THERATEST LABS
Innovations in Immunoassays

EL-aCL™

An enzyme immunoassay for the detection and measurement of anticardiolipin autoantibodies

(screening and isotype specific measurement-IgM,IgG,IgA)

For Professional Use Only

Instruction Manual

EL-aCL™ screen	**Catalog No. 202-202**
EL-aCL™ IgM, IgG, IgA	**Catalog No. 201-202**

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TABLE OF CONTENTS

INTRODUCTION

Intended Use

FOR IN VITRO DIAGNOSTIC USE

The TheraTest EL-aCL™ is an in vitro diagnostic test for the detection and measurement of autoantibodies in human serum directed against the phospholipid cardiolipin.

Summary and Explanation

Autoantibodies against phospholipids have been associated with thrombosis, fetal wastage, and thrombocytopenia and have been detected by three basic methods.[1] The first is a group of tests termed the Standard Tests for Syphilis (Venereal Disease Research Laboratory Test, rapid plasma reagin test, and Wasserman test). The second method is defined by the ability of antiphospholipid antibodies to prolong in vitro clotting, such as in tests for the partial thromboplastin or prothrombin times (i.e., the lupus anti-coagulant). The third method is the anticardiolipin test, which utilizes solid phase radioimmunoassay or ELISA techniques to detect antibodies that bind to cardiolipin-coated wells. Anticardiolipin antibodies may be IgM, IgG, or IgA. The presence of IgG anticardiolipin antibody appears to be the most predictive and specific test for thrombosis, recurrent fetal loss, and thrombocytopenia.[1-7] However, patients with "anticardiolipin associated clinical features" often have elevated IgM and IgA anticardiolipin antibodies [4-6,] therefore, it has become clinically relevant to determine the presence of each of these immunoglobulin (Ig) classes of autoantibodies in patient serum. The TheraTest EL-aCL™ is a diagnostic test that can be used to screen sera for the presence of anticardiolipin antibodies, and further, to measure the levels of IgM, IgG, and IgA anticardiolipin in sera that are found positive in the aCL screening test.

Method Description

The TheraTest EL-aCL™ test is a solid phase enzyme immunoassay based on a 96-well plate format. The cardiolipin-coated wells are incubated with either dilute serum Specimens or dilute Positive and Negative Controls, including Calibrator(s). During the incubation, anticardiolipin antibodies present in the test sample are bound to the solid phase antigen. The wells are washed and horseradish peroxidase-labeled anti-human immunoglobulin antibody is added. After incubation, unbound labeled antibody is removed by aspiration and washing. A chromogenic substrate is added and the presence of antibodies to cardiolipin is detected by a color change read by a spectrophotometer. The absorbance value of a diluent blank well is subtracted from all the values obtained in the wells with human serum.

First, sera are screened for the presence of anticardiolipin antibodies in a test using a polyvalent antibody against human IgM, IgG, and IgA. If the test is positive, the level of each anticardiolipin antibody class is determined in a test using class-specific anti-(human)Ig antibodies: anti-IgM, anti-IgG, and anti-IgA. Alternatively, the Ig class specific evaluation may be performed without prior screening.

REAGENTS

Upon receipt, store all reagents at 2º - 8°C. Do not freeze. Allow all reagents to equilibrate to room temperature (18º - 25°C) prior to use. All reagents except the Wash Buffer must be prepared immediately prior to use and discarded thereafter. When stored at 2º - 8°C, the diluted Wash Buffer is stable for 8 weeks.

1. Antigen Coated Wells

Each plate contains twelve strips, defined as a length of eight wells. For each assay planned, the frame and the required number of strips are removed from the pouch; any remaining unused strips should be promptly placed in the metalized pouch with desiccant; this assembly is then placed in a zip-seal pouch, which is sealed and stored refrigerated (2º - 8ºC). The frame should also be saved. Unused wells stored in a previously opened pouch should be used within 3 months.

2. aCL Specimen Diluent

Buffer containing serum and preservative.

3. aCL Wash Buffer (10X)

10X concentrated buffer containing preservative

4. aCL Ig Enzyme Tracer (for aCL™ screen kit only) (*Note*: Enzyme Tracer may also be called Enzyme Conjugate.)
Anti-Human Ig antibody (IgM, IgG, and IgA) conjugated with horseradish peroxidase. The tracer has been diluted and is ready for use. Solution is colored red.

5. aCL IgM Enzyme Tracer (for aCL™ IgM, IgG, IgA kit only)
Anti-Human IgM antibody ($Fc_{5\mu}$ specific) conjugated with horseradish peroxidase. The tracer has been diluted and is ready for use. Solution is colored blue.

6. aCL IgG Enzyme Tracer (for aCL™ IgM, IgG, IgA kit only)
Anti-Human IgG antibody (Fcγ specific) conjugated with horseradish peroxidase. The tracer has been diluted and is ready for use. Solution is colored green.

7. aCL IgA Enzyme Tracer (for aCL™ IgM, IgG, IgA kit only)
Anti-Human IgA antibody (α-chain specific) conjugated with horseradish peroxidase. The tracer has been diluted and is ready for use. Solution is colored brown.

8. Chromogen
Buffered substrate and chromogenic indicator 3,3',5,5'-tetramethylbenzidine [TMB]. Ready for use. Protect from light.

9. Stop Reagent
2 mol/L phosphoric acid

10. aCL IgM Positive Control* (for aCL™ IgM, IgG, IgA kit only)
Human serum containing IgM antibodies to cardiolipin, and preservative. See enclosed Data Sheet for performance characteristics.

11. aCL IgG Positive Control* (for aCL™ IgM, IgG, IgA kit only)
Human serum containing IgG antibodies to cardiolipin, and preservative. See enclosed Data Sheet for performance characteristics.

12. aCL IgA Positive Control* (for aCL™ IgM, IgG, IgA kit only)
Human serum containing IgA antibodies to cardiolipin, and preservative. See enclosed Data Sheet for performance characteristics.

13. aCL Negative Control*
Human serum containing preservative. See enclosed Data Sheet for performance characteristics.

14. aCL Calibrators*: a) screen; b) IgM; c) IgG; d) IgA
Human serum, diluted in buffer, contains IgM, IgG, and/or IgA antibodies to cardiolipin, as well as preservative. See enclosed Data Sheet for performance characteristics.

*Reagents Containing Sodium Azide

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE.

Reagents Containing Human Source Material

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 1/2. 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," 5th ed., 2009. HHS Publication (NIH and CDC). Web site: http://www.cdc.gov/biosafety/publications/index.htm

Stop Reagent (2 mol/L Phosphoric Acid)

May cause severe irritation on 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 Community Hazardous Substance Risk Phrases (Council Directive 88/79/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 (8).

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. aCL Wash Buffer, Chromogen, and Stop Reagent are interchangeable among the kits. All other reagents are kit and lot specific and therefore not interchangeable.
2. The Stop Reagent can irritate eyes and mucous membranes.
3. Do not allow Chromogen to come in contact with metal or oxidizing agents.
4. Handle patient sera and kit reagents with appropriate precautions. Do not pipette by mouth.
5. Do not use test components beyond expiration date.
6. Avoid microbial contamination of the reagents. If solutions become turbid, they should not be used.
7. Avoid exposure of reagents to excessive heat or light during storage.
8. Use disposable labware or wash all glassware and plasticware thoroughly according to standard laboratory practice.

SPECIMEN REQUIREMENTS

Collection and Storage of Serum

A whole blood specimen should be obtained (in a red top tube) using accepted medical techniques to avoid hemolysis. The blood should be allowed to clot and the serum separated by centrifugation. Unseparated blood can be stored at 18º - 25°C for 24 hours before the separation of serum. The test serum should be clear and non-hemolyzed. Serum samples may be stored at 2º - 8°C for up to 14 days prior to testing. If testing cannot be completed within 14 days of collection, the separated serum must be stored at -20°C. Allow serum to equilibrate to room temperature (18 - 25°C) prior to testing. Do not use serum that has been thawed more than once or that has been heat inactivated.

PROCEDURE

Materials and Reagents Supplied with Each Kit

Materials	**EL-aCL screen**	**EL-aCL IgM, IgG, IgA**
aCL Plate, 96 wells each	2	2
aCL Specimen Diluent	2 x 100 mL	1 x 100 mL
aCL Wash Buffer (10X)	1 x 100 mL	1 x 100 mL
aCL Ig Enzyme Tracer	1 x 25 mL	
aCL IgM Enzyme Tracer		1 x 15 mL
aCL IgG Enzyme Tracer		1 x 15 mL
aCL IgA Enzyme Tracer		1 x 15 mL
Chromogen	1 x 27 mL	1 x 27 mL
Stop Reagent	1 x 27 mL	1 x 27 mL
aCL Ig Positive Control	1 x 0.35 mL	
aCL IgM Positive Control		1 x 0.35 mL
aCL IgG Positive Control		1 x 0.35 mL
aCL IgA Positive Control		1 x 0.35 mL
aCL Negative Control	1 x 0.35 mL	1 x 0.35 mL
aCL screen Calibrator	2 x 1.5 mL	
aCL IgM Calibrator		1 x 1.5 mL
aCL IgG Calibrator		1 x 1.5 mL
aCL IgA Calibrator		1 x 1.5 mL

Materials Required but not Provided

In addition to the reagents supplied with the TheraTest EL-aCL™ kit, the following are required:

1. Calibrated adjustable micropipettors:
   a) multichannel pipettors: 12 channels, 50-300 µL (±5%)
   b) single-channel micropipettor, 5-50 µL (±5%)
   c) single-channel micropipettor, 200-1000 µL (±5%)
   d) 8-channel repeat pipettor, 50-200µL (±10%) - for plate washing (optional)
2. Glass or plastic pipettes (1 mL, 5 mL, and 10 mL)
3. Pipette tips (blue, yellow, plugged)
4. 1.2 mL minitubes (cluster tubes or dilution tubes)
5. Timer
6. Multichannel pipette reagent reservoirs
7. Single (450 nm) or dual (450 nm test, 620 - 690 nm reference) wavelength spectrophotometer (ELISA reader) for 96-well microtiter plates, capable of linear absorbance readings from 0.02 to 2.5
8. Deionized water (resistivity >1MOhm) or purified water for irrigation, USP
9. Clean wash bottle and automated plate washer (optional)
10. 1 liter graduated cylinder (Class B or better)
11. Absorbent paper towels

Reagent Preparation

1. 1X Wash Buffer
   The aCL 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 the diluted Wash Buffer thoroughly. When stored at 2º - 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.***
2. **Enzyme Tracers**
   The aCL Enzyme Tracers have been optimally diluted and are ready for use. The aCL Ig Enzyme Tracer can be used initially for screening. The positive samples subsequently are tested with use of the class-specific aCL Ig Enzyme Tracers.
3. **Specimens, Positive Control, and Negative Control**
   The Specimens and Controls must be diluted 1:101 prior to use. For example, pipette 10 µL of the appropriate Specimen into 1 mL of aCL Specimen Diluent. The unused diluted Specimen is discarded when the assay is completed.
4. **aCL Calibrators**
   The Calibrators are prediluted and ready for use

Screening for Anticardiolipin Antibody

1. Use **the EL-aCL™ screen** kit for this purpose. Allow all reagents and patient sera to equilibrate to room temperature (18º - 25°C).
2. When screening for the presence of anticardiolipin antibody, Blanks, Calibrator, Positive and Negative Controls **must** be included on each run. In the blank wells, Specimen Diluent may be added or not; the wells may be left empty until the next step (tracer). If desired, the test can be run in duplicate.
3. Determine the number of strips needed. Place the remaining unused strips in the metallized pouch with desiccant; place this assembly in the zip-seal pouch and store refrigerated (2º - 8ºC) for future use.
4. Mark the position of the samples (i.e., Calibrator, Positive Control, Negative Control, Blank and Specimens) on a copy of the plate layout shown on the enclosed Data Sheet.
5. Dilute all samples (but not the Calibrator) 1:101 in aCL Specimen Diluent (see Reagent Preparation section) and mix well. Pipette 100 µL of the prediluted Calibrator and 100 µL of each of the diluted samples (i.e., Positive Control, Negative Control, and patient Specimens), as well as 100 µL of Specimen Diluent, into the appropriate wells. All wells of each plate should be pipetted within 5 minutes.
6. Incubate the plate for 30±5 minutes at room temperature (18º - 25°C).
7. Remove the sample fluid from the wells by aspiration with a vacuum device or by manually decanting the liquid from the plate into a container or sink designated for biohazardous waste. Fill all wells with 1X Wash Buffer (approximately 250 - 350 µL per well) but do not overflow wells. Remove Wash Buffer from wells by aspiration or by decanting liquid into an appropriate disposal container or sink. Repeat two more times for a total of three washes. Remove all residual liquid from the wells by tapping the inverted plate on absorbent paper.. (*Note: a semi-automated plate washer may be used to accomplish the washing procedure.)*
8. Immediately pipette 100 µL of Ig Enzyme Tracer into each well. Complete this step within 5 minutes.
9. Incubate the plate for 30±5 minutes at room temperature (18º - 25°C).
10. Remove the Enzyme Tracer from the wells by aspiration with a vacuum device or by manually decanting the liquid from the plate into a container or sink designated for biohazardous waste. Wash all wells three times as described above in Step **7**.
11. Immediately pipette 100 µL of Chromogen into each well. Incubate the plate for 15±1 minutes at room temperature (18º - 25°C). Protect plate from exposure to direct light. The cardiolipin-coated wells that have been incubated with the Positive Control and the Calibrator will develop a blue color.

12. At the end of the incubation period, pipette 100 µL of Stop Reagent into each well and mix by gently tapping the plate. The blue color will immediately change to yellow.
13. Firmly seat 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.

Class specificity and levels of anticardiolipin antibodies

1. Use the **EL-aCL™ IgM,IgG,IgA** kit for this purpose**.** Allow all reagents and patient sera to equilibrate to room temperature (18º - 25°C).
2. Determine the number of cardiolipin-coated strips required. Each antibody class determination requires the use of separate Blank**,** Calibrator, Controls and Specimen wells. For best performance, do each test in duplicate. The plate map on the Data Sheet provided with the kit can be used to mark the location of the samples.
3. Dilute all samples 1:101 (but not the Calibrators) in aCL Specimen Diluent (see Reagent Preparation section) and mix well. Dispense 100 µL of the Calibrator (as supplied), and 100 µL of each of the diluted Controls and Specimens as well as 100 µL of Specimen Diluent into the appropriate wells.
4. Follow steps **6 - 13** as described in the "**Screening for Anticardiolipin Antibody**" section with the following exceptions: IgM, IgG and IgA Enzyme Tracers are used instead of an Ig Enzyme Tracer. and separate IgM, IgG, & IgA isotype specific Positive Controls are used instead of a single Positive Control. Each antibody tracer is provided ready for use. As delineated on the Data Sheet, add the desired antibody tracer to the appropriate wells. Repeat- and/or multichannel-pipettors are suggested for timely distribution of the tracer solutions.

Procedural Comments

1. **Storage**
   The wells that are not used during the assay should be promptly placed in the metallized pouch with desiccant; this assembly is then placed in the provided zip-seal pouch, which is sealed and stored at 2º - 8°C. Wells stored in a previously opened pouch should be used within 3 months.
2. **Washing**
   Each column of wells may be washed using a multichannel pipettor or a repetitive pipettor. The wells may be aspirated or decanted into an appropriate disposal container or sink. Alternatively, commercial semi-automated washing systems may be used. When using either washing technique, the wells must be blotted thoroughly on absorbent paper after the last wash.
3. **Pipetting**
   To avoid cross-contamination and sample carryover, the Specimen Diluent, Positive Controls, Negative Control, Calibrators and test Specimens **must** be pipetted using separate pipette tips. When testing multiple Specimens, a calibrated multichannel pipettor should be used to pipette the Enzyme Tracer, Wash Buffer, Chromogen, and Stop Reagent. Avoid cross-contamination of these reagents by using separate tips also.
4. **Measurement of Absorbance Values**
   Absorbance values should be measured within 30 minutes after completion of an assay. If the absorbance value for a well exceeds the limit of detection for the instrument, an approximate value may be obtained as follows: remove 100 µL from the well and add 100 µL of deionized or distilled water to the well; determine the net absorbance value and then multiply that value by 2. This dilution procedure may be repeated. The absorbance values obtained from this dilution procedure are only approximations of antibody bound to the well.

QUALITY CONTROL

1. The **Blank, Calibrator** and **Positive** and **Negative Controls <u>must</u>** be run each time the assay is performed.

2. **Controls:** In order to validate the assay, the **Positive** and **Negative Control** values **must** be within the ranges stated on the enclosed Data Sheet. If the values obtained fall outside the specified ranges, the entire run should be discarded and the test repeated.
3. **Blanks:** The absorbance value(s) of the Blank(s) should be <0.200.
4. **Calibrator:** The net absorbance values of the Calibrators should fall within the ranges given on the Data Sheet.

RESULTS

Determination of Net Absorbance Values

The net absorbance value for each sample is calculated by subtracting the raw absorbance value of the Diluent Blank well from the raw absorbance value of the sample well.

Example:

Absorbance for Blank well = 0.150
Absorbance for sample well = 1.150
Net Absorbance for sample is 1.150 - 0.150 = 1.000

NOTE: *If the absorbance of the Diluent Blank well is higher than the absorbance of the sample well, the net absorbance should be considered 0. When measurements are done in duplicate, then the mean absorbance value for the duplicate wells is determined.*

Calculation of Anticardiolipin Antibody Activity with a One-Point Calibrator

When the Kits are used either for screening a test Specimen or for determining specific antibody class, anticardiolipin antibody activity in Units/mL is calculated as follows:

$$\text{Conversion Factor} = \frac{\text{Calibrator aCL activity value}}{\text{net Abs of Calibrator}} \quad \text{(See Data Sheet for Calibrator activity)}$$

Sample aCL activity = Conversion Factor x net Absorbance of Sample

The **EL-aCL™ IgM,IgG,IgA** kit contains a separate Calibrator for each Ig isotype (IgM Calibrator, IgG Calibrator, and IgA Calibrator) and a separate Positive Control for each Ig isotype. Therefore, the isotype specificity of the Antibody Tracer used **must match** the isotype specified on the label of the Calibrator (or Positive Control). For example, the IgM Tracer must be used with the aCL IgM Calibrator and with the aCL IgM Positive Control. The values for the Calibrators are reported on the enclosed Data Sheet. The Conversion Factor for each Calibrator must be calculated every time the Calibrator is used for testing. If the absorbance value of the Specimen exceeds the limits of the reader and an endpoint is needed, the sample should be diluted further in aCL Specimen Diluent (e.g., 1:500, 1:1000, and 1:2500) and re-tested. The calculated autoantibody Units are then multiplied by the utilized additional dilution factor to provide the final value.

Limitations of the Procedure

1. The TheraTest EL-aCL Test should not be performed on grossly hemolyzed, microbially contaminated, or grossly lipemic samples. This method has been tested with serum samples only. The performance using other specimen types has not been determined.
2. Except for SLE, the clinical significance of a positive test is unknown and under investigation.
3. Despite a negative aCL test, if clinically indicated, the Lupus anticoagulant should be measured.
4. Diagnosis should not be made solely on the basis of a positive test result. The results must be interpreted in conjunction with all clinical information available to the physician (i.e., history, physical exam and other diagnostic procedures).

5. Treatment of a patient should not be initiated solely on the basis of a positive aCL test. Supportive clinical information should be available.
6. A high percentage of confirmed active or seropositive syphilis patients will have elevated aCL levels. Confirmatory procedures should be performed to rule out syphilis in aCL-positive individuals.
7. Rheumatoid factor can interfere with IgM aCL determinations.

EXPECTED VALUES

The TheraTest EL-aCL is designed to measure the class (Ig isotype) and level of anticardiolipin antibodies in human serum.

Normals

100 healthy blood donors were tested.

Example:

Assay	Percentile	Normal Values	Equivocal	Abnormal
EL-aCL Screen	95	≤ 8 aCL Units		≥ 9
EL-aCL IgM	95	≤ 10 MPL	11-13	≥ 14
EL-aCL IgG	98	≤ 15 GPL	16-20	≥ 21
EL-aCL IgA	99	≤ 7 APL	8-10	≥ 11

(Normal values should also be determined by the individual laboratory, since values may vary with age groups.)

The Units for IgG and IgM have been determined according to the SSC Subcommittee for the Standardization of Lupus Anticoagulants. The test's sensitivity was evaluated on 100 SLE patients and on 80 patients with recurrent spontaneous abortions. We identified 62% as positive for IgG; a meta-analysis of 29 published studies comprising over 1000 SLE patients determined that on the average 44% of SLE patients have IgG anti-cardiolipin antibodies.[7]

TABLE I. Percentages of abnormal levels of IgG, IgM and IgA anticardiolipin antibodies in patients with recurrent abortions (N=80) and in patients with systemic lupus erythematosus (SLE, N=100)

% Abnormal

Abortions			SLE		
GPL	***MPL***	***APL***	***GPL***	***MPL***	***APL***
25%	18%	4%	62%	59%	22%

GUIDE TO INTERPRETATION

1. The TheraTest EL-aCL is a semi-quantitative assay that measures autoantibodies in serum at a standard dilution of 1:101.
2. The detection of IgG anticardiolipin antibody at moderate to high levels is associated with increased risk for thromboembolic events[1] or fetal loss.[2,3,4]
3. The presence of IgA anticardiolipin antibody also may be associated with increased risk of fetal loss, but insufficient data exist at this time for a definitive statement.
4. The presence of IgM anticardiolipin antibody may be nonspecific.

PERFORMANCE DATA

Within-Run Precision

Within-run precision was determined by assaying, in at least 10 wells, a Specimen containing autoantibodies for each antibody class. The coefficient of variation was found to be 11% for IgM, 6% for IgG and 9.5% for IgA.

Between-Run Precision

Between-run precision was determined by assaying a specimen containing autoantibodies in 16 separate runs (plates) by two different technologists on two separate lots over a period of two months. The coefficient of variation was found to be 18% for IgM, 14% for IgG and 14% for IgA.

Specificity

Soluble cardiolipin was shown to inhibit the detection of anticardiolipin antibodies by greater than 80%.

TROUBLESHOOTING

Problem	Possible Causes	Solution
Positive and/or Negative Control 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 & reread.
	5. Wavelength of filter incorrect.	5. Change filter to 450 ± 5 nm.
	6. Blank OD > 0.200.	6. a) An excess of Tracer was added to the wells. b) the incubation time was too long. c) insufficient washing. d) a damaged or dirty well. e) Chromogen is contaminated; replace.
Calibrator OD values out or 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. Wavelength of filter incorrect.	2. Change filter to 450 ± 5 nm.
	3. Contamination of Calibrator in well.	3. Repeat test; pipette carefully.
	4. Blank OD out of range.	4. a) an excess of 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 & reread. e) Chromogen is contaminated; replace.
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. Antigen-coated plate inactive.	3. Check for obvious moisture in unused wells. Rerun test with Controls only for activity check..
All test results yellow.	1. Contaminated Chromogen.	1. Check absorbance of unused Chromogen
	2. Contaminated buffers & reagents.	2. Check all reagents 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.	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;	3. Develop consistent uniform technique and avoid

inconsistency in timing intervals, air bubbles.	splashing or use multi-tip device or autodispenser to decrease reagent delivery time.
4. Air currents blowing over plate during incubations.	4. Cover plate or place in 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 of wash cycle.	7. Use only acceptable washing devices. Lengthen timing delay on automated washing devices. 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.

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Abbreviated Test Procedure

1. **Dilute Controls and Specimens 1:101 with aCL Specimen Diluent.**
2. **Pipette 100 µL of *pre*-diluted Calibrator into each of the designated wells.**
3. **Pipette 100 µL of *Specimen Diluent*, diluted Controls and diluted Specimens into the designated wells.**
4. **Incubate for 30±5 minutes at room temperature (18° - 25°C).**
5. **Wash the wells three times with 1X aCL Wash Buffer.**
6. **Add 100 µL of the appropriate Tracer (Enzyme Conjugate) to each well.**
7. **Incubate for 30±5 minutes at room temperature (18° - 25°C).**
8. **Wash the wells three times with 1X aCL Wash Buffer.**
9. **Add 100 µL Chromogen to each well.**
10. **Incubate for 15±1 minutes at room temperature (18° - 25°C).**
11. **Add 100 µL Stop Reagent to each well.**
12. **Read the absorbance at 450 nm, reference 620-690 nm, within 30 minutes.**

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