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Anti-Histone

ORG 507

96 Tests

**Immunometric Enzyme Immunoassay for
the quantitative determination of IgG
autoantibodies to Histone**

Instruction for use

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NAME AND INTENDED USE

Anti-Histone is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies to histone in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of Systemic Lupus Erythematosus (SLE) or lupus-like disorders.

SUMMARY AND EXPLANATION OF THE TEST

All of the nucleic acids (DNA) of eukaryotic cells is associated with proteins. The complex structure of DNA and its associated small basic proteins, which are called histones is known as chromatin. Both, The histones comprises about 50 percent of the total mass of eukaryotic chromosomes. The complex of DNA and the histones can be dissociated by treatment of the eukaryotic chromatin with salt or diluted acids. Five different types of histones are known. They are called H1, H2A, H2B, H3 and H4. Their molecular weights range from 11 to 21 kD. In correlation to their fundamental function in the organisation of chromatin, the structure of all histones in all eukaryotes is highly conserved. They contain a lot of basic amino acid residues which presumably interact with the negative charged groups of DNA. Furthermore they contain apolar amino acid residues which may be important for their interaction among each other.

Antibodies to histones usually produce a homogeneous, rim or speckled pattern of nuclear staining in indirect immunofluorescence. Antibodies against the histones dimers H2A-H2B are observed within 20 to 50 percent in spontaneous systemic Lupus erythematoses and in 50 to 90 percent in procainamide induced SLE. Compared to other autoantibodies anti-histone antibodies are relatively rare in spontaneous SLE. Autoantibodies to histones are not specific for SLE but are found also in drug induced LE and rheumatoid arthritis (RA). In drug induced LE histone antibodies are found in a three times higher incidence than in SLE.

Most of the anti-Histone antibodies in drug induced LE are of temporary character. They mainly disappear within a few month after treatment with the inducing drug.

Determination of anti-histone antibodies is indicated in:

- drug induced Lupus (mainly after treatment with Procainamide, Isoniazide or Hydralazine)
- drug induced ANA without clinical SLE symptoms
- spontaneous Systemic Lupus erythematoses
- ANA positive and seropositive rheumatoid arthritis
- ANA positive scleroderma

PRINCIPLE OF THE TEST

Highly purified total histones are bound to microwells. Antibodies to these antigens, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically bind to the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg and HIV by FDA approved methods. No test can guarantee the absence of HBsAg or HIV, and so all human serum-based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which contains hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.)
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not Eat, Drink, Smoke or Apply Makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified histones (H1, H2A, H2B, H3 and H4). Ready to use.
6 vials, 1.5 ml each	Anti-Histone Calibrators (A-F) in a serum/buffer matrix (PBS, BSA, NaN ₃ <0,1% (w/w)) containing: 0; 12.5; 25; 50; 100; and 200 U/ml. Ready to use.
2 vials, 1,5 ml each	Anti-Histone Controls in a serum/buffer matrix (PBS, BSA, NaN ₃ <0,1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN ₃ <0,1% (w/w)), yellow, concentrate (5x)
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 <0,5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN ₃ <0,1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8°C
2. Keep microplate wells sealed in a dry bag with desiccants
3. The reagents are stable until expiration of the kit
4. Do not expose test reagents to heat, sun or strong light during storage and usage
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- data reduction software

Preparation of reagents

- distilled or deionized water
- graduated cylinder for 100 and 1000 ml
- plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis
2. Allow blood to clot and separate the serum by centrifugation
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity
6. Testing of heat-inactivated sera is not recommended

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates
2. Do not interchange kit components from different lots
3. All materials must be at room temperature (20-28°C)
4. 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.
5. Perform the assay steps only in the order indicated
6. Always use fresh sample dilutions
7. Pipette all reagents and samples into the bottom of the wells
8. To avoid carryover contamination change the tip between samples and different kit controls
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28°C)
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well
6. Incubate for 15 minutes at room temperature
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution
8. Dispense **100 µl** of TMB substrate solution into each well
9. Incubate for 15 minutes at room temperature
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed color is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ORGENTEC Anti-Histone ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not met, the results are invalid and the test should be repeated.

Calculation of results

For Anti-Histone IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Histone test:

Anti-Histone IgG
Cut-Off: 40 U/ml

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum anti-Histone antibodies. The above reference ranges should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Histone kit. The assay showed linearity over the full measuring range.

Precision (Reproducibility)

Statistics were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision and the run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

Intra-Assay			Inter-Assay		
Sample No	Mean [U/ml]	CV [%]	Sample No	Mean [U/ml]	CV [%]
1	23	4.1	1	25	5.3
2	52	3.8	2	54	4.6
3	120	4.6	3	124	4.9

Sensitivity

The lower detection limit for Anti-Histone was determined at 1 U/ml.

Specificity

The microplate is coated with highly purified total histones (H1, H2A, H2B, H3, H4). The Anti-Histone test kits recognises only autoantibodies specific to histones. No crossreactivity was observed to DNA antibodies.

Calibration

Since no international reference preparation for anti-histone autoantibodies is available, the assay system is calibrated against the WHO reference preparation for human anti nuclear factor (homogenous), MRC 66/233. With the Anti-histone kit this preparation is determined at a concentration of 100 U/ml.

LIMITATIONS OF PROCEDURE

The Anti-Histone ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples be avoided.

REFERENCES

1. Slor, Hanoch, Sobe, Tama and Shoenfeld, Yehuda Quantification of Anti-Nuclear Autoantibodies by Enzyme Immunoassay (EIA): Specificity, Correlation with Other Methods, and Clinical Significance. Dept. of Human Genetics, Sackler School of Medicine, Tel Aviv University; and Research Unit for Autoimmune diseases, Soroka, Medical Center; Faculty of Health Sciences, Ben Gurion University, Beer Sheva, Israel. BioHyTech (Israel) LTD; reprinted from Quarterly Medical Review 1987; Vol. 1, no. 4.
2. Cohnen, G. Arzneimittelinduzierte Lupus-erythematodes-ähnliche Syndrome. Allergologie 1985; Jahrgang 8, Nr. 7: 282 - 92.
3. Kalden, J. R. Klinik, Diagnostik und Behandlung des Lupus erythematodes. Internistische Welt 1980; 4: 119 - 25.
4. Weiner, E.M., Martinho, C., Genth, E. Histon-Antikörper-Nachweis mittels Immunoblot und ELISA bei rheumatischen Erkrankungen. Zeitschrift f. Rheumatologie 1992; 51, suppl. 2: 76.
5. Schoenfeld, Y. and Segol, O. Anti-Histone antibodies in SLE and other autoimmune diseases. Clin. and Exp. Rheum. 1989; 7: 265 - 271.
6. Cohen, M. G. and Welb, J. Anti-histone antibodies in Rheumatoid arthritis and Felty's syndrome. Arthr. and Rheum. 1989; 32, no. 10.

INCUBATION SCHEME

- 1** Pipet **100 μ l** calibrator, control or patient sample
→ Incubate for **30 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 μ l** wash solution
- 2** Pipet **100 μ l** enzyme conjugate
→ Incubate for **15 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 μ l** wash solution
- 3** Pipet **100 μ l** substrate solution
→ Incubate for **15 minutes** at room temperature
- 4** Add **100 μ l** stop solution
→ Leave untouched for **5 minutes**
→ Read at **450 nm**