ReQuest® ANA SCREENING

REF 01-310H 96-Test Set IVD

Intended Use: The SeraQuest Antinuclear Antibody Screening test is a qualitative enzyme Immunoassay (EIA) intended to screen for the presence of antinuclear antibodies (ANAs) in human serum as an aid in the diagnosis of certain systemic rheumatic diseases. This assay collectively detects In one well, total ANAs against double stranded DNA (dsDNA, nDNA), histones, SS-A/Ro, SS-B/La, Sm, Sm/RNP, SCL-70, Jo-1, and centromeric antigens, along with sera positive for immunofluorescent (IFA) HEp-2 ANAs.

For in vitro diagnostic use only.

Principle

Purified antigens (dsDNA, histones, SB-A/Ro, SS-B/La, Sm, Sm RNP, Scl-70, Jo-1, centromere and other antigens extracted from the HEp-2 nucleus) are bound to microwells. Antibodies to these antigens, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human lgG immunologically binds to the bound patient antibodies forming a "conjugate - antibody – antigen" sandwich. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of the color is measured photometrically at 450

Summary and Explanation

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases (1). Although these antibodies were first associated with systemic lupus erythematosus (SLE), the list of implicated diseases has expanded and many rheumatic diseases are characterized by the presence of one or more of these ANAs. For instance, anti-SS-A/Ro and anti-SS-B/La antibodies are associated with SLE and Sjogren's Syndrome (SS), anti-dsDNA and anti-Sm antibodies with SLE, anti-histone antibodies with SLE and Drug Induced Lupus, anti-RNP antibodies with mixed connective tissue disease (MCTD) and SLE, anti-Scl-70 antibodies with scleroderma (progressive systemic sclerosis [PSS]), anti-Jo-1 antibodies with polymyositis and dermatomyositis and anti-centromere antibodies with CREST syndrome (2-4).

The IFA has been used as the standard method in the detection of ANAs (5). Although the IFA is a sensitive test, it is laborious when testing large numbers of patient samples and is subject to errors from human interpretation and from variability in fluorescent microscopes (1). The IFA HEp-2 ANA test is also subject to the following concerns: it is sometimes insensitive to certain sera containing antibodies to SS-A, SS-B, Sm, or dsDNA (6) and it tends to find sera positive in a large number of patients who do not develop systemic rheumatic diseases within a follow up two year period (7). The EIA test system is an excellent alternative to the IFA test system for screening patient's serum for the presence of ANAs of clinical significance. The EIA test system efficiently screens large numbers of patient samples and reduces human error.

The ReQuest® ANA SCREENING test collectively detects, in one well, total ANAs against double stranded DNA (dsDNA, nDNA), histones, SS-A/Ro, SS-B/La, Sm, Sm RNP, Scl-70, Jo-1, and centromeric antigens, along with sera positive for IFA HEp-2 ANAs. Sera positive on the EIA ANA Screening test should be tested for the specific autoantibodies indicative of various systemic rheumatic diseases.

Reagents

Component	Content
01-311 Coated Wells	Plastic Microwells: Coated with purified antigens of dsDNA, histones, SS-A/Ro, SS-B/La, Sm, Sm RNP, Scl-70, Jo-1, centromere and other antigens extracted from the HEp-2 nucleus.
01-312 Conjugate	Goat Anti-Human immunoglobulin labeled with HRP.
01-313 Negative Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-314 Cut-Off Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-315 Positive Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-316 Sample Diluent	Saline with a protein stabilizer.
01-317 Substrate	**TMB (**3,3′,5,5′-Tetramethylbenzidine**) CAS#*54827-17-7***
01-318 Wash Concentrate	**Phosphate-buffered saline** with **Tween 20**, pH 8.0. Not subject to GHS, US HCS, EC CLP, and analogous global GHS-based regulatory requirements in this product mixture and concentration.
01-319 Stop Solution	**Sulfuric Acid CAS#** 7664-93-9

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

Materials Required

1. EIA Reader
2. Pipettors for dispensing 10 and 100 µl
3. 8-channel repeating pippetor (for washing)

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4. Deionized water
5. Pipettes (1 ml & 10 ml)
6. Dilution tubes or microwells
7. 1 liter container (for Wash Solution)
8. Timer

Recommended Materials

1. Automatic Washer
2. 100µ18-Channel Micropipettor (for reagent delivery).
3. 1ml Mini-Tubes (for sample dilution).

Precautions / Warnings

1. The Stop Solution contains a dilute add solution. Use with care to avoid contact with skin and eyes. Avoid exposure to bases, metals, or other compounds which may react with acids. Spills should be cleaned up immediately.
2. Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
3. Do not smoke, eat, or drink in areas where patient samples and kit reagents are handled.
4. Do not pipette by mouth.
5. Wear personal protective equipment while handling all reagents and samples and while operating the washer and reader.
6. Dispose of all wastes in accordance with applicable national and/or local regulations.
7. Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
8. Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.
9. Chemical reagents should be handled in accordance with Good Laboratory Practices.
10. Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
11. Do not use kit beyond its expiration date. The date is printed on kit boxes.
12. This product uses human serum in the manufacture of the Cutoff and Controls. Each unit was tested by FDA accepted methods and found non-reactive for HIV-t, HIV-2, Hepatitis B (HBV), Hepatitis C (HGV) and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the Cutoff and Controls with the same precautions used with patient specimens.
13. Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
14. Never mix the contents from different bottles of the sane reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
15. Approximately 30 minutes before beginning the assay, remove the kit from the refrigeration (2-8 OC) and allow the kit components to come to room temperature (18-27°C). Mix reagents thoroughly by gently swirling the container several times before use. Return the assay materials to 2-6 °c after use.
16. Do not interchange reagents between kit lots.

Specimen Collection

Use serum for the test. Consider any materials of human origin as infectious and handle them using typical biosafety procedures. Collect blood aseptically in untreated tubes. Allow blood to clot, separate serum immediately. Avoid use of lipemic, hemolyzed or contaminated sera. Store sera at 2-6°C. Freeze sera at -20°C if not tested within 24 hours; avoid repeated freezing.
Caution: serum samples should not be heat-inactivated as this may cause false positive results.

Stability and Storage of Reagents

The kit is stabilized for ambient shipment. All kit components should be stored at 2-8°C and can be used until the expiration data printed-on-the labels.

Procedure Notes

1. All materials must be at room temperature (18-27 °C) before beginning the assay.
2. Do not use Cutoff or Controls from different kit lots. Do not use expired reagents.
3. Avoid contamination of reagents, dispensing pipettes, and microtiter wells. Use new dispensing pipettes for air samples. Do not interchange caps. Always keep bottles capped when not in use. Do not reuse the microtiter wells or pipettes. Avoid pipettes contaminated with peroxidase.
4. All wells should be handled in the same sequence and the same manner throughout the test. The test should be performed without interruptions.
5. Gently and completely swirl each bottle of liquid reagent and sample before use.
6. Make reliable1:40 dilutions.
7. Make all dilutions in uncontaminated Sample Diluent. Prepare all dilutions before starting test. Always use fresh sample dilutions.
8. Always run an ANA Positive Control, an ANA Cutoff Control, and a Negative Control. Always blank against Sample Diluent.
9. Humidity affects the antigen-coated wells; do not open pouch until it reaches room temperature. Calculate the number of wells required for the current assay, remove them from the room temperature foil pout, align them on the EIA Frame, then add samples immediately. Unused wells should be returned immediately to the resealed toil pouch with desiccant.
10. Incubation times affect EIA results. Do not allow any of the controls, samples or Conjugate to incubate in the strip wells for more than 40 minutes. For best results, use 1 ml mini-tubes to prepare sample dilutions. Transfer all solutions into wells with an 8-channel Micropipettor.
11. After each incubation, thoroughly wash the microtiter wells with -200 µL Wash Solution per well. Be sure to remove all liquid before proceeding to next step. Fill wells, then invert and rapidly flick away the liquid. After complete washing, blot the plate on a paper towel.

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12. Transfer to a graduated test tube 1 ml of Conjugate for each strip to be run. Discard excess transferred Conjugate.
13. Transfer to a graduated test tube 1 ml of Substrate for each strip to be run. Discard excess transferred Substrate.

Preparation and Storage of Reagents

The kit is stabilized tot ambient shipment. Alt kit components should be stored at 2-8 °C and can be used until the expiration dale printed on the labels.
• Collect all reagents, samples and dilutions necessary before starting assay.
• Assign and record wells hr controls and samples.

ANA Microplate
1. Ready to use.
2. After opening the foil pouch, the wells are stable for 30 days if immediately returned to resealed toil pouch with desiccant.

EIA Frame
Retain for future use.

Wash Solution
Because the Wash Concentrate contains salt, crystals may form in the concentrated solution. For proper preparation of the Wash Solution, complete the following steps:
1. Empty contents of the 60 ml Wash Concentrate bottle, including any crystals, into a 1 bottle.
2. If any crystals remain in the Wash Concentrate bottle, remove them by adding some deionized water to the bottle; mix and pour all contents into the 1 bottle.
3. Add deionized water to the t bottle to bring the final volume of the solution to 1 liter.
4. Place a stir bar in the 1 bottle and place on a stir plate. Stir the diluted Wash Solution for a few minutes until all crystals are dissolved. If no stir plate is available, cover the top of the Wash Solution and gent y invert back and forth until the crystals am dissolved. Avoid excessive bubbles.

Diluted Wash Solution is stable for 14 days at 2-8 QC. Retain for future use.

Sample Diluent
1. Ready to use.
2. Allow Sample Diluent to reach room temperature before use.
3. Mix thoroughly
4. Avoid unnecessary contamination.

Conjugate, Substrate and Stop Solution
Ready to use.

1:40 Working Solutions
Prepare as Follows:
1. Dilute 10 µl of patient's sera in 0.4 ml of Sample Diluent.
2. Dilute 10 µl of ANA Positive Control in 0.4 ml of Sample Diluent.
3. Dilute 10 µl of ANA Cutoff Control in 0.4 ml of Sample Diluent.
4. Dilute 10 µl of Negative Control in 0.4 ml of Sample Diluent.
5. Discard excess working solutions after use.

Indications or Instability or Deterioration of Reagents

Do not use any reagents that show signs of leakage.

Procedure

Assay Steps

1. Apply diluted samples and controls to wells:
   • Controls - Apply 100 µl of diluted controls (1:40th Sample Diluent) to assigned wells. Add 100 µl of Sample Diluent as a blank control.
   • Patient samples -Apply 100 µl of diluted patient serum (1:40 in Sample Diluent) to assigned wells.
2. Incubate wells - Shake plate gently, then incubate for 30 minutes at room temperature (18-27 °C). (Do not incubate diluted sera in wells for more than 40 minutes.)
3. Discard incubated samples -After 30 minute incubation, discard samples by inverting plate and rapidly flicking the fluid away from the plate.
4. Wash wells - Gently fill 5X with -200 µl of Wash Solution and discard. Remove all liquid before proceeding.
5. Apply Conjugate - Add 100 µl Conjugate to all wells. Discard excess transferred Conjugate after use.
6. Incubate wells - Shake plate gently, then incubate tot 30 minutes at room temperature (18-27°C). (Do not incubate Conjugate in wells for more than 40 minutes.)
7. Discard incubated Conjugate - After 30 minute incubation, discard Conjugate by inverting plate and rapidly flicking the liquid away from the plate.
8. Wash wells gently fill 5X with -200 µl of Wash Solution and discard. Remove all liquid before proceeding.
9. Develop color -Add 100 µl of Substrate to each well. Discard excess transferred Substrate after use.
10. Incubate Shake or tap plate gently to disperse color. Incubate hr 30 minutes at room temperature (18-27 °C).
11. Stop color development- After 30 minute color development, add 100 µl of Stop Solution to each well to stop the color development.
12. Read results - Read wells within 30 minutes with an EIA reader set to 450 nm. Zero the reader on the Sample Diluent Blanking Control well, then read the color of the control and patient wells. The ANA Positive Control well should show yellow color. The .A Cutoff Control well should show yellow color The ANA Negative Control well should show moderate color The Sample Diluent Blanking Control well should show little color or no color.

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Calculation of Results

Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Test Sample Absorbance}}{\text{Index* ANA IgG Cut off Control Absorbance}} \quad \text{Test Sample}$$

*ANA index are qualitative. They are units defined by Quest International.

If the Cut off control is run in duplicate, use the average absorbance value to calculate results.

Quality Control

1. The Controls, ANA IgG Positive Control, ANA IgG Cut off Control and ANA IgG Negative Control must be included in each test run.
2. The absorbance value of the Sample Diluent OD (reagent blank) should be less than 0.200 against air.
3. The ANA IgG Positive Control and ANA IgG Cut off Control must have absorbance values within the specified range printed on the quality control card included with each kit lot number.
   If any of these criteria are not met, the test is invalid and should be repeated.

Interpretation of Results

The following is intended as a guide to interpretation of ReQuest® ANA SCREENING test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Index Value	Interpretation
<1.0	Negative for anti-ANA IqG antibody.
> 1.0	Positive for anti-ANA IgG antibody.

Limitations

1. The results obtained with the ReQuest® ANA Screening test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Confirmative testing for specific antibodies should be run if a positive assay is obtained. A positive result suggests certain diseases and should be confirmed by clinical findings.
3. The assay performance characteristics of ReQuest® ANA Screening have not been established for matrices other than serum.
4. The performance characteristics of the ReQuest® ANA Screening test with automated analyzers have not been established.

Performance Characteristics

Sensitivity

Sensitivity can be defined as the ability of the test to give a positive result for serum samples that should be positive. The sensitivity performance of the Bio-Rad EIA ANA Screening Test was established in the following manner:

Sera with monospecific antibodies of clinical significance (n = 59) obtained from a variety of clinical sources were tested on the EIA ANA Screening Test. 100% of these ANA monospecific sera were positive on the EIA ANA Screening Test.
IFA HEp-2 ANA positive sera (n = 371) obtained from a variety of clinical sources were tested on the EIA ANA Screening Test. The results are summarized below:

IFA HED-2 ANA Titer	EIA ANA Screen Results	Number of Samples	.%.
≥1:160	Positive	220	91%
≥1:160	Negative	22	9%
1:40-1:80	Positive	72	56%
1:40- 1:80	Negative	57	44%

Lupus patient sera (n = 38) obtained from a variety of clinical sources were tested on the EIA ANA Screening Test. 100% of the Lupus patient sera were positive on the EIA ANA Screening Test.

Specificity

Specificity can be defined as the ability of the test to give a negative result for "normal" sera. The specificity performance of the Bio-Rad Autoimmune EIA ANA Screening Test was established using 70 'normal" sera obtained from a volunteer blood donor testing facility. One donor had antibodies to dsDNA and was thus not considered to be "normal". Of the remaining 69 sera, 64 were negative on the EIA ANA Screening Test, thus yielding a 92.8% specificity.

Accuracy

Sera (n = 180) obtained from a variety of clinical sources were tested on four different predicate devices and the EIA ANA Screening Test for comparison purposes. The predicate devices were:
1) EIA ENA Plus Screening Test (for the detection of antibodies to SS-A, SS-B, Sm, Sm RNP, Scl-70 or Jo-i), 2) EIA anti-dsDNA. 3) EIA anti-

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Histones, and 4) IFA HEp-2 ANA used for the purpose of detecting anti-centromeric antibodies. Of the sera tested, 110 were positive on one or more of these predicate assays, while 70 were "normal' sera negative on all four of the predicate assays.

• Sera (n = 469) obtained from a variety of clinical sources were tested on the IFA HEp-2 ANA and the EIA ANA Screening Test for comparison purposes. The overall agreement was 86.1%.

Precision

Intra-assay precision was determined by testing a strong positive control and a weak positive control with a replication of 18; the CVs were 6.6% and 9.5% respectively.
Inter-assay precision was determined by testing a strong positive control and a weak positive control in a total of 24 assays; the CVs were 6.8% and 8.3% respectively.

References

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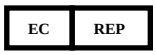
Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.

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