
Type 2 diabetes	54	48	88.9
Autoimmune Thyroiditis	19	19	100
Renal failure	26	24	92.3
Apparently Healthy	153	152	99.3
Total	277	268	96.8

High-Dose Hook

No high-dose hook effect was seen for antinuclear antibodies concentrations up to 8000 AU/mL.

Method Comparison

A comparison of the ANA Screen assay with a commercially available immunoassay, gave the following correlations (AU/mL):

Number of samples measured: 106

Passing-Bablok: y=1.0253x-1.4056, τ=0.913.

The clinical specimen concentrations were between 11.9 and 397 AU/mL.

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SYMBOLS EXPLANATIONS

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

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