AVISE® CTD

Comprehensive Connective Tissue Disease Assessment

		Specimen	Test Order	Patient	**Sample, Susan S.**
Order ID 739813		*Collected* 07/21/2025	*Created* 07/22/2025	*Gender* Female	
Provider Exagen Provider MD		*Received* 07/22/2025	*Reported* 07/24/2025	*MRN* AB123450	*DOB* 01/01/1996

AVISE Lupus Result: **Tier 1 Positive**

Negative — Indeterminate — Positive — **Tier 1 Positive**

-5 -4 -3 -2 -1 0 1 2 3
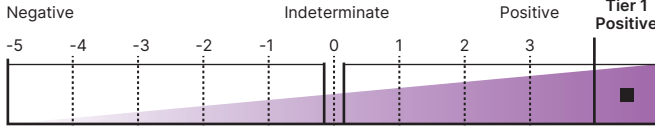
INDEX INTERPRETATION: Tier 1 positive results are associated with an increased likelihood of SLE and are greater than 96% specific for SLE against other autoimmune CTDs. These results are driven by elevated markers that are highly specific for SLE (anti-dsDNA, anti-smith, and/or strong positive EC4d/BC4d). Results should be interpreted by a provider conjunction with all available clinical findings.

Lupus Index Score Biomarkers	Value	Interpretation	Reference Range
+ Anti-dsDNA IgG (ELISA)	865.61 IU/mL	**POSITIVE**	<201 - Negative I 201-<302 - Equivocal I ≥302 - Positive
Confirmation by Crithidia luciliae (IFA)		Positive anti-dsDNA confirmed by Crithidia	
Anti-Smith IgG (ELFA)	1.2 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
+ CB-CAP: EC4d - Erythrocyte-bound C4d (FC)	18 Net MFI	**POSITIVE**	<15 - Negative I 15-75 - Positive I >75 - Strong Positive
CB-CAP: BC4d - B-lymphocyte-bound C4d (FC)	52 Net MFI	Negative	<61 - Negative I 61-200 - Positive I >200 - Strong Positive
++ ANA IgG (ELISA)	120.88 Units	**STRONG POSITIVE**	<20 - Negative I 20-<60 - Positive I ≥60 - Strong Positive
Anti-SSB/La IgG (ELFA)	1.7 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Scl-70 IgG (ELFA)	0.8 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-Centromere Protein B (CENP) IgG (ELFA)	1.0 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)	1.4 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

T Cell Biomarkers	Value	Interpretation	Reference Range
+ CB-CAP: TC4d (FC)	215 Net MFI	**POSITIVE**	<200 - Negative I ≥200 - Positive
+ T Cell autoantibody: TIgG (FC)	190 Net MFI	**POSITIVE**	<170 - Negative I ≥170 - Positive
+ T Cell autoantibody: TIgM (FC)	250 Net MFI	**POSITIVE**	<230 - Negative I ≥230 - Positive

ANA (Immunofluorescence)	Value	Interpretation	Reference Range
+ ANA by HEp-2 (IFA)	Titer: 1:640	**POSITIVE**	<1:80 - Negative I ≥1:80 - Positive
	Nuclear Pattern: Homogeneous		
	Cytoplasmic Pattern: Not Observed		

Comments:

- EC4d and TC4d are markers of classical complement activation with greater than 95% specificity against healthy individuals. In the positive range, EC4d is present in 46% of SLE patients and in low percentages of patients with Sjögren's disease, myositis, systemic sclerosis, RA, APS, and vasculitis. EC4d correlates with SLE disease activity. When positive, TC4d is observed in 58% of SLE patients. It is also present in 28% of Sjögren's disease, 13% of spondyloarthropathies, 13% of PsA, and 5% of RA patients. [Kyttaris et al. 2025]. **A positive result alone does not establish a diagnosis. Results must be interpreted by a healthcare provider in the context of all available clinical findings.**

- TIgG and TIgM autoantibodies are 95% specific against healthy individuals. When positive, TIgG is observed in 31% of SLE patients. It is also present in 10% of Sjögren's disease and 10% of RA patients. When positive, TIgM is observed in 30% of SLE patients. It is also present in 14% of Sjögren's disease, 4% of PsA, 7% of fibromyalgia, and 6% of RA patients. Additionally, TIgM is elevated in up to 21% of patients exposed to COVID-19, though levels typically do not persist beyond six months in mild (non-hospitalized) cases. [Kyttaris et al. 2025 and Perez-Diez et al. 2024]. **A positive result alone does not establish a diagnosis. Results must be interpreted by a healthcare provider in the context of all available clinical findings.**

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 Flourescent 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).

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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)	1.4 U/mL	Negative	<7 - Negative I 7-10 - Equivocal >10 - Positive
Anti-PAD4 IgA (ELISA)	3.6 U/mL	Negative	<25 - Negative I ≥25 - Positive
Anti-PAD4 IgG (ELISA)	4.1 U/mL	Negative	<13 - Negative I ≥13 - Positive
Anti-RA33 IgA (ELFA)	0.6 U/mL	Negative	<5 - Negative I ≥5 - Positive
Anti-RA33 IgG (ELFA)	1.8 U/mL	Negative	<8 - Negative I ≥8 - Positive
Anti-RA33 IgM (ELFA)	0.9 U/mL	Negative	<42 - Negative I ≥42 - Positive
Rheumatoid Factor IgA (ELFA)	1.3 U/mL	Negative	<14 - Negative I 14-20 - Equivocal I >20 - Positive
Rheumatoid Factor IgM (ELFA)	<1.1 U/mL	Negative	<3.5 - Negative I 3.5-5 - Equivocal I >5 - Positive

Sjögren's Disease Biomarkers	Value	Interpretation	Reference Range
Anti-SSA/Ro52 IgG (ELFA)	0.4 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)	1.7 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)	1.5 U/mL	Negative	<5 - Negative I 5-10 - Equivocal I >10 - Positive
Anti-RNP70 IgG (ELFA)	1.1 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Antiphospholipid Syndrome Biomarkers	Value	Interpretation	Reference Range
+ Anti-Cardiolipin IgG (ELFA)	93.6 GPL	**POSITIVE**	<10 - Negative I 10-40 - Weak Positive I >40 - Positive
Anti-Cardiolipin IgM (ELFA)	1.7 U/mL	Negative	<10 - Negative I 10-40 - Weak Positive I >40 - Positive
Anti-β2 Glycoprotein 1 IgG (ELFA)	1.9 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive
Anti-β2 Glycoprotein 1 IgM (ELFA)	<2.4 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

Systemic Sclerosis Biomarkers	Value	Interpretation	Reference Range
Anti-Scl-70 IgG (ELFA)	0.8 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)	1.0 U/mL	Negative	<7 - Negative I 7-10 - Equivocal I >10 - Positive

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. PAD4 autoantibodies are highly specific (>95%) for RA against healthy individuals. In a cohort of 379 RA patients (220 seropositive and 159 seronegative) IgA and IgG antibodies were collectively observed in 50% of seropositive RA and 14% of seronegative RA.

AViSE CTD

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	07/21/2025	07/22/2025	Female		
Provider					
Exagen	*Received*	*Reported*	*MRN*		*DOB*
Provider MD	07/22/2025	07/24/2025	AB123450		01/01/1996

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.

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)	15 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

FC: Flow Cytometry

IFA: Immunofluorescence Assay

ELISA: Enzyme-Linked Immunosorbent Assay

ELFA: Enzyme-Linked Fluorescent Assay

Case Remarks:

Signed by: Prashanti Reddy, MD

Date: 10/25/2024

References
1. Kyttaris V, et al. Front Immunol. 2025; 16:1518208. doi: 10.3389/fimmu. 2025.1518208.
2. Concoff A, Warsi T, Taghavi S, et al. Arthritis Rheumatol. 2024; 76 (suppl 9). Abstract #2232.
3. Concoff A, Warsi T, Taghavi S, et al. Arthritis Rheumatol. 2024; 76 (suppl 9). Abstract #2233.
4. Ramsey-Goldman R, et al. ACR Open Rheumatol. 2021 Feb;3(2):116-123. doi: 10.1002/acr2.11219.
5. Arriens C, et al. Lupus Sci Med. 2020 Apr; 7(1). doi: 10.1136/lupus-2019-000377.
6. Ramsey-Goldman R, et al. Arthritis Rheumatol. 2020 Jan;72(1):78-88. doi: 10.1002/art.41093.
7. Hui-Yuen J, et al. Lupus. 2018 Dec;27(14):2262-2268. doi: 10.1177/0961203318809181.
8. Sieghart D, Platzer A, Studenic P, et al. Front Immunol. 2018;9:876. doi:10.3389/fimmu.2018.00876.
9. Wallace D, et al. Lupus Sci Med. 2016 Feb;3(1):e000127. doi: 10.1136/lupus-2015-000127.
10. Putterman C, et al. Lupus Sci Med. 2014 Oct;1(1):e000056. doi: 10.1136/lupus-2014-000056.

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# What's NEW in this AVISE CTD Report

NEW Anti-PAD4 Biomarkers for Rheumatoid Arthritis

Peptidylarginine deiminases (PADs) play a crucial role in the pathogenesis of RA by generating citrullinated proteins, which are the hallmark autoantigens of the disease. Anti-PAD4 autoantibodies specifically target one of these enzymes involved in this pathogenic process. **Unlike traditional markers, anti-PAD4 can be present in patients who are seronegative by RF and anti-CCP**, helping clinicians identify RA earlier in the disease course.

Anti-PAD4 autoantibodies are observed in 14% of seronegative RA patients and 50% of seropositive RA patients.

14%
of seronegative RA patients

50%
of seropositive RA patients
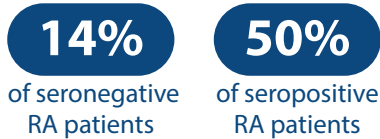
Anti-PAD4 autoantibodies are also **highly specific against healthy individuals (>95%) and other autoimmune rheumatic diseases** including systemic lupus erythematosus (SLE), psoriatic arthritis (PsA), and Sjögren's disease.
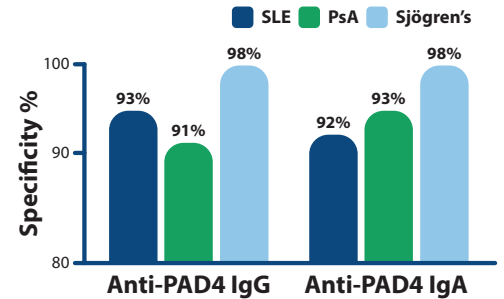
Anti-PAD4 autoantibodies can develop pre-clinically and **are associated with heightened risk for severe joint damage**. The use of seronegative RA biomarkers with high specificity provides an opportunity to substantiate clinical suspicion of RA, aiding earlier diagnosis and treatment.

Other Recently Added Biomarkers for RA and SLE

Unique Value of T Cell Biomarkers for Systemic Lupus Erythematosus

T Cell-bound C4d (TC4d) and antibodies targeting T Cells (TIgM and TIgG) demonstrate superior diagnostic performance compared to conventional SLE biomarkers such as anti-dsDNA, anti-Smith, and complement C3/C4. **TC4d is elevated in approximately 60% of SLE patients** and is positive in about 50% of SLE patients who test negative for all conventional SLE biomarkers. TIgM and TIgG autoantibodies are also primarily sensitive for SLE, with **approximately 30% of SLE patients showing positive TIgM and TIgG**. TC4d, TIgM, and TIgG are 95% specific for SLE against healthy individuals and are rarely elevated in patients with other autoimmune diseases.

Unique Clinical Value of RA33 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, PAD4 and anti-CarP can provide evidence of systemic autoimmunity when conventional biomarkers are negative. 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 32% of seropositive RA and 16% of seronegative RA patients.