MAYO CLINIC LABORATORIES

Test Definition: ADNAP

Double-Stranded DNA (dsDNA) Antibodies Panel, IgG, Serum

Overview

Useful For

Evaluation of patients at-risk of systemic lupus for erythematosus (SLE) with abnormal antinuclear antibodies

Management of affected individuals

Distinguishing SLE from other autoimmune diseases

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
ADNA1	dsDNA Ab, IgG, S	Yes	Yes
CRTHS	dsDNA Ab IFA Screen, IgG, S	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CRTHT	dsDNA Ab IFA Titer, IgG, S	No	No

Testing Algorithm

If the double-stranded DNA (dsDNA) IgG antibodies *Crithidia luciliae* immunofluorescence screen is positive, then the dsDNA IgG antibodies *Crithidia luciliae* immunofluorescence titer will be performed at an additional charge.

For more information see Connective Tissue Disease Cascade.

Method Name

ADNA1: Enzyme-Linked Immunosorbent Assay (ELISA)
CRTHS, CRTHT: Indirect Immunofluorescence Assay (IFA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 0.8 mL Serum
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

See Specimen Required

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK
Heat-Treated	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	7 days	
	Frozen	21 days	

Clinical & Interpretive

Clinical Information

Anti-double-stranded DNA (anti-dsDNA) antibodies are highly specific for systemic lupus erythematosus (SLE) and are offered in clinical laboratories for the evaluation at-risk patients and management of affected individuals.(1) Anti-dsDNA antibodies are also included in the classification criteria for SLE.(2,3) In addition to their use in SLE diagnostic evaluation, these autoantibodies are also associated with disease activity and lupus nephritis (LN).(4-7)These antibodies are detected and quantified using various methods and analytes to dsDNA.(8) The immunological response to dsDNA has been demonstrated to be diverse with different results reported between test systems probably due to multiple factors not limited to disease heterogeneity and the absence of assay harmonization.(4-8)

The frequently used methods for detection and quantification of anti-dsDNA antibody in the clinical laboratory include the solid-phase immunoassays (SPAs) such as the enzyme linked immunosorbent assay, fluoro-enzyme immunoassay, chemiluminescence immunoassay, multiplexed bead-based assays and indirect immunofluorescence test using *Crithidia luciliae* (CLIFT) as substrate. Due to its high specificity but low sensitivity, the CLIFT is widely used as a confirmatory test following a positive anti-dsDNA IgG result obtained in a SPA.(4-8) However, discordance of positivity between SPAs as well as SPAs and CLIFT for anti-dsDNA antibodies is relatively common (4-8), occurring in a fifth of patients overall and a

third of visits (6). Although the performance between specific SPA and CLIFT vary, SPA positivity is generally associated with LN less often than CLIFT positivity.

With the significant discordance of results between anti-dsDNA antibody assays, obtaining both CLIFT and SPA may be beneficial for diagnosis and routine monitoring of SLE. Using two different methods in a panel for detecting anti-dsDNA antibodies at diagnosis is useful because it improves diagnostic sensitivity while maintaining high specificity, providing complementary information that a single assay alone might miss.(4-8) This combined approach enhances the overall diagnostic yield for SLE, particularly in identifying specific clinical features like LN.

Reference Values

DOUBLE-STRANDED DNA (dsDNA) ANTIBODIES, IgG:
<100 IU/mL (Negative)
> or =100 IU/mL (Positive)
Negative is considered normal.
Reference values apply to all ages.

DOUBLE-STRANDED DNA (dsDNA) IgG ANTIBODIES, *CRITHIDIA LUCILIAE* IMMUNOFLUORESCENCE SCREEN
Negative (<1:10)
Reference values apply to all ages.

DOUBLE-STRANDED DNA (dsDNA) IgG ANTIBODIES, *CRITHIDIA LUCILIAE* IMMUNOFLUORESCENCE TITER
Negative (<1:10)

Interpretation

The presence of anti-double stranded DNA (anti-dsDNA) IgG antibody is a diagnostic criterion of systemic lupus erythematosus (SLE). Utilization of two different methods to detect anti-dsDNA antibodies has the potential to optimize the diagnosis of SLE and management of affected patients.

Negative results for both the anti-dsDNA IgG by enzyme linked immunosorbent assay and indirect immunofluorescent assay do not exclude a diagnosis of SLE.

Cautions

Discrepancy between the two methods for detecting anti-double stranded DNA (anti-dsDNA) IgG antibodies is not uncommon. This may be related to prior use of immunosuppression or differences in sensitivities of methods for certain epitopes.

Some patients with early or inactive systemic lupus erythematosus may be positive for anti-dsDNA IgG by enzyme linked immunosorbent assay (ELISA) but negative in the anti-dsDNA indirect immunofluorescent assay using *Crithidia luciliae* as substrate (CLIFT). If the CLIFT result is negative but the patient has a positive ELISA and clinical suspicion remains, consider antinuclear antibody testing. In addition, a subset of patients may test positive by CLIFT and negative in the ELISA test.

Clinical Reference

1. Kavanaugh AF, Solomon DH; American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines. Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-DNA antibody tests. Arthritis

Rheum. 2002;47(5):546-555. doi:10.1002/art.10558
2. Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64(8): 2677-2686. doi:10.1002/art.34473
3. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for systemic lupus erythematosus. Arthritis Rheumatol. 2019;71(9):1400-1412
4. Compagno M, Rekvig OP, Bengtsson AA, et al. Clinical phenotype associations with various types of anti-dsDNA antibodies in patients with recent onset of rheumatic symptoms. Results from a multicentre observational study. Lupus Sci Med. 2014;1(1):e000007
5. Enocsson H, Sjowall C, Wirestam L, et al. Four anti-dsDNA antibody assays in relation to systemic lupus erythematosus disease specificity and activity. J Rheumatol. 2015;42(5):817-825
6. Sarbu MI, Salman-Monte TC, Munoz PR, Lisbona MP, Bernabe MA, Carbonell J. Differences between clinical and laboratory findings in patients with recent diagnosis of SLE according to the positivity of anti-dsDNA by the Crithidia luciliae method. Lupus. 2015;24(11):1198-1203
7. Zaminski D, Saxena A, Izmirly P, Buyon JP, Belmont HM. Clinical implications of discordance between anti-dsDNA antibodies by multiplex flow immunoassay and Crithidia luciliae assay in a multiethnic racial cohort of patients with SLE. Lupus Sci Med. 2023;10(2):e001012
8. Cockx M, Van Hoovels L, De Langhe E, et al. Laboratory evaluation of anti-dsDNA antibodies. Clin Chim Acta. 2022;528:34-43

Performance

Method Description

Double-Stranded DNA Antibodies, IgG:
The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with double-stranded DNA (dsDNA). In the first reaction step, diluted patient samples, calibrators and controls are incubated in the wells. Anti-dsDNA antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, rabbit anti-human IgG-HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have human IgG binding to the dsDNA antigen. The wells are washed to remove any unbound HRP enzyme conjugate. 3,3,5,5, tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.(Package insert: Anti-dsDNA-NcX ELISA (IgG). EUROIMMUN; 7/8/2020)

Crithidia luciliae Immunofluorescence Test Screen:
Patient serum is diluted 1:10 and allowed bind to the kinetoplast of the non-pathogenic hemoflagellate *Crithidia luciliae* adhered to the slide. The *Crithidia luciliae* contains a dense, pure concentration of circular double-stranded DNA that provides a unique substrate free from contaminating nuclear antigens like histones or single-stranded DNA. Following binding, unbound diluted patient serum is washed off the slide and fluorescent-conjugated antiserum added to the

substrate on the slide and incubated. A second washing step is performed to remove excess conjugate. The substrate is cover slipped and reviewed for fluorescent patterns with a fluorescent microscope. Observation of specific fluorescent patterns on the substrate indicate the presence of autoantibodies in the test sample.(Package insert: Immuno Concepts IgG ANTI-nDNA Fluorescent Test System. Immuno Concepts; Rev 4.0, 2023)

Crithidia luciliae Immunofluorescence Test Titer:
Patient serum with a positive screen at the 1:10 dilution is titered up to 1:640 and allowed to bind to the kinetoplast of the non-pathogenic hemoflagellate *Crithidia luciliae* adhered to the slide. The *Crithidia luciliae* contains a dense, pure concentration of circular double-stranded DNA that provides a unique substrate free from contaminating nuclear antigens like histones or single-stranded DNA. Following binding, unbound diluted patient serum is washed off the slide and fluorescent-conjugated antiserum added to the substrate on the slide and incubated. A second washing step is performed to remove excess conjugate. The substrate is cover slipped and reviewed for fluorescent patterns with a fluorescent microscope. Observation of specific fluorescent patterns on the substrate indicate the presence of autoantibodies in the test sample. Positive results are reported with an end point titer up to 1:640. Results higher than 1:640 are reported as greater than 1:640.(Package Insert: Immuno Concepts IgG ANTI-nDNA Fluorescent Test System, Immuno Concepts, Rev 4.0 2023)

PDF Report
No

Day(s) Performed
ADNA1: Monday through Saturday
CRTHS, CRTHT: Monday through Friday

Report Available
2 to 4 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA

requirements.

CPT Code Information

ADNA1-86225
CRTHS-86255

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ADNAP	dsDNA Ab Panel, IgG, S	97564-9

Result ID	Test Result Name	Result LOINC® Value
ADNA1	dsDNA Ab, IgG, S	33799-8
623426	dsDNA Ab IFA Screen, IgG, S	6457-6
623463	dsDNA Interpretation	69048-7