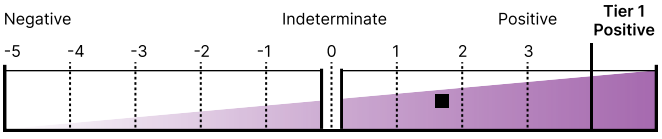
AVISE® CTD

Comprehensive Connective Tissue Disease Assessment

Order ID 739813	Specimen	Test Order	Patient	**Sample, Susan S.**	
Provider Exagen Provider MD	*Collected* 10/22/2024	*Created* 10/23/2024	*Gender* Female		
	Received 10/23/2024	*Reported* 10/25/2024	*MRN* AB123450	*DOB* 01/01/1996	

AVISE Lupus Result: **Positive - Index: 1.8**



INDEX INTERPRETATION: A Lupus Index score of 1.8 is associated with an increased likelihood of SLE. Positive results are driven by ANA (ELISA) positivity as well as classical complement activation, as indicated by the level of EC4d and/or BC4d. Results should be interpreted by a provider in conjunction with all available clinical findings.

Lupus Index Score Biomarkers	Value	Interpretation	Reference Range
Anti-dsDNA IgG (ELISA)	18.04 IU/mL	Negative	<201 - Negative I 201 - <302 - Equivocal I ≥302 - Positive
Confirmation by Crithidia luciliae (IFA)		N/A	
Anti-Smith IgG (ELFA)	<0.7 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
CB-CAP: EC4d - Erythrocyte-bound C4d (FC)	11 Net MFI	Negative	<15 - Negative I 15-75 - Positive I >75 - Strong Positive
+ CB-CAP: BC4d - B-lymphocyte-bound C4d (FC)	192 Net MFI	**POSITIVE**	<61 - Negative I 61-200 - Positive I >200 - Strong Positive
+ ANA IgG (ELISA)	22.93 Units	**POSITIVE**	<20 - Negative I 20-<60 - Positive I ≥60 - Strong Positive
Anti-SSB/La IgG (ELFA)	0.8 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Scl-70 IgG (ELFA)	<0.6 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Centromere Protein B (CENP) IgG (ELFA)	0.7 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Jo-1 IgG (ELFA)	<0.3 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-CCP IgG (ELFA)	1.2 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

T Cell Result: **Abnormal**

T Cell Biomarkers	Value	Interpretation	Reference Range
+ CB-CAP: TC4d (FC)	236 Net MFI	**POSITIVE**	<200 - Negative I ≥200 - Positive
+ T Cell autoantibody: TIgG (FC)	198 Net MFI	**POSITIVE**	<170 - Negative I ≥170 - Positive
+ T Cell autoantibody: TIgM (FC)	277 Net MFI	**POSITIVE**	<230 - Negative I ≥230 - Positive

ANA (Immunofluorescence)	Value	Interpretation	Reference Range
+ ANA by HEp-2 (IFA)	Titer: 1:320	**POSITIVE**	<1:80 - Negative I ≥1:80 - Positive
	Cytoplasmic Pattern: Speckled		
	Cytoplasmic Pattern: Not Observed		

Comments:

- BC4d and TC4d are markers of classical complement activation. In the positive range, BC4d is present in 53% of SLE patients and in low percentages of patients with Sjogren's disease, myositis, systemic sclerosis, RA, APS, and vasculitis. In the positive range, TC4d is present in 59% of SLE patients and in low percentages of patients with Sjogren's disease, spondyloarthropathies, PsA, fibromyalgia, RA, and chronic localized pain.
- TIgG and TIgM autoantibody formation against T Cell antigens is common in SLE. In the positive range, TIgG is present in 32% of SLE patients and in low percentages of patients with Sjogren's disease, spondyloarthropathies, PsA, fibromyalgia, RA, and chronic localized pain. In the positive range, TIgM is present in 31% of SLE patients and in low percentages of patients with the previously mentioned diseases.

Signed by: Prashanti Reddy, MD Prashanti Reddy MD **Date: 10/25/2024**

Results were obtained using Flow Cytometry for complement C4d fragment bound to erythrocytes (EC4d), B-lymphocytes (BC4d), T Cells (TC4d), T Cell-bound IgG (TIgG) and T Cell-bound IgM (TIgM) autoantibodies. Results were obtained by Enzyme Linked Immunosorbent Assay (ELISA) and Indirect Immunofluorescence Assay (IFA) for determination of Antinuclear Antibodies (ANA). Results were obtained by HEp2-ANA (by IFA) for the semi-quantitative determination of ANA. ANA by ELISA was used for the index calculation. In a study of 794 subjects comprising 304 SLE patients, 285 patients with other rheumatic diseases and 205 normal healthy controls, positivity for Tier 1 markers (anti-dsDNA by ELISA, confirmed with Crithidia by IFA, anti-Sm by Enzyme Linked Fluorescent Immunoassay (ELFA) or elevated EC4d and BC4d by Flow Cytometry yielded a sensitivity of 46% and a specificity of 97% for SLE vs. other autoimmune rheumatic diseases (ORD). Among the 164 SLE subjects negative in Tier 1, a positive index score composite of ANA (by ELISA), EC4d/BC4d and positivity for anti-CCP, SS-B/La, CENP, Jo-1 or Scl-70 (by ELFA) resulted in sensitivity of 62% for SLE and specificity of 89% for SLE vs. ORD. The overall two-tier algorithm result yielded 80% sensitivity for SLE and 86% specificity vs. ORD diseases (98% specificity for SLE vs. healthy individuals). RA33 autoantibodies are highly specific (>95%) for RA against healthy individuals. In a cohort of 161 diagnosed RA patients (60 seropositive and 101 seronegative based anti-CCP and RF), IgG, IgM, and IgA RA33 autoantibodies were collectively present in 32% of seropositive RA patients and 16% of seronegative RA patients.

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Patients: Positive individual biomarker results may not imply a positive disease diagnosis. Please discuss these test results with your provider in the context of all clinical information.

Rheumatoid Arthritis Biomarkers	Value	Interpretation	Reference Range
Anti-CCP IgG (ELFA)	1.2 U/mL	Negative	<7 - Negative I 7-10 - Equivocal >10 - Positive
Anti-RA33 IgG (ELFA)	1.5 U/mL	Negative	<8 - Negative I ≥8 - Positive
Anti-RA33 IgM (ELFA)	2.1 U/mL	Negative	<42 - Negative I ≥42 - Positive
Anti-RA33 IgA (ELFA)	0.8 U/mL	Negative	<5 - Negative I ≥5 - Positive
Anti-Carbamylated Protein (CarP) IgG (ELISA)	1.5 Units	Negative	<20 - Negative I ≥20 - Positive
Rheumatoid Factor IgM (ELFA)	<0.6 IU/mL	Negative	<3.5 - Negative I 3.5-5 - Equivocal I >5 - Positive
Rheumatoid Factor IgA (ELFA)	0.9 IU/mL	Negative	<14 - Negative I 14-20 - Equivocal I >20 - Positive

Sjögren's Disease Biomarkers	Value	Interpretation	Reference Range
Anti-SSA/Ro52 IgG (ELFA)	0.5 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-SSA/Ro60 IgG (ELFA)	<0.4 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-SSB/La IgG (ELFA)	0.8 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Mixed Connective Tissue Disease Biomarkers	Value	Interpretation	Reference Range
+ Anti-U1RNP IgG (ELFA)	22.8 U/mL	**POSITIVE**	<5 - Negative I 5-10 - Equivocal I >10 - Positive
Anti-RNP70 IgG (ELFA)	0.3 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Antiphospholipid Syndrome Biomarkers	Value	Interpretation	Reference Range
Anti-Cardiolipin IgM (ELFA)	<0.9 MPL	Negative	<10 - Negative I 10-40 - Weak Positive I >40 - Positive
+ Anti-Cardiolipin IgG (ELFA)	19.3 GPL	**POSITIVE**	<10 - Negative I 10-40 - Weak Positive I >40 - Positive
Anti-β2 Glycoprotein 1 IgM (ELFA)	<2.4 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
+ Anti-β2 Glycoprotein 1 IgG (ELFA)	82.6 U/mL	**POSITIVE**	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Systemic Sclerosis Biomarkers	Value	Interpretation	Reference Range
Anti-Scl-70 IgG (ELFA)	<0.6 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-RNA Pol III IgG (ELFA)	<0.7 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Centromere Protein B (CENP) IgG (ELFA)	0.7 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

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Myositis Biomarkers	Value	Interpretation	Reference Range
Anti-Jo-1 IgG (ELFA)	<0.3 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Thyroid Biomarkers	Value	Interpretation	Reference Range
Anti-Thyroglobulin IgG (ELFA)	<12 IU/mL	Negative	<40 - Negative I 40-60 - Equivocal I >60 - Positive
Anti-Thyroid Peroxidase IgG (ELFA)	<4 IU/mL	Negative	<25 - Negative I 25-35 - Equivocal I >35 - Positive

Case Remarks:

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Exagen®

1261 Liberty Way, Vista CA 92081
CLIA# 05D1075048
CAP# 7201051 I NYSDOH PFI# 8369

Laboratory Director:
Prashanti Reddy, MD

Provider Relations: 888.452.1522

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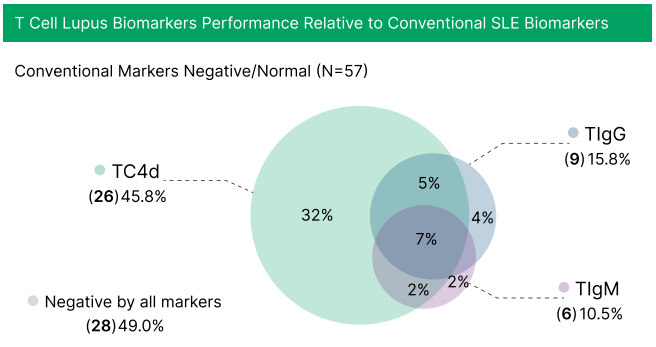
What's NEW in this AVISE CTD Report

1. NEW T Cell Biomarkers for Systemic Lupus Erythematosus

Conventional SLE biomarkers such as anti-dsDNA, anti-Smith, and complement C3/C4 have limited discriminatory power characterized by poor sensitivity in incipient cases. T Cell bound C4d (TC4d) and autoantibodies bound to T Cells (TIgG and TIgM) have superior diagnostic accuracy compared to conventional SLE biomarkers and can be informative when conventional markers are negative.

Unique Clinical Value

Supported by scientific evidence, newly available T Cell biomarkers provide unique and unmatched diagnostic accuracy when conventional biomarkers are uninformative, facilitating earlier diagnoses and treatment decisions.



TC4d

TC4d is a measure of cell-bound complement on T Cells. Elevated levels of TC4d are sensitive for SLE and present in approximately 60% of SLE patients. Approximately 20% of SLE patients test TC4d positive while testing negative for all conventional SLE biomarkers. TC4d is rarely elevated in patients with other autoimmune diseases and healthy individuals.

TIgM and TIgG

Autoantibodies (IgG and IgM) bound to T Cells are frequently present in SLE patients. Approximately 40% to 50% of SLE patients have elevated TIgM and TIgG, respectively. TIgM and TIgG are rarely elevated in patients with other autoimmune diseases and healthy individuals.

2. NEW Biomarkers for Rheumatoid Arthritis

Seronegative RA makes up approximately 30% of diagnosed RA and up to 50% of early RA cases. Novel RA biomarkers such as anti-RA33 and anti-CarP have narrowed the seronegative gap by providing evidence of systemic autoimmunity when conventional biomarkers are negative.

Unique Clinical Value

Patients with inflammatory arthritis who test negative for conventional RA biomarkers face a complex differential diagnosis and potential treatment delay. The use of seronegative RA biomarkers with high specificity provides an opportunity to substantiate clinical suspicion of RA, aiding earlier diagnosis and treatment. Given the prevalence of such patients, seronegative RA biomarkers are a critically important part of a comprehensive Connective Tissue Disease evaluation.

RA33 IgG, RA33 IgM, RA33 IgA

Autoantibodies to the RA33 antigen are highly specific (>95%) for RA against healthy individuals. Collectively, IgG, IgM, and IgA antibodies to RA33 are found in 16% of RA patients testing negative for anti-CCP and RFs.

Anti-Carbamylated Protein IgG

Autoantibodies to the carbamylated protein (anti-CarP) are present in 13% of RA patients testing negative for anti-CCP and RFs. RA patients testing positive for anti-CarP tend to have more erosive joint damage compared to anti-CarP negative RA patients.

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SA 1684 (11/24 v3)