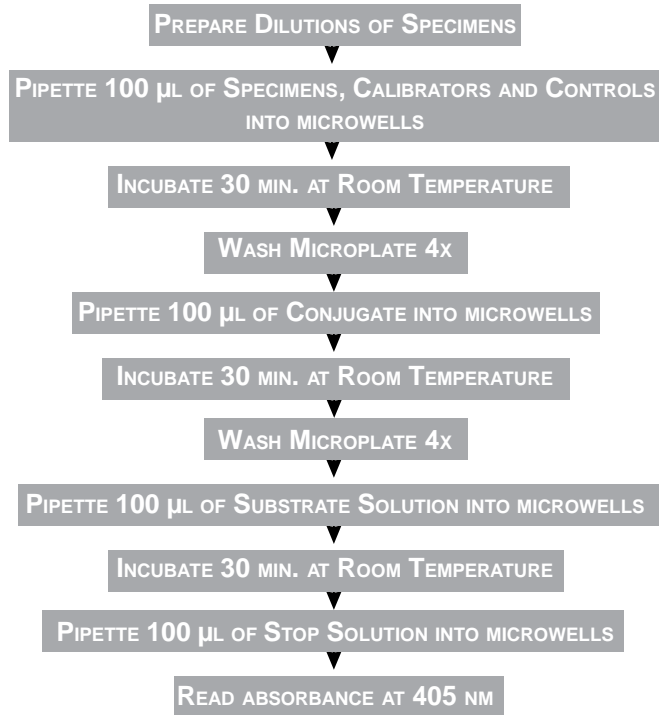


ImmuLisa™ PROCEDURE AT A GLANCE



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ImmuLisa™ Anti-Histone Antibody (AHA) ELISA

For *in vitro* Diagnostic Use
CLIA Complexity: High
CDC Analyte Identification Code: 0437
CDC Test System Identification Code: 28354

PRODUCT INSERT

Catalog No. 1119

96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-histone antibodies in human serum.

SUMMARY AND EXPLANATION

Antibodies to histone, a protein associated with DNA in the nucleus of the eukaryotic cells, occur in a number of clinical conditions but primarily in *systemic lupus erythematosus* (SLE), drug induced LE and in drug induced antinuclear antibody positive patients¹⁻⁸. There are several assays for the detection of anti-histone antibodies but indirect immunofluorescence and ELISA seem to be more specific and have been used more frequently¹⁻⁸. Indirect immunofluorescence methods, even though specific for anti-histone antibodies, can not be used with sera that contain anti-DNA antibodies⁵. In addition, anti-histone antibodies to H3 and H4 are not detected by the indirect immunofluorescence method. Because of the limitations of the latter assay, the ELISA method has been the method of choice for the detection of anti-histone antibodies.

Anti-histone antibodies occur in approximately 50% of unselected SLE sera and in 83% of active SLE patients⁶. Almost all patients with drug induced LE and 22% of patients with drug induced ANA are positive for anti-histone antibodies¹. In addition to idiopathic and drug induced LE, anti-histone antibodies also occur in approximately 10-15% of patients with rheumatoid arthritis and in patients with mixed connective tissue disease (MCTD) and scleroderma. The anti-histone antibody levels are much higher in idiopathic and drug induced LE as compared to rheumatoid arthritis, MCTD and scleroderma.

With the advent of the ELISA method, anti-histone antibodies have been found to be of both IgG and IgM isotypes. In idiopathic SLE both IgG and IgM anti-histone antibodies occur. A correlation between IgG anti-histone antibody levels and the severity of lupus has also been reported³⁻⁶. However, in drug induced LE the incidence of anti-histone antibodies of each class varies. For example, in procainamide and hydralazine induced LE, the anti-histone antibodies are predominantly of the IgM class⁴.

PRINCIPLES OF PROCEDURE

The anti-histone antibody test is performed as a solid phase immunoassay (ELISA). Microwells are coated with various types of histone antigen followed by blocking the unreacted sites to reduce non-specific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-histone antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding a polyvalent enzyme labeled anti-human conjugate to the wells. The enzyme conjugated antibodies bind specifically to the human immunoglobulin. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to histone is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. The results are expressed in ELISA units (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. **Do not freeze.**

Do not use, if reagents are not clear or if a precipitate is present. All reagents must be brought to room temperature (20°-25°C) prior to use. When stored at 2°-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

Precautions

For *in vitro* Diagnostic Use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials¹².

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO DIAGNOSTICS. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

NOTES

REFERENCES

1. Epstein A, Greenberg M, Halbert S, Kramer L and Barland P. The clinical application of an ELISA technique for the detection of anti-histone antibodies. *J Rheumatol* 13:304-307, 1986.
2. Cohen MG, Pollard KM, Webb J. Antibodies to histone in systemic lupus erythematosus, prevalence, specificity and relationship to clinical and laboratory features. *Ann Rheum Dis* 51:61-66, 1992.
3. Gompertz NR, Isenberg DA, Turner BM. Correlation between clinical features of systemic lupus erythematosus and levels of anti-histone antibodies of the IgG, IgA, and IgM isotypes. *Ann Rheum Dis* 49:524-527, 1990.
4. Rubin RL. Antihistone antibodies. In "Systemic Lupus Erythematosus", Lahita RG, Ed, Wiley, New York, 271-289, 1987.
5. Portanova JP, Rubin RL, Joslin FG, Agneloo VD, Tan EM. Reactivity of antihistone antibodies induced by procainamide and hydralazine. *Clin Exp Immunol* 25:67-79, 1982.
6. Gioud M, Aitkaci M, Monier JC. Histone antibodies in systemic lupus erythematosus. *Arthr Rheum*: 25:407-413, 1982.
7. Costa O, Monier JC. Anti-histone antibodies detected by ELISA and immunoblotting in systemic lupus erythematosus. *J Rheumatol* 13:722-725, 1986.
8. Aitkaci, Monier JC, Mamelle N. Enzyme-linked immunosorbent assay for anti-histone antibodies and their presence in systemic lupus erythematosus sera. *J Immun Methods* 44:311-322, 1987.
9. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control, National Institutes of Health, (HHS Pub. No. (CDC) 93-8395) 1993.

Materials provided

ImmuLisa™ Anti-Histone (AHA) Antibodies

Catalog No. 1119

Kit contains sufficient reagents to perform 96 determinations.

- 12 x 8** Ready to use **Microplate** with individual breakaway microwells coated with AHA antigen.
- 1 x 1.5 ml** *Ready to use **Positive Control** (*red cap*). Contains human serum positive for AHA antibodies. The expected concentration range in EU/ml is printed on the label.
- 1 x 1.5 ml** *Ready to use **Negative Control** (*white cap*). Contains human serum.
- 4 x 1.5 ml** *Ready to use **set of 4 Calibrators**; Calibrator A (*green cap*), Calibrator B (*violet cap*), Calibrator C (*blue cap*) and Calibrator D (*yellow cap*). Human serum containing antibodies to histone antigen. Concentrations in EU/ml are printed on the labels.
- 1 x 12 ml** *Ready to use **anti-human Alk. Phos. Conjugate**. Color coded pink.
- 1 x 60 ml** *Ready to use **Serum Diluent**. Color coded blue.
- 1 x 12 ml** *Ready to use **Enzyme Substrate**. Contains pNPP. **Protect from light.**
- 1 x 12 ml** Ready to use **Stop Solution**.
- 2 vials** Powder **Wash Buffer**. Reconstitute to one liter each.
- 1 x extra** Frame Holder
- 2 x** Protocol Sheets
- *CAUTION - Contains <0.1% NaN₃

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper towels
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 630 nm.
- Automatic microplate washer capable of dispensing 200 µl

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

PERFORMANCE CHARACTERISTICS

Precision:

The intra-assay and inter-assay coefficient of variation of the AHA test system was determined by taking positive samples of varying AHA levels and were found to be between 5-10%.

	AHA EU/ml	% C.V.
intra-assay	66.0	6.9
	93.0	9.5
	72.0	7.2
inter-assay	83.8	9.3
	73.3	5.2

The Immulisa™ AHA test kit was compared with another commercially available anti-histone ELISA test kit. A total of 129 sera identified as positive or negative for histone antibody immunofluorescence by a clinical reference laboratory were obtained and tested simultaneously with both kits. The clinical conditions of the patients was not known. All sera were tested according to the performance and quality control procedures recommended by the manufacturer. The relative specificity, relative sensitivity and agreement are shown in the following Table:

Comparison between AHA ELISA Methods

		Immulisa AHA		
		Positive	Negative	Total
Other ELISA	Positive	36	4	40
	Negative	14	75	89
	Total	50	79	129

Agreement: 86%
Sensitivity: 90%
Specificity: 84%

Interpretation

The cutoff values were obtained by testing sera on 100 normal blood donors for AHA. The cutoff levels were established by determining the mean EU/ml. Values less than 2 SD of this normal were considered negative and values between 2-3 SD as equivocal. The following serves only as a guide in the interpretation of the laboratory results. Each laboratory must determine its own normal values. These may vary with the population examined.

AHA value	Interpretation
< 20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

LIMITATIONS OF THE PROCEDURE

CLIA Complexity: High. CDC Analyte Identification Code: 0497. CDC Test System Identification Code: 2828. The Immulisa™ AHA Test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only. The results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves.

EXPECTED VALUES

The incidence of AHA in various disease conditions is summarized in the following Table:

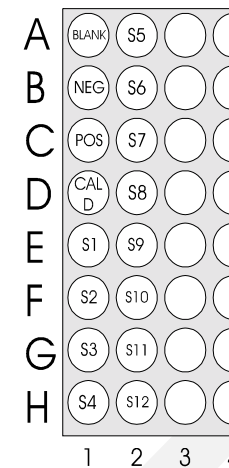
Incidence of Anti-Histone Antibodies

Disease	Incidence %
Systemic lupus erythematosus	42
Drug induced LE	100
Drug induced ANA	22
Rheumatoid arthritis	15
Scleroderma	10
Mixed connective tissue disease	15
Sjögren's syndrome	28
Normal patients	0

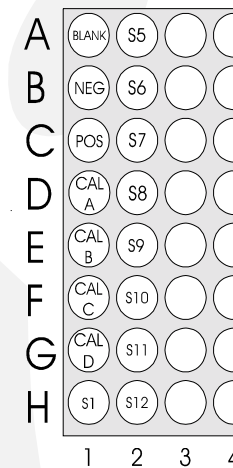
Test Method

- Step 1** Let all reagents and specimens equilibrate at room temperature.
- Step 2** Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.
- Step 3** For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).
- or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

QUALITATIVE DETERMINATION



SEMI-QUANTITATIVE DETERMINATION



- Step 4** Prepare a **1:101** dilution of the patient samples by mixing **5 µl** of the patient sera with **0.5 ml** of Serum Diluent.
- Step 5** Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder .
- Step 6** Pipette **100 µl** of Ready to use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.
Note: Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank.
- Step 7** Incubate **30 minutes** (± 5 min) at room temperature.

- Step 8** Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.
- Step 9** Pipette **100 µl** of Conjugate into microwells.
- Step 10** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 11** Wash all microwells as in Step 8.
- Step 12** Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.
- Step 13** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 14** Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance values within 1 hour from adding Stop Solution.
- Step 15** Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. The Calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be less than 20 EU/ml. If the test is run in duplicate, the mean of the two readings should be taken for determining the concentration of histone antibodies. While performing Qualitative determinations, the optical density of the Calibrator D must be greater than that of the negative control and lesser than the absorbance of the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial.

RESULTS

Calculations

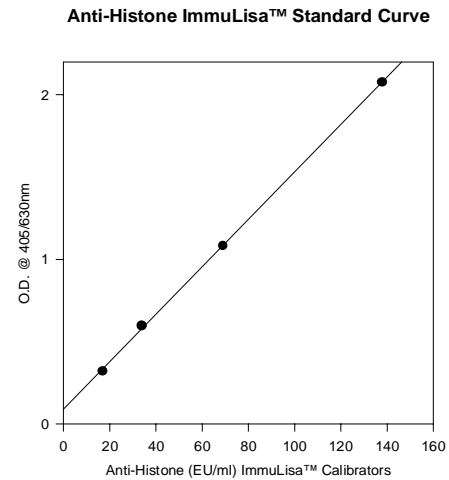
The concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator D}} \times \text{EU/ml of Calibrator D} = \text{EU/ml Test Sample}$$

2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.



Calibrator

The Ready to Use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum sample should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.