Test Definition: ADNAR

dsDNA Ab with Reflex, IgG, S

Overview

Useful For

Evaluating patients with clinical features or at-risk for systemic lupus erythematosus with additional testing by indirect immunofluorescence to clarify cases of borderline enzyme-linked immunosorbent assay results

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
CRITH	dsDNA Ab by Crithidia IFA, IgG, S	No	No

Testing Algorithm

If the double-stranded DNA (dsDNA) result is borderline, then the dsDNA by immunofluorescence will be performed at an additional charge.

Special Instructions

- Connective Tissue Disease Cascade (CTDC)

Method Name

ADNAR: Enzyme-Linked Immunosorbent Assay (ELISA)

CRITH: Indirect Immunofluorescence

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Specimen Volume: 0.5 mL

Specimen Minimum Volume

0.35 mL

Reject Due To

Gross hemolysis	OK

Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

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

Clinical and Interpretive

Clinical Information

Of the systemic lupus erythematosus (SLE)-specific antibodies outlined in the immunology domain of the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria for SLE,(1) antibodies to double-stranded DNA (dsDNA) is the most common. It is also included in the Systemic Lupus International Collaborating Clinics classification criteria (SLICC) for SLE.(2) Detection of IgG antibodies to dsDNA is the most used isotype clinically.(3-5) The diagnostic performance of dsDNA IgG antibodies in SLE is variable and dependent on several factors; notably the immunological method used for their detection, the structure of the DNA, the patientâ€™s disease state (early or active vs inactive) including specific clinical manifestations and demographics.(3-7) Weak-positive dsDNA IgG antibodies have low affinity and low avidity with variable clinical correlations for SLE.(3)

Testing for IgG antibodies to dsDNA is indicated in patients positive for anti-cellular antibody (ie, antinuclear antibody: ANA) homogeneous pattern using HEp-2 substrate by indirect immunofluorescence assay (IFA) along with clinical features compatible with SLE.(1,2,8). A minority of SLE patients may test negative using HEp-2 by IFA for nuclear antibodies.(8,9) Testing antibodies associated with HEp-2 IFA cytoplasmic pattern such as ribosomal P IgG autoantibodies may be useful if features of neuropsychiatric disease are present. Alternatively, patients may be tested for Smith, ribonucleoprotein, SSA-52, and SSA-60 antibodies.(8,9)

The levels of antibodies to dsDNA may fluctuate with SLE disease activity. Increasing antibody levels may be associated with flares while decline or negative results may indicate response to treatment or disease remission.

Reference Values

<30.0 IU/mL (negative)

30.0-75.0 IU/mL (borderline)

>75.0 IU/mL (positive)

Negative is considered normal.

Reference values apply to all ages.

Interpretation

A positive result for double-stranded DNA (dsDNA) IgG antibodies in the appropriate clinical context is suggestive of systemic lupus erythematosus (SLE). The performance characteristics of dsDNA IgG antibodies in SLE is dependent on the immunological method used for their detection, the patientâ€™s disease state including clinical

manifestations, and demographics.

Weak-positive dsDNA IgG antibody results have a low-positive predictive value for SLE.

Negative results do not rule out a diagnosis of SLE.

Cautions

Measurements of IgG antibodies to double-stranded DNA (dsDNA) are semiquantitative. Slight changes in the levels of these antibodies should not be relied upon to predict changes in the clinical course of patients with systemic lupus erythematosus (SLE). Clinical flares of disease in patients with SLE may not be accompanied by changes in the levels of dsDNA antibodies. Thus, antibody levels alone are not sufficient to guide disease management.

Weak-positive results may not correlate with a diagnosis of SLE. Confirmation with *Crithidia luciliae* indirect immunofluorescence test (CLIFT), which is more specific for SLE may be useful to make diagnosis in certain circumstances.

A weak-positive dsDNA IgG result by enzyme-linked immunosorbent assay and a CLIFT-negative result may suggest early disease, remission, or false-positive results.

False-positive results are usually of low titers.

A negative result does not exclude a diagnosis of SLE.

Clinical Reference

1. 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 Sep;71(9):1400-1412. doi: 10.1002/art.40930

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 Aug;64(8):2677-86. doi: 10.1002/art.34473

3. Infantino M, Manfredi M, Merone M, et al: Analytical variability in the determination of anti-double-stranded DNA antibodies: the strong need of a better definition of the old and new tests. Immunol Res. 2018 Jun;66(3):340-347. doi: 10.1007/s12026-018-8992-9

4. Fox BJ, Hockley J, Rigsby P, Dolman C, Meroni PL, Ronnelid J, et al: A WHO Reference Reagent for lupus (anti-dsDNA) antibodies: international collaborative study to evaluate a candidate preparation. Ann Rheum Dis. 2019 Dec;78(12):1677-1680. doi: 10.1136/annrheumdis-2019-21584

5. Ambrose N, Morgan TA, Galloway J, et al: Differences in disease phenotype and severity in SLE across age groups. Lupus. 2016 Dec;25(14):1542-1550. doi: 10.1177/0961203316644333

6. Rekvig OP: Autoimmunity and SLE: Factual and semantic evidence-based critical analyses of definitions, etiology, and pathogenesis. Front Immunol. 2020;11:569234. doi: 10.3389/fimmu.2020.569234

7. Bragazzi NL, Watad A, Damiani G, Adawi M, Amital H, Shoenfeld Y: Role of anti-DNA auto-antibodies as biomarkers of response to treatment in systemic lupus erythematosus patients: hypes and hopes. Insights and implications from a comprehensive review of the literature. Expert Rev Mol Diagn. 2019 Nov;19(11):969-978. doi: 10.1080/14737159.2019.1665511

8. Damoiseaux J, Coelho Andrade LE, Carballo OG, et al: Clinical relevance of HEp-2 indirect immunofluorescent

patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis. 2019 Jul;78(7):879-889. doi: 10.1136/annrheumdis-2018-214436

9. Choi MY, Clarke AE, St Pierre Y, et al: Antinuclear antibody-negative systemic lupus erythematosus in an international inception cohort. Arthritis Care Res (Hoboken). 2019 Jul;71(7):893-902. doi: 10.1002/acr.23712

Performance

Method Description

Microwells are pre-coated with calf thymus double-stranded DNA (dsDNA) antigen. The calibrators, controls, and diluted patient samples are added to the wells, and autoantibodies recognizing the dsDNA antigen bind during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labeled goat anti-human IgG conjugate is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualized with 3,3â€™,5,5â€™ tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to a concentration of autoantibody in the sample. Sulfuric acid is added to each well to stop the reaction. This produces a yellow end point color, which is read at 450 nm.(Package insert: QUANTA Lite dsDNA SC ELISA, INOVA Diagnostics Inc; 08/2014)

Confirmatory testing for borderline dsDNA results by enzyme-linked immunosorbent assay (ELISA) testing is performed by immunofluorescence assay (IFA). Autoantibodies in a test sample directed against dsDNA bind to antigens in the substrate placed on the slide-which, in this case, is *Crithidia luciliae*. Washing removes excess serum from the substrate. Fluorescein-conjugated (FITC) antiserum added to the substrate attaches to the bound autoantibody. After a second washing step to remove excess conjugate, the substrate has a coverslip added, and it is viewed for fluorescent patterns with a fluorescent microscope. Observation of specific fluorescent patterns on the substrate indicates the presence of autoantibodies in the test sample.(Package insert: Bio-Rad Kallestad Crithidia luciliae Substrate. Bio-Rad Laboratories; 06/2015)

PDF Report

No

Day(s) Performed

Monday through Saturday

Report Available

2 to 3 days

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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

86225

86225-CRITH (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
ADNAR	dsDNA Ab with Reflex, IgG, S	33799-8

Result ID	Test Result Name	Result LOINC Value
ADNAR	dsDNA Ab with Reflex, IgG, S	33799-8