THERATEST LABS
Innovations in Immunoassays

EL-ANAscr™

An enzyme immunoassay for the screening of human serum
to detect antinuclear antibodies (ANAs)

Instruction Manual

Catalog Nos.:
100-002, 100-004

TheraTest Laboratories, Inc.
1120 N. DuPage Ave.
Lombard, IL 60148 USA
Tel: 1-800-441-0771
Fax: 1-630-627-4231
e-mail: support@TheraTest.com
www.TheraTest.com

TABLE OF CONTENTS

Page

INTRODUCTION.....3

REAGENTS.....3

WARNINGS & PRECAUTIONS.....4

SPECIMEN REQUIREMENTS 5

PROCEDURE 6

QUALITY CONTROL.....8

RESULTS 8

EXPECTED VALUES.....9

PERFORMANCE DATA.....10

TROUBLESHOOTING 11

REFERENCES.....13

ABBREVIATED TEST PROCEDURE.....15

INTRODUCTION

Name: TheraTest **EL-ANAScr™**

Intended Use

FOR IN VITRO DIAGNOSTIC USE ONLY

The EL-ANAScr™ (EL-ANA Screen) is an enzyme immunoassay intended to screen human serum for the presence of antinuclear antibodies (ANAs). The test detects the presence of antibodies to double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), Sm, RNP/Sm, SSA (Ro), SSB (La), Histones, Scl-70, and Jo-1. It also detects other autoantibodies that show, on an IFA test, various immunofluorescent patterns on HEp-2 cells such as: speckled, nucleolar, homogeneous, peripheral, and centromere.

Summary and Explanation

Autoantibodies directed against nuclear and cytoplasmic constituents have been used for the differential diagnosis of various systemic rheumatic diseases including systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), C.R.E.S.T., mixed connective tissue disease (MCTD), Sjögren's syndrome, and rheumatoid arthritis (RA).[1,2] The most widely used method for detection of ANAs is immunofluorescence with HEp-2 cells (FANA). Although very sensitive, IFA can be a labor-intensive and subjective method with results varying according to microscope optics, reagent quality, and the experience of the technologist. Although the FANA test generally provides a dependable method to screen for systemic rheumatic disease, it has been demonstrated that the FANA cannot provide 100% assurance of autoantibody detection. Studies have shown that sera containing antibodies directed against native DNA, SSA/Ro and ENA have, in some instances, demonstrated "false negative" results on FANA.[4,6] The ELISA format facilitates the efficient, objective testing of multiple samples while optimizing sensitivity and specificity.[7]

Although many biochemical attributes contribute to pathogenicity of autoantibodies (e.g., class, avidity, charge, complement fixation), the IgG isotype is clearly linked to the pathogenesis of autoimmune diseases. The presence of low levels of IgM class autoantibodies have been detected in transient (non-rheumatic disease) conditions, in non-symptomatic relatives of rheumatic disease patients, and in normal, healthy elderly individuals.[2,5,8] Therefore, testing for IgG specific antinuclear antibodies reduces the number of "false positive" test results.

Method Description

The TheraTest EL-ANAScreen is a dual well solid phase enzyme immunoassay test. Polystyrene microwells have been coated with both purified nuclear antigens and HEp-2 cell extract in two separate microwells: A (ScrA) and B (ScrB), respectively. Calibrators A and B, Controls and patient Specimens are tested in both the A and B wells. During the incubation, antinuclear antibodies, if present in the patient Specimen, bind to the solid phase. The microwells are washed and horseradish peroxidase-labeled goat anti-human IgG (Fcγ specific) is added to the wells. After incubation, the plate is washed, and a chromogenic substrate is added. Autoantibody binding is detected by a color change that is measured using a spectrophotometric microplate reader. All Specimens that yield absorbance values higher than the Calibrator A or B for the corresponding A and/or B well(s) are considered positive. The positive Specimens should be tested further to determine the specific autoantibody(s) present. FANA (a fluorescence antinuclear antibody test) may be performed if pattern identification is desired, but specific antinuclear antibody panels provide the most complete clinical information. Specimens with values lower than the Calibrators are not considered to contain significant levels of autoantibody. If clinical findings dictate, the test may be repeated in 4 - 6 weeks.

REAGENTS

EL-ANAScr™ Kit (Catalog Number 100-002), sufficient for 96 determinations.
EL-ANAScr™ Kit (Catalog Number 100-004), sufficient for 192 determinations.

Upon receipt, store reagents at 2-8°C. Do not freeze. Allow all reagents to equilibrate to room temperature (18-25°C) prior to use. Return all reagents to refrigeration (2-8°C) immediately after use.

1. Coated Wells (A wells) and (B wells)
Wells identified as A (ScrA) are coated with purified nuclear antigens. Wells identified as B (ScrB) are coated with a HEp-2 cell preparation. Prior to use, allow wells and holder to equilibrate to room temperature (18-25°C) in the metallized pouch to protect wells from condensation. Coated wells are stable for three months at 2-8°C once the pouch has been opened if the pouch is promptly resealed with enclosed desiccant.

2. EL-ANA Specimen Diluent
Phosphate buffer with serum proteins, containing preservative and a yellow dye.

3. ANAscr IgG Enzyme Conjugate (Tracer)
Anti-human IgG (Fcγ specific) conjugated with horseradish peroxidase; containing preservative and a green dye.

4. Chromogen
Buffered Substrate and Chromogen (3,3', 5,5' Tetramethylbenzidine [TMB]). Protect from light.

5. EL-ANA Wash Buffer (10X)
10X concentrated buffer.

6. Stop Reagent
2M Phosphoric Acid.

7. ANAscr A Calibrator *
Human sera with preservative.

8. ANAscr B Calibrator *
Human sera with preservative.

9. Positive Control *
Human sera with preservative.

10. Negative Control *
Human sera with preservative.

*Reagents containing Sodium Azide

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

Reagents Containing Human Source Material

CAUTION: Controls and Calibrators contain Human Serum. Treat as potentially infectious.

Each serum/plasma donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HBsAg, antibody to HCV and antibody to HIV 1 and 2. While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source material for which there is no approved test. Because no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all products containing human source material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 4th ed., 1999. HHS Publication (NIH and CDC); web site: http://bmbl.od.nih.gov/

Stop Reagent (2M Phosphoric Acid)

May cause severe burns upon contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes.

European Communities Hazardous Substance Risk Phrases (Council Directive 88/379/EEC)
R34: Causes burns
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical attention.

S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Chromogen

Irritant! This product contains 3,3',5,5'-tetramethylbenzidine (TMB) (≤0.05%), a chromogenic indicator of horseradish peroxidase activity. It has shown neither mutagenic nor carcinogenic effects in laboratory experiments (10).

Hazardous Substance Risk & Safety Phrases:
R36/37/38 – Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S24/25 – Avoid contact with skin or eyes.
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36 – Wear suitable protective clothing.
S51 – Use only in well-ventilated areas.

Reagents Containing Sodium Azide

Calibrators and Controls contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drain with large quantities of water to prevent azide build-up.

Hazardous Substance Risk & Safety Phrases:
R22 - Harmful if swallowed.
R36/37/38 - Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 - After contact with skin, wash immediately with plenty of water.
S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection
S46 - If swallowed, seek medical advice immediately and show this container label.

General Precautions and Information

1. The Stop Reagent can irritate eyes and mucous membranes.
2. Do not allow Chromogen to come in contact with metal or oxidizing agents.
3. Handle patient sera and kit reagents with appropriate precautions. Do not pipette by mouth.
4. Do not use test components beyond expiration date.
5. Avoid microbial contamination of the reagents. If solutions become turbid, they should not be used.
6. Avoid exposure of reagents to excessive heat or light during storage.
7. Use disposable material or wash all glass and plastic thoroughly according to standard laboratory practice.

SPECIMEN REQUIREMENTS

A whole blood specimen should be obtained in a red top tube with use of acceptable medical techniques. The blood should be allowed to clot and the serum separated by centrifugation. Serum samples may be stored at 2-8°C for up to 14 days prior to testing. If testing cannot be completed within 14 days of collection, store the sera at –20°C or below. Do not use sera that have been thawed more than once or that have been heat-inactivated. Hemolysis and lipemia do not significantly alter the results.

PROCEDURE

Materials and Reagents Supplied with Each Kit

	Cat. No. 100-002 96 tests	Cat. No. 100-004 192 tests
1. Antigen Coated Wells (A Set)	1	2
2. HEp-2 Coated Wells (B Set)	1	2
3. Specimen Diluent	100 mL	2 x 100 mL
4. EL-ANA Wash Buffer (10X)	100 mL	100 mL
5. ANAScr IgG Enzyme Conjugate (Tracer)	27 mL	2 x 27 mL
6. Chromogen	27 mL	2 x 27 mL
7. Stop Reagent	27 mL	2 x 27 mL
8. Positive Control	0.3 mL	0.35 mL
9. Negative Control	0.3 mL	0.35 mL
10. ANAscr A Calibrator	0.3 mL	0.35 mL
11. ANAscr B Calibrator	0.3 mL	0.35 mL

Materials Required but not Supplied

[ ] Clean wash bottle and automated plate washer (optional).
[ ] Calibrated adjustable multichannel pipette (50-300 µL).
[ ] Pipette tips.
[ ] Distilled or deionized water.
[ ] Test tubes (12 x 75 mm) or 1.2 mL minitubes (cluster tubes, dilution tubes).
[ ] Calibrated adjustable micropipettors (5-20 µL, 20-1000 µL) or autodilutor, and pipette tips.
[ ] Timer (60 minute range).
[ ] Reagent Reservoirs.
[ ] Absorbent paper towels.
[ ] Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA reader) for 96-well microtiter plates.

Reagent Preparation

1. EL-ANAScreen Plate

The plate is comprised of twelve rows of printed microwells. The assay may be run in the preconfigured format as independent tests—ScrA and ScrB on separate plates. Alternatively, the wells can be easily removed and reconfigured to allow for running A and B wells simultaneously on one plate.

NOTE: Serum Specimens, as well as Calibrators and Control materials, must be tested with ***BOTH*** A and B wells.

2. 1X Wash Buffer

The EL-ANA Wash Buffer (10X) must be diluted 1:10 prior to use. Prepare 1X Wash Buffer by pouring the contents of the 10X Wash Buffer into a clean one-liter volumetric container. Rinse the bottle with deionized or distilled water to remove residual buffer. Add the rinse to the one-liter container. Add deionized or distilled water until a total volume of 1.0 liter is reached; mix thoroughly. When stored at 2-8°C, the diluted Wash Buffer is stable for 8 weeks. ***Note*: *Prior to use, check the 10X Wash Buffer for any crystals formed during storage in the cold. If crystals are present, warm the bottle until the crystals dissolve. Failure to take this precaution will cause test failure.***

3. Calibrators, Positive, Negative Controls, and Specimens

The Calibrators (A and B), Controls (Negative and Positive) and Specimens must be diluted 1:101 prior to use. Pipette 10 µL of the appropriate specimen into 1 mL of EL-ANA Specimen Diluent. If positive Specimens will be evaluated within 5 hours by a TheraTest ANA Profile, prepare an adequate volume of diluted Specimen (e.g. 2 mL), otherwise discard diluted Specimens when the assay is complete.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature. Upon removal from 2-8°C storage, allow the wells to reach room temperature in the sealed metallized pouch to protect wells from condensation.

2. **PREPARE SERA AND REAGENTS** as described in the previous section.

3. **REMOVE WELLS** from the metallized pouches being careful not to touch the optical surfaces on the bottom of the wells. Wells should be used only if the desiccant included in the pouch is blue. Remove appropriate number of wells (using one A well and one B well for each Control and Specimen and two of each A and B wells for the corresponding Calibrators A and B). Place the remaining wells in the metallized pouch with the desiccant, reseal and store refrigerated (2-8^{o}C) for future use.

4. **SPECIMEN ADDITION:** Pipette 100 µL of each diluted Control and Specimen into each of the two wells, A and B. Pipette 100 µL of diluted ANAscr A Calibrator into duplicate A wells and 100 µL of diluted ANAscr B Calibrator into duplicate B wells

For the **Blank**, pipette 100 µL of Specimen Diluent into each of the two wells, A and B, then subsequently treat as a specimen well. All samples for one assay must be pipetted within 5 minutes. The use of multichannel pipettors and 96-well format minitubes facilitates this process.

5. **SPECIMEN INCUBATION:** Incubate the plate undisturbed at room temperature (18-25°C) for 30-35 minutes.

6. **DISCARD WELL CONTENTS** by decanting fluid into a designated disposal container or designated sink`.

7. **WASH:** Completely fill all wells with 1X Wash Buffer (~300 µL per well). Empty by shaking into disposal container or aspirate. Repeat two more times for a total of three washes. Remove all residual liquid from wells by inverting and blotting the plate on absorbent paper. *Note: A semi-automated plate washer may be used in place of a manual wash.*

8. **ANAScr IgG ENZYME CONJUGATE (TRACER) ADDITION:** Pipette 100 µL of Conjugate into each well with the aid of a multichannel pipette. Incubate the plate undisturbed at room temperature (18-25°C) for 30-35 minutes.

9. **WASH:** Discard Conjugate from wells and wash plate as described in step 7. Remove all residual liquid from the wells by inverting and blotting the plate on absorbent paper.

10. **CHROMOGEN ADDITION:** Add 100 µL of Chromogen to each well using a multichannel pipette. Incubate the plate undisturbed at room temperature (18-25°C) for 15±1 minutes. DO NOT WASH PLATE! The wells containing samples with antibody will develop a blue color.

11. **STOP COLOR DEVELOPMENT:** To stop the reaction at the end of the Chromogen incubation, pipette 100 µL of Stop Reagent into all of the wells at the same regular intervals and in the same order that the prepared Chromogen was added. Gently tap the plate to disperse the Stop Reagent. The color will change from blue to yellow.

12. **READ ABSORBANCE:** Firmly set the microtiter plate in the reader and measure the absorbance of each well at 450 nm. Absorbance values should be measured within 30 minutes of completing the assay. If a dual wavelength ELISA reader is used, set the test wavelength at 450 nm and the reference at 620 to 690 nm.

Procedural Comments

1. Handling of Microwells

The metallized pouch must be cut at one end so that it may be resealed. Promptly replace unused microwells in the pouch with the desiccant, seal the pouch and store at 2-8°C. To avoid false positive readings, ensure that the bottoms of the microwells are clean.

2. Specimen Dilution

Make sure that the diluted Specimens are mixed well.

3. Washing

Each row of wells may be washed using a multichannel pipettor or a repetitive pipettor. The wells may be aspirated or shaken into a disposal container. Alternatively, a commercial automated or semi-automated washing system may be used following the instructions provided by the manufacturer. When using either washing technique, the wells should be blotted thoroughly on absorbent paper after the last wash.

ATTENTION: Incomplete washing of wells may lead to decreased precision.

4. Pipetting

If the test is performed manually, in order to avoid cross-contamination and sample carry-over, the Calibrators A and B, Positive and Negative Controls and test Specimens should be pipetted using separate pipette tips. When testing multiple Specimens, a multichannel pipettor should be used to pipette the ANAScr IgG Enzyme Conjugate, Wash Buffer, Chromogen, and Stop Reagent.

QUALITY CONTROL

1. ANAscr A and B Calibrators, Positive and Negative Controls must be included with each test run.

2. Positive and Negative Controls

The Unit values for the Positive and Negative Controls should fall within the ranges provided on the Data Sheet. If the values are not in agreement with the values on the Data Sheet, the assay is not valid and the results should not be reported.

3. Blank

The absorbance value should not exceed the value listed on the Data Sheet. If the value is greater than that value listed, consult the TROUBLESHOOTING section and repeat the assay.

4. Calibrators

The net absorbance values for the ANAscr A and B Calibrators must fall within the range listed on the Data Sheet. If the values are outside the listed range, consult the TROUBLESHOOTING section and repeat the assay.

RESULTS

A. Calculating Net Absorbance Values

To determine net absorbance, the absorbance value of the Specimen Diluent Blank is subtracted from the raw absorbance values of Calibrators A and B, Controls and Specimens. This may also be accomplished by setting the instrument to blank on the respective Specimen Blank well.

B. Calculating Autoantibody Unit Activity

Autoantibody activity is calculated as follows. The Unit value for the ANAscr A and B Calibrators is listed on the Data Sheet. Conversion factors (C.F.) for A and B Calibrators are calculated separately. Multiply C.F. by the net absorbance (Abs) for Specimen to yield final Unit value.

Example Calculation of Unit Value for A and B Wells

Units assigned to ANAscr A Calibrator = 20

Net Abs values for ANAscr A Calibrator in duplicate A wells = 0.220 and 0.180

Mean value = (0.220 + 0.180) / 2 = 0.200

$$\text{C.F. for A wells} = \frac{\text{Calibrator A Unit value}}{\text{Mean net Abs Calibrator A}} = \frac{20}{0.200} = 100$$

Specimen "X" net Abs in well A = 0.600
Unit value for Specimen X (well A) = C.F. x Specimen net Abs = 100 x 0.60 = 60 Units
(i.e., a positive result)

Unit value for ANAscr B Calibrator in duplicate B wells is calculated in the same manner:
Units assigned to ANAscr B Calibrator = 15

$$\text{C.F. for B wells} = \frac{\text{Calibrator B Unit value}}{\text{Mean net Abs Calibrator B}} = \frac{15}{0.250} = 60$$

Specimen "X" net Abs in well B = 0.200
Unit value for Specimen X (well B) = C.F. x Specimen net Abs = 60 x 0.200 = 12 Units
(i.e., a positive result)

If one or both unit values (A and/or B well) is/are above the cutoff value of 10 Units, then the Specimen is positive. Either both values or the higher of the two may be reported. Computers attached to spectrophotometric readers may perform all calculations. If computers are used, verify data reduction algorithm prior to use by comparison with manual calculation.

Interpretation of Results

Specimens with unit values of 10 or less are not considered to have a significant level of autoantibody. Further testing for specific antibodies is generally considered unnecessary. However, the clinical condition of the patient may dictate additional serological testing. Normal values may differ with the population tested and each laboratory should establish their own "normal range". The elderly and relatives of patients with SLE are more likely to have non-disease related positive ANA results.
Specimens with values >10 Units are considered abnormal. Judgments as to the level of specific autoantibodies based on the level of absorbance should not be made. Positive Specimens may be tested for pattern and titer by FANA and/or for antibody specificity by a panel of individual autoantibody tests.

Limitations of the Procedure

The EL-ANAScr should not be performed on microbially contaminated or heat-inactivated serum. This method has been tested using serum samples only. The performance of other types of specimen has not been determined.
Diagnosis should not be made solely on the basis of a laboratory test result. The data provided by the patient history and other clinical information available to the physician is essential in the diagnosis of autoimmune disease. Antinuclear antibodies are found in many autoimmune diseases or are induced by certain drugs, therefore the result should be considered in proper clinical context.[3]
A negative result does not exclude the diagnosis of rheumatic disease.

EXPECTED VALUES

An abnormal result is present if the absorbance value for the test sample exceeds the absorbance value of the Calibrators. A study of 100 normal random blood bank donors showed that six exceeded 10 Units by EL-ANAScr and 11 by FANA with an IgG specific FANA test. All 100 SLE patients tested (with either active or inactive disease state) had values over 10 Units and were also deemed positive by the FANA test.

TABLE 1. Frequencies of positive antinuclear antibodies by EL-ANA Screen in various rheumatic disease populations

Disease (n)*	% Positive
Blood bank donors (100)	6
SLE (100)	100
Sjögren's (10)	80
Scleroderma (27)	78
Rheumatoid arthritis (51)	37
MCTD (9)	100
Polymyositis (27)	92
Fibromyalgia (49)	4
Osteoarthritis** (39)	25

*Number tested **Mostly over the age of 60 years

PERFORMANCE DATA

Within-run coefficient of variation (CV) for positive Specimens tested 20 times was under 10%. Between-run CV for different positive Specimens tested 20 separate times was between 8% and 17%.
The following reference sera from the Centers for Disease Control and Prevention also appeared abnormal: anti-dsDNA, Sm, RNP, SSA, SSB, Scl-70, Nucleolar, Centromere and Jo-1. In addition, a collection of Specimens containing primarily anti-Histone or anti-ssDNA also reacted in the ANAScreen.

TABLE 2. Comparison of results obtained for 100 normals and 100 SLE patients using the EL-ANAScreen and IFA (AFT^R System HEp, Behring Diagnostics Inc.)

		EL-ANAScreen	
		Positive	Negative
AFT^R	+	101	10
	-	5	84

TABLE 3. Comparison of the sensitivity, specificity and predictive value between the EL-ANAScreen and IFA (AFT^R) with respect to SLE

	EL-ANAScreen	AFT^R
Sensitivity	100 / (100 + 0) = 100%	100 / (100 + 0) = 100%
Specificity	94 / (94 + 6) = 94%	89 / (89 + 11) = 89%
Predictive Value	100 / (100 + 6) = 94%	100 / (100 + 11) = 90%

TROUBLESHOOTING

Problem	Possible Causes	Solution
Positive and/or Negative Control Unit values out of range.	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test.
	2. Cross-contamination of Controls.	2. Pipette carefully.
	3. Improper dilution.	3. Repeat test.
	4. Optical pathway not clean.	4. Check for moisture or dirt. Wipe bottom and reread.
	5. Wavelength of filter incorrect.	5. Change filter to 450 ± 5 nm.
	6. Overdilution of Calibrators A and/or B.	6. Recheck procedure. Repeat test.
	7. Blank OD.> value listed on Data Sheet.	7. a) an excess volume of ANAScr IgG Enzyme Conjugate (Tracer) was added to the wells; b) the incubation time was too long; c) insufficient washing; d) a damaged or dirty well.
Calibrator(s) OD values out of range	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test.
	2. Improper dilution / wrong Calibrator added to A/B wells	2. Repeat test.
	3. Contamination of Calibrators.	3. Repeat test; pipette carefully.
	4. Wavelength of filter incorrect.	4. Change filter to 450 ± 5 nm.
	5. Blank OD out of range.	5. a) an excess volume of Conjugate (Tracer) was added to the wells. b) the incubation time was too long. c) insufficient washing. d) a damaged or dirty well; clean optical surface and reread.
All test results negative.	1. One or more reagents not added, or added in wrong sequence.	1. Recheck procedure. Check for unused solutions. Repeat test.
	2. Improper dilution of Wash Buffer.	2. Repeat test.
	3. Coated well(s) inactive.	3. Check for obvious moisture in unused wells. Rerun test with Controls only for activity.
All test results yellow.	1. Contaminated Chromogen solution.	1. Check absorbance of unused Chromogen solution.
	2. Contaminated buffers / reagents.	2. Check all solutions for turbidity.
	3. Wash Buffer (1X) contaminated.	3. Use clean container. Check quality of water used to prepare buffer.
	4. Improper dilution of serum.	4. Repeat test.
Poor precision (Duplicate abs. readings greater than 20% of their mean).	1. Pipettor delivery CV greater than 5% or samples not added slowly.	1. Check calibration of pipettor. Use reproducible technique.
	2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long; inconsistency in timing intervals; air bubbles.	3. Develop consistent uniform technique and avoid splashing or use multitip device or autodispenser to decrease time.
	4. Air currents blowing over plate during incubations.	4. Cover plate and place inside chamber.
	5. Optical pathway not clean.	5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt.
	6. Instrument not equilibrated before readings were taken.	6. Check instrument manual for warm-up procedure.
	7. Washing not consistent; trapped bubbles; liquid left in wells at end	7. Use only acceptable washing devices. Lengthen timing delay on automated

of wash cycle.	washing devices or increase number of wash cycles. Check that all wells are filled and aspirated uniformly. Dispense Wash Buffer above level of reagents previously added to wells.
8. Improper pipetting.	8. Avoid air bubbles in pipette tips.
9. Residual liquid not removed from wells.	9. Blot inverted plate on absorbent paper.

REFERENCES

1. Tan EM, AS Cohen, et. al., "The 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus," **Arthritis Rheum.,** 25:1271-1277 (1982).
2. Nakamura RM and WL Binder, "Current Concepts and Diagnostic Evaluation of Autoimmune Disease," **Arch. Pathol. Lab. Med.,** 112:869-877 (1988).
3. Shiel WC and M Jason, "The Diagnostic Associations of Patients with Antinuclear Antibodies Referred to a Community Rheumatologist," **J. Rheumatol.,** 16:782-785 (1989).
4. Bridges A, et. al., "Antinuclear Antibody Testing in a Referral Laboratory," **Lab Medicine,** 24:345-349 (1993).
5. Nakamura RM and EM Tan, "Update on Autoantibodies to Intracellular Antigens in Systemic Rheumatic Diseases," **Clin. Lab. Med.,** 12:1-23 (1992).
6. Bylund DJ and RM Nakamura, "Importance of Detection of SS-A/Ro Autoantibody in Screening Immunofluorescent Tests for Autoantibodies to Nuclear Antigens," **Journ Clin. Lab. Anal.,** 5:212-218 (1991).
7. Jaskowski TD., et. al., "Screening for Antinuclear Antibodies by Enzyme Immunoassay," **Am. Journ. Clin. Path.,** 105:468-473 (1996).
8. Teodorescu M and C Froelich, "Advanced Immunoassays in Rheumatology," **CRC Press,** Boca Raton, Florida, (1994).
9. Teodorescu M and C Froelich, "Laboratory Evaluation of Systemic Lupus Erythematosus". In R. Lahita (ed.) Systemic Lupus Erythematosus. Acad. Press, 3rd edition, 477-505 (1999).
10. Garner RC. Testing of some benzidine analogues for microsomal activation to bacterial mutagens. Cancer Lett 1975, 1:39-42.

NOTES:

Abbreviated Test Procedure

1. Dilute Calibrators A and B, Negative and Positive Controls, and Specimens 1:101 with Specimen Diluent.
2. Pipette 100 µL of Calibrator A into two A wells and 100 µL of Calibrator B into two B wells.
3. Note: total Calibrator wells per run must equal 4 (duplicate A wells and duplicate B wells).
4. Pipette 100 µL of Negative and Positive Controls and Specimens into A & B wells.
5. Incubate for 30-35 minutes at room temperature (18-25°C).
6. Wash the wells three times with 1X Wash Buffer.
7. Add 100 µL ANAScr IgG Enzyme Conjugate (Tracer) to each well.
8. Incubate for 30-35 minutes at room temperature (18-25°C).
9. Wash the wells three times with 1X Wash Buffer.
10. Add 100 µL Chromogen to each well.
11. Incubate for 15 ±1 minutes at room temperature (18-25°C).
12. Add 100 µL Stop Reagent to each well.
13. Read the absorbance at 450 nm, reference 620-690 nm within 30 minutes.

TheraTest Laboratories, Inc.
1120 N. DuPage Ave.
Lombard, IL 60148
Tel: 1-800-441-0771
1-630-627-6069
Fax: 1-630-627-4231
e-mail: support@TheraTest.com
www.TheraTest.com