PROBLEM	POSSIBLE CAUSE	SOLUTION
3) Low ANA test results	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

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ANA SCREEN *i*Clia

Chemiluminescence microparticle immunoassay for the Qualitative Detection of Anti-nuclear antibodies in Human Serum/Plasma

1. INTRODUCTION

The antibodies that target "normal" proteins within the Anti-nuclear antibodies (ANA) are found in a variety of autoimmune and rheumatic diseases. dsDNA antibodyies is highly specific for SLE, with a positive rate of 40-90%. Anti-sm antibody is with about 5-30% positive rate for SLE. Anti-nucleosome antibody is commonly detected in SLE (50-100%) and autoimmune hepatitis (40-50%). Anti-nucleosome antibody can exist independently of anti-dsDNA antibody for which 18% of SLE patients' serum only react with nuc but not with dsDNA. Anti-histone antibody is more common (95% positive) in SLE induced by drugs such as procainamide, hydrazine or other drugs. Or it happens in 30-70% of disseminated lupus and 15-50% of patients with rheumatoid arthritis. Anti-ribosomal P0 can be detected in 10-20% of SLE patients and is found to be associated with complications of central nervous system, kidney or other organs. Anti-PCNA antibody has a high specificity but very low sensitivity for SLE, the positive rate is only 3%, which is rarely seen in other diseases.

Anti-SS-A antibody is associated with various autoimmune diseases, most commonly in Sjogren's syndrome (40-80%), systemic lupus erythematosus (30-40%), primary biliary cirrhosis (20%), rheumatoid arthritis (3-5%), and occasionally in chronic active hepatitis. It will 100%happen in newborns with lupus erythematosus. Anti-Ro52 antibody is found in serum of patients with various autoimmune diseases, such as Sjogren's syndrome, systemic lupus erythematosus, dermatomyositis, etc. Anti-SS-B antibody is almost exclusively found in women with Sjogren's syndrome (40-80%) and SLE (10-20%), with a male-to-female ratio of 1:29. Anti-SS-A and anti-SS-B are often present together in Sjogren's syndrome. Anti-Scl-70 is a specific marker in patients with scleroderma (specificity is 98-100%). In patients with Progressive Systemic Sclerosis (PSS), the positive detection rate is 40-60%; in patients with Systemic Sclerosis, the positive detection rate is 20-40%. The presence of anti-Scl-70 is often associated with diffuse skin involvement and pulmonary fibrosis. Anti-PM-Scl is mainly found in patients with polymyositis and dermatomyositis, with a positive rate of 50-70%, it can also be found in patients overlapping with scleroderma (a positive rate of 50-70%). Anti-Jo-1 is commonly found in 25-35% patients with polymyositis (PM), 25% in PM/DM and less than 10% in DM alone. High titer of anti-nRNP/Sm (mainly U1-RNP) is a marker of mixed connective tissue disease (MCTD), with a positive rate of 95-100%. Its titer is associated with disease activity. It can also be detected in some patients with SLE, always accompanied by anti-SM.

Anti-CENP-B is associated with progressive systemic sclerosis and primary biliary cirrhosis (PBC) (positive rates of 80-95%), and is particularly important for the diagnosis of CREST subtype in systemic scleroses (i.e., calcification, Raynaud's syndrome, esophageal motility disorders, stiff fingers, and telangiectasia). Anti-CENP-B is also detected in the serum of Raynold syndrome, tumors, and other rheumatic diseases, such as SLE, Sgregren's syndrome or rheumatoid arthritis. AMA-M2 is highly specific for primary biliary cirrhosis (PBC) and is detectable in approximately 90% of PBC patients, independent of disease activity. Low titer of AMA-M2 is seen in other chronic liver diseases (30%) and progressive systemic sclerosis (7-25%).

2. INTENDED USE

ANA Screen CLIA Kit is a chemiluminescent microparticle immunoassay intended for the in vitro qualitative measurement of anti- Sm, dsDNA, Nuc, His, P0, PCNA, SSA, Ro52, SSB/La, Scl-70, Jo-1, PM/SCl, CENP-B, nRNP/Sm, AMA-M2 antibodies in human serum/plasma as an aid in the diagnosis of Anti-nuclear antibodies related disease. This kit is only operational in connection with J.Mitra CLIA Analyzer.

3. PRINCIPLE

ANA Screen iCLIA is a chemiluminescent microparticle immunoassay based on the "indirect " Principle

In the first step, ANA antigen labeled magnetic microparticle, human serum/plasma and a assay buffer are mixed and incubated in an assay cup, which allows patient specific anti- Sm/dsDNA/Nuc/His/P0/PCNA/SSA/Ro52/ SSB/La/Scl-70/ Jo-1/PM/SCl/CENP-B/nRNP/Sm/AMA-M2 antibodies to bind to microparticle. After sample matrix is removed by washing, anti-human IgG conjugated acridinium ester (AE Conjugate) is added and combined, and the Microparticle-ANA antigen/ antibodies immune complex is kept with the help of a magnetic separator. Excess acridinium ester conjugate is removed by washing and finally the bound acridinium ester is detected by addition of chemiluminescent substrate. The relative light unit (RLU) intensity is proportional to the amount of anti-ANA IgG. According to the anti-ANA IgG RLU-concentration standard curve, the RLU tested can be interpreted to anti-ANA IgG concentration in the sample expressed as RU/mL.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in European Standard EN ISO 15223-1:2021.

Manufactured By	In vitro diagnostic medical device
No. of tests	Instruction for use
Lot Number Batch Number	Temperature Limitation
Manufacturing Date	Caution, see instruction for use
Expiry Date	Catalogue Number
Keep away from sunlight	Do not use if package is damaged
Contains biological Material of Human Origin	Contains biological Material of Animal Origin
Country of Manufacture	Keep Dry

5. KIT PRESENTATION

- 50 Tests
- 100 Tests

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic microparticles coated with ANA antigen with preservatives.
Sample Diluent (RB)	Buffer containing protein stabilizers and antimicrobial. agents as preservative.
Assay Buffer (RC)	Buffer containing protein stabilizers & antimicrobial agents as preservative.
AE Conjugate (RD)	Anti-human IgG linked to acridinium ester with protein stabilizers.
Calibrator-1 (C0)	Purified human-derived PM-Scl-IgG antibody in buffer.
Calibrator-2 (C1)	Purified human-derived PM-Scl-IgG antibody in buffer.
Control-1 (Q1)	Purified human-derived PM-Scl-IgG antibody in buffer.
Control-2 (Q2)	Purified human-derived PM-Scl-IgG antibody in buffer.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The kit should be stored at 2-8ºC in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8ºC.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **J. Mitra CLIA Analyzer**

All materials and analyzer to be used for running the ANA Screen iClia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.

3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer.
4. Specimens should be free of microbial contamination and may be stored at 2-8ºC for one week, or frozen at -20ºC or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

ANA Screen iClia test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. Centrifuge the sample at 10,000 rpm for 15 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8ºC.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microparticles that may have settled during transport or storage.
5 Once reagents are opened, reagent plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7 To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
8. Once a reagent plug has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
9. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent plugs) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent plugs placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run control-1 & control-2 in each assay to evaluate validity of the kit.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Avoid strong light exposure during the assay.
13. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia Analyzer user manual for detailed information on preparing the analyzer.
2. Before loading the ANA Screen iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticles buffer that may have settled during transporation/ storage. Once the microparticles have been loaded, no further mixing is required.

   Important Note: Swirl the microparticle (RA) bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. *Once the microspheres have been resuspended, remove the cap and place the the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB) and (RD) bottles and place the reagent plugs before use as follow:*

   (RA) & (RB) : Natural color plug

   (RC) : Purple color plug

   (RD) : Brown color plug
3. Load the ANA Screen iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 μL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 20 μl.
7. Ensure sample positons are properly define at the time of loading in the analyzer.
8. The ANA test-specific parameters are stored in barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Mix ANA Screen iClia calibrator and controls by gentle inversion before use. Open the cap and place the calibrator and control-1 & control-2 vials into each respective assigned positions. Read the barcode for calibrator and controls provided with the kit.
10. Run calibration as mentioned in heading calibration below.
11. Press Run. The test result for first sample will be obtained at 20 minutes.
12. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Calibration

1. Test Calibrators in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that calibrator and controls values are within the validity range specified in this instruction for use.
2. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless, recalibration is required.
3. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and pre-trigger/ Trigger solution/wash buffer).
   b) Every 15 days and/or at the time of any component to be changed.
   c) Controls are out of validation range.
   d) Required by pertinent regulations.
   e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the ANA iClia.

14. RESULT CALCULATION:

The analyzer calculates the cut-off value based on the RLU of the calibrator. The results are automatically calculate by Clia Analyzer for each sample based on cut-off value and given in RU/ml.

a) RESULT INTERPRETATION

If the test sample unit is < 20 RU/ml then interpret the sample as Negative for ANA antibodies and If the test sample unit is > 20 RU/ml then interpret the sample as Positive for ANA antibodies. The results of ANA Screen iCLIA can only be used as part of the overall clinical evaluation of the patient, and the clinical diagnosis should be combined with clinical symptoms and other diagnostic methods.

Determination of Reference Interval

Cut-off value of ANA Screen iCLIA is considered as < 20.00 RU/mL for healthy individuals, which is established referring to receiver operating characteristic curve and literatures, based on the rest results of more than 60 clinical samples.

Due to the differences in geography, race, gender or age, it is suggested each laboratory should establish its own reference interval or conduct verification of the existing reference interval.

15. PERFORMANCE CHARACTERISTICS

A) In-house Evaluation:

The Performance of the ANA Screen iClia with reference to sensitivity and specificity was evaluated in-house with 06 negative and 24 anti-nuclear antibodies positive samples. The results of all the positive and negative samples were compared with Roche ANA Clia. The results of the in-house study done are as follows:

Sensitivity: 100% **Specificity:** 100%

Precision: Precision is checked by running ANA Screen iClia test in 10 replicates (Intraassay variation, Inter assay variation) and Inter Machine variation with Kitcontrols(Control 1& Control 2) and 2 ANA positive samples; one strong positive and one weak positive .The CV% in Sample RLU and both the controls and positive samples is within 10.0%.

Intra Assay Variation

Within run variation was determined by 5 replicate measurements of two different ANA Screen control sera(Low) and (High) in one assay in 3 different lots. The within assay variability is <10 %.

Inter Assay Variation

Between run variation was determined by 5 replicate measurements in 10 sequential days of two different control sera (Low) and (High) in 3 different lots.The between assay variability is <10.0%.

Intra-Assay, n=5			Inter-Assay, n=5×2		
Control	Mean (RU/mL.)	CV	Sample	Mean (RU/mL.)	CV
1	10.35	5.67%	1	10.34	9.03%
2	83.13	5.63%	2	81.86	9.52%

Inter machine(CLIA Analyzer) Variation

Between machine variation was determined by 3 replicate measurements of two different ANA Screen control sera (Low) and (High)in 3 different lots in 3 different J. Mitra CLIA Analyzer. The between machine variability is <15%.

Linearity

The linearity was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP6-A requirements.

The linearity range was verified by more than 6 concentration levels which encompass or be equal to the minimum and the maximum values of linearity range and duplicate assays in triplicate in single run for each lot at all 6 levels. The ANA Screen iClia kit has been demonstrated to be linear from 5.00 RU/mL to 1000 RU/mL, regression (R^2) of more than >0.990.

Specificity

Interference

A study was performed based on guidance from CLSI EP7-A2.

Potentially interfering substances were evaluated to determine whether AFP concentrations were affected when using the ANA Screen iClia assay kit. Samples containing the potential interferents were prepared at two ANA concentrations. The samples were assayed, and the ANA concentrations of the spiked samples were compared to the reference samples.

Potential Interferent	Interferent Concentration	% Interferent Bias
Bilirubin	20 mg/dL	±10%
Hemoglobin	500 mg/dL	±10%
Triglyceride	1000 mg/dL	±10%

15. LIMITATION OF THE TEST

- The test should be used for detection of Anti-nuclear Antibodies in serum or plasma only and not in other body fluids.
- Clinical diagnosis should not be made on the findings of a single test result, but should be integrated with all clinical and laboratory findings.
- Hemoglobin < 500 mg/dL, triglyceride < 1000 mg/dL and bilirubin < 20 mg/dL will have no significant interference for the results.

17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

18. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8ºC.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8ºC.
	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
2) High ANA test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.