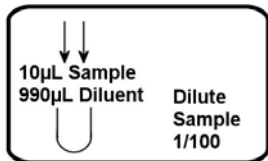
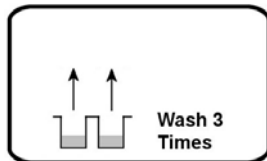


Test Procedure

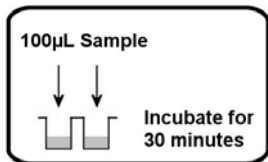
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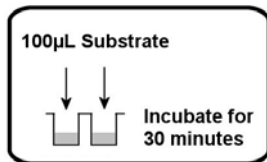
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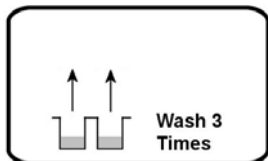
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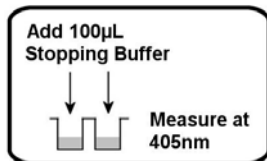
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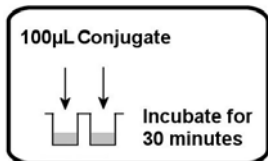
3.



7.



4.



AUTOZYME™ ACL

ACL Screen Z4796

Instructions for Use

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Bibliography

Asherson, R.A. *et al*, (1987) Recurrent stroke and multi-infarct dementia in systemic lupus erythematosus: association with anti-phospholipid antibodies. *Ann. Rheum. Diseases*, **46**, 605-611.

Gharavi, A.E. *et al*, (1987) Anti-cardiolipin antibodies - isotype distribution and phospholipid specificity. *Ann. Rheum. Diseases*, **46**, 1-6.

Harris, E.N. *et al*, (1983) Anti-cardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*, 1211-1214.

Harris, E.N. *et al*, (1985) Anti-phospholipid antibodies. *Clin Rheum Dis*, 11/3, 591-609.

Valesini, G. *et al*, (1992) A new player in the anti-phospholipid syndrome: the β_2 -Glycoprotein-1 co-factor, *Autoimmunity* **14**, 105-110.

Loizou, S. *et al*, (1985) Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardisation and quantitation of results, *Clin. Exp. Immunol.* **62**, 738-745.

Hughes, G.R.V. *et al*, (1989). Anti-phospholipid syndrome linking many specialities, *Ann. Rheum. Diseases* **45**, 355-356.

Unander, A.M. *et al*, (1987) Anti-cardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am. J. Obstet. Gynecol*, **156**/1, 114-119.

Molina, J. F. *et al*, (1997) Variability of anti-cardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J. Rheumat*, **24**/2, 291-296.

Wilson, W. A. *et al*, (1999) International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. *Arthritis & Rheumatism*, **42**/7, 1309-1311.

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Kit Contents Symbols

CAL	Calibrator
CONTROL -	Negative Control
CONTROL +	Positive Control
BUF WASH	Wash Buffer
DIL SPE	Sample Diluent
CONJ	Conjugate solution
SUB	Substrate solution
STOP	Stop Solution
SORB	Solid Phase – Antigen Coated Wells

1. Intended Use

The AUTOZYME™ Anti-cardiolipin (ACL) Screen is a sandwich immunoassay for the qualitative detection of the combined IgA, IgG and IgM class anti-cardiolipin autoantibodies in human serum or plasma. AUTOZYME™ ACL Screen results are expressed in U/mL. AUTOZYME™ ACL Screen is intended as an aid in the diagnosis of an increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or lupus like disorders and the assay is suitable for use on open automated immunoassay systems.

2. Background

The anti-phospholipid syndrome (APS) or Hughes' syndrome is an autoimmune disease with variable and diverse clinical manifestations characterised by arterial and/or venous thrombosis, recurrent foetal loss and elevated titres of anti-phospholipid antibodies. The syndrome may be either primary or may occur in the setting of an associated disease (secondary) most frequently SLE or other autoimmune diseases. Lupus anticoagulants and anti-cardiolipin antibodies to IgG and/or IgM classes at medium to high titres are laboratory indicators of the condition as recommended by the Sapporo Criteria. Patients with secondary APS associated with SLE have more episodes of arthritis and livedo reticularis, and more frequently exhibited thrombocytopenia. ACL is seen in approximately 88% of patients. Furthermore, ACL antibodies have been found in some non-thrombotic neurological disorders like cerebrovascular insufficiency, cerebral ischemia or chorea and in myocardial infarction. Recent studies indicate that elevated levels of ACL IgA associated with respect to different ethnic groups as well as

13. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use only.

The **substrate** contains ABTS™ which is harmful if swallowed in large quantities and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water.

The sera used for **calibrators and controls** contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen (HbsAg) using FDA approved assays, no test can guarantee their absence.

Therefore, the calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

Safety data sheets are available on request.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic acid) is a trademark of Roche Diagnostics.

c. Analytical Specificity and Sensitivity:

No cross-reactivity with Rheumatoid Factor or dsDNA antibodies that may be present in patients with Systemic Lupus Erythematosus (SLE) was observed. Neither was there cross-reactivity from myeloma sera samples.

There is no interference from bilirubin at 0.5 mg/mL, ascorbate at 2 mg/mL, haemoglobin at 5 mg/mL, lipids at 10% w/v or from the use of anticoagulants.

Measurement of over 700 positive and negative sera samples on AUTOZYME™ ACL Screen and on the AUTOZYME™ ACL IgG, IgM and IgA kits gave the following sensitivity and specificity results:

Sensitivity	100.0%
Specificity	94.8%
Positive predictive value	95.6%
Negative predictive value	100.0%
Agreement	97.6%

A negative result should not be used as a sole criterion to rule out APS or other autoimmune disease, but must be taken in relation to other clinical observations and diagnostic tests.

The precision of the ACL Screen kit is sufficient to allow samples to be measured in single determinations when the assay protocol is adhered to.

ACL IgG and IgM are found frequently in these patient groups. The determination of IgM antibodies is a valuable indicator in the diagnosis of the beginning of autoimmune diseases and there is a significant association between ACL IgM and haemolytic anaemia. A serum cofactor is needed for the detection of ACL which is identified as a 50kD protein known as β 2-glycoprotein 1 and this acts to facilitate the binding of ACL antibodies to cardiolipin antigen.

3. Principle

The AUTOZYME™ ACL Screen employs a unique antigen-coated microwell technology, which is ideal for the batch-screening of both large and small numbers of samples for ACL.

First incubation:

AUTOZYME™ ACL wells are provided coated with purified antigen (cardiolipin and β 2-glycoprotein 1 cofactor). When calibrators or diluted sera are added, any ACL present will bind to the well surface. The wells are then washed in wash buffer to remove unbound serum antibodies.

Second incubation:

Goat anti-human IgA/IgG/IgM peroxidase conjugate is added to the wells, which will bind to any captured ACL. Unbound conjugate is removed by washing in wash buffer.

Third incubation:

A colourless to pale green enzyme substrate is then added to the wells which reacts with the bound conjugate. The intensity of the green colour formed is directly proportional to the concentration of IgA/IgG/IgM ACL antibodies bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

- 1 vial calibrator, 1.5 mL (ready-to-use)
- 1 vial wash buffer concentrate (PBS), 67 mL
- 1 vial sample diluent (PBS), 100 mL
- 1 vial conjugate (anti-IgA/IgG/IgM HRP), 15 mL
- 1 vial substrate (ABTS), 15 mL
- 1 vial stopping buffer (oxalic acid), 15 mL
- 1 foil sachet, containing 1 set of antigen-coated microwells
- 1 vial Positive control, 1.5 mL (ready-to-use)
- 1 vial Negative control, 1.5 mL (ready-to-use)
- 1 instruction leaflet
- 1 QC certificate

5. Storage

The kit should be stored refrigerated at 2-8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight. Keep microplate wells sealed in the foil bag with desiccant until required.

6. Sample Handling

AUTOZYME™ ACL Screen must be performed with human serum or plasma samples. Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing is not advisable as it may give variable loss of autoantibody activity. Do not heat treat samples prior to assay.

The ACL calibrators are calibrated against the original Rayne Institute Reference Standards supplied by Dr E N Harris now at the Anti-phospholipid Standardisation Laboratory, Division of Rheumatology, University of Louisville, Kentucky 40292, USA.

12. Performance Characteristics

a. Precision data:

	Intra-assay precision			n
	Mean ratio	SD	CV %	
Sample 1	1.68	0.046	2.7	20
Sample 2	1.00	0.035	3.5	20
Sample 3	0.39	0.017	4.4	20

	Inter-assay precision			n
	Mean ratio	SD	CV %	
Sample 1	2.98	0.074	2.5	5
Sample 2	1.72	0.063	3.7	5
Sample 3	0.45	0.011	2.4	5

b. Reference values:

AUTOZYME™ ACL Screen was used to determine the ACL of 100 blood donor serum samples measured in duplicate. The mean ratio plus two standard deviations gave a cut-off value of 0.9. It is recommended that each laboratory determines its own reference range.

Any sample giving a value of greater than 0.9 U/mL can be considered positive. Further differentiation and typing should be carried out using the quantitative Anti-cardiolipin IgG, IgM and/or IgA kits.

Example:

$\frac{\text{OD patient sample}}{\text{OD calibrator}} = \text{Ratio U/mL}$

Mean Calibrator absorbance = 0.600

Sample	Mean Absorbance	Value	Interpretation
1	0.200	0.3	negative
2	1.400	2.3	positive
3	0.700	1.2	positive

Refer to QC certificate for positive control, negative control and calibrator absorbance specifications.

The ACL Screen assay recognises the sum of IgA/IgG/IgM class anti-cardiolipin autoantibodies. Samples that give borderline negative results in the individual ACL IgA, IgG or IgM assays may, due to additive effects, give a positive result on ACL Screen.

11. Quality Control

Good laboratory practice requires that quality control specimens be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

7. Additional Reagents and Equipment Required

Deionised or freshly distilled water.

Precision micropipettes to deliver 10 - 1000 μL .

Multichannel micropipette or repeating dispenser to deliver 100 μL .

1000 mL measuring cylinders for reagent preparation.

Automated plate washer (optional).

96-well microplate reader with 405 nm filter (reference filter at 492nm).

Software package (optional).

8. Procedural Precautions

Allow all reagents to equilibrate to room temperature (18°C to 25°C) before use for a minimum of 2 hours. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results. Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change tips between different calibrators, samples or control sera to prevent sample carryover.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be colourless to pale green. Any green colouration above an OD of 0.200 indicates substrate contamination and should be discarded. The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

AUTOZYME™ ACL Screen has been designed so that AUTOZYME™ ACL IgA and ACL IgG and ACL IgM and Screen can be run simultaneously on the same ACL plate if required. All reagents are common with the exception of calibrators, controls and conjugates.

Do not use the kit beyond the expiry date given on the label. Unused reagents and reconstituted wash buffer are stable at 2 - 8°C for 1 month after first opening the container. However, multiple re-use could increase the risk of reagent contamination.

9. Assay Procedure

1. Prepare the following reagents:
Wash buffer: dilute contents of wash buffer concentrate vial to 1000 mL (1/15) with deionised water or proportionally less if not using the whole kit.
2. Dilute the patient samples by 1/100 using the sample diluent e.g. 10 µL sample added to 990 µL diluent. The **calibrator and kit controls** do not require dilution.
3. Remove the antigen-coated microwells from the foil sachet and seal any unrequired wells in the resealable pouch, along with the desiccant sachet.
4. Dispense 100 µL of each calibrator or diluted patient sample into appropriate wells. Incubate for 30 minutes at room temperature (18 - 25°C). It is recommended that the calibrator and controls be tested in duplicate. The patient samples can be tested as singles.

5. Using the diluted wash buffer, wash the wells at least three times either with an automated plate washer set to at least 300 µL per well, or by adding 300 µL to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 µL conjugate to each well and incubate for 30 minutes at room temperature (18 - 25°C).
7. Wash the wells at least three times using the same procedure as in step 5.
8. Dispense 100 µL substrate into each well, ensuring that it is initially pale green and incubate for 30 minutes at room temperature (18 - 25°C).
9. Stop the reaction by adding 100 µL of stopping buffer.
10. Measure the absorbance at 405 nm on a 96-well microplate reader and calculate the results. Dual wavelength measurement may be performed with a reference at 492nm.

The assay procedure detailed above is appropriate for use with open automated ELISA processors as well as manual operation.

10. Calculation of Results

From a laboratory study of 100 blood donor samples, a cut-off level has been determined at 0.9 times the mean calibrator absorbance.

Calculate the ratio of samples to the calibrator by dividing the mean sample absorbance by the mean calibrator absorbance.