AVISE®

Interpretation Guide

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AVISE Interpretation Guide

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	Marker (method)	AVISE CTD	AVISE Lupus	SLE Monitor	SLE Prognostic	AVISE APS	AVISE Vasculitis	Associated Disease	Sensitivity	Interpretation
SLE Associated Markers	EC4d (FACS)	●	●	●				Systemic Lupus Erythematosus (SLE)	46% [1]	Cell-Bound Complement Activation Products (CB-CAPs) EC4d & BC4d are measures of classical complement activation. CB-CAPs are primarily associated with SLE with 66% of patients having one or both CB-CAPs elevated [1]. EC4d significantly correlates with fluctuations in SLE disease activity[2].
	BC4d (FACS)	●	●					SLE prognostic	53% [1]	
	C3 (IT)			●				SLE	33% [1]	C3/C4 proteins are integral components of the complement system. Abnormally low concentrations of C3/C4 associate with SLE primarily with 44% of SLE patients having one or both proteins abnormally low.
	C4 (IT)			●				SLE prognostic	32% [1]	
	Anti-C1q IgG (ELISA)			●	●			Lupus Nephritis/ disease activity	58% [4]	Antibodies against the complement protein C1q are found in 58% of SLE patients with active lupus nephritis. Anti-C1q levels associate with renal activity [2]. Low levels of anti-C1q antibodies have been found in up to 8% of patients with other diseases, such as infection and normal healthy individuals.
	Anti-dsDNA IgG (ELISA and IFA)	●	●					SLE	33% [1]	Anti-dsDNA antibodies provide high positive predictive value due to high specificity for SLE. In the AVISE Lupus algorithm, patients are initially screened with an ELISA assay, and all patients testing positive are reflexed to Crithidia luciliae IFA testing for confirmation.
	Anti-dsDNA (CIA)			●				SLE/disease activity	46% [24,25]	The anti-dsDNA by CIA method measures quantitative levels of anti-double stranded deoxyribonucleic acid with a superior correlation to disease activity compared to other methods.
	Anti-Nuclear Antibodies IgG (ANA) (ELISA and IFA)	●	●					Autoimmune Diseases	89% in SLE [1]	ANA has high negative predictive value for ruling out SLE due to its high sensitivity for SLE. However, ANA is found in many autoimmune disorders and a significant proportion of apparently healthy individuals.
	Anti-Ribosomal P IgG (ELISA)				●			Neuropsychiatric Lupus	9% [5]	Antibodies against ribosomal-P are highly specific for SLE and can be present in anti-dsDNA or anti-Sm negative patients. Anti-Ribosomal-P antibodies have been shown to associate with neuropsychiatric SLE manifestations.
	Anti-Smith IgG (ELISA)	●	●					SLE	14% [1]	Anti-Smith antibodies are highly specific, but comparatively low sensitivity marker for SLE. One of the ACR criteria for SLE.
	Marker (method)							**Associated Disease**	**Sensitivity**	**Interpretation**
ENA Markers	Anti-CENP IgG (ELISA)	●	●					CREST Syndrome	20-60% [6]	Antibodies to (CENP) protein-B are found in 20-60% of patients with CREST syndrome, a limited form of scleroderma.
	Anti-Jo-1 IgG (ELISA)	●	●					Polymyositis/ Dermatomyositis (PM/ DM)	20-30% [7]	Marker to aid diagnosis of PM/DM. Found in about 25% of patients with PM/DM.
	Anti-RNP70 IgG (ELISA)	●						Mixed Connective Tissue Disease (MCTD)	90% [8]	RNP70 is a 70 kDa protein of the U1-snRNP complex. RNP70 antibodies are highly sensitive for MCTD.
	Anti-Scl-70 IgG (ELISA)	●	●					Scleroderma	28-70% [9]	Up to 70% of scleroderma patients are positive. Anti-Scl70 is highly specific for diffuse cutaneous scleroderma and associates with interstitial lung disease (ILD).
	Anti-Ro52 IgG (CIA)	●						Myositis, SLE, Sjögren's Syndrome & Scleroderma	20-70% [10]	Ro52 antibodies are found in multiple autoimmune conditions. Anti-Ro52 has been shown to associate with interstitial lung disease (ILD) in patients with Sjögren's syndrome or scleroderma.
	Anti-Ro60 IgG (CIA)	●						SLE, Sjögren's Syndrome, Myositis & Scleroderma	20-65% [10]	Ro60 antibodies are found in multiple autoimmune conditions. Anti-Ro60 is commonly found in both SLE and Sjögren's syndrome.
	Anti-RNA Pol III IgG (ELISA)	●						Systemic Sclerosis	5-22% [11]	Antibodies against RNA polymerase III are specific for systemic sclerosis often found in the absence of anti-Scl70 and anti-CENP antibodies. RNA Pol III antibodies associate with diffuse cutaneous scleroderma.
	Anti-SS-B/La IgG (ELISA)	●	●					Sjögren's Syndrome	39% [1]	Anti-La antibodies are a low sensitivity but highly specific marker for Sjögren's Syndrome.
	Anti-U1-RNP IgG (ELISA)	●						MCTD	95-99% [8]	Anti-U1-RNP antibodies are highly sensitive for MCTD. Absence of anti-U1-RNP antibodies is used to rule out MCTD.
	Anti-Histone IgG (ELISA)							Drug-Induced Lupus (DIL)	95% [12]	Up to 95% of drug-induced lupus and 50% of SLE patients exhibit elevated levels of histone antibodies. Histone antibodies have also been found in RA, DM, and SS placing added importance on clinical presentation.

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	Marker (method)	AVISE CTD	AVISE Lupus	SLE Monitor	SLE Prognostic	AVISE APS	AVISE Vasculitis	Associated Disease	Sensitivity	Interpretation
RA Markers	Anti-Cyclic Citrullinated Peptide IgG (ELISA)	●	●					Rheumatoid Arthritis (RA)	70-90% [13]	Antibodies to Cyclic Citrullinated Peptides (CCP) aid in the diagnosis of Rheumatoid Arthritis (RA). Anti-CCP antibodies are highly specific for RA. Anti-CCP is included in the AVISE Lupus algorithm to help with the differential diagnosis of RA vs. SLE.
	Anti-Carbamylated Protein IgG (ELISA)							RA	33% [14]	Anti-CarP antibodies serve as a marker of more severe prognosis in RA, independent of anti-CCP or RF status. Studies have shown anti-CarP found in early RA associates with future erosive damage. The clinical significance of positive anti-CarP in the absence of RA has not been established.
	Rheumatoid Factor IgM & IgA (ELISA)	●						RA	70-90% [13]	Quantitative measurement of IgM and IgA rheumatoid factors (RF) to aid in the diagnosis of rheumatoid arthritis. Presence of IgA RF isotype may be associated with more severe RA prognosis.
APS Markers	**Marker (method)**							**Associated Disease**	**Sensitivity**	**Interpretation**
	Anti-β2-Gylcoprotein I IgG, IgM & IgA (ELISA)	●			●	●		Antiphospholipid Syndrome (APS)	45% [15]	Antibodies to Beta 2 Glycoprotein 1 (ß 2 GP1) exhibit higher specificity than anti-cardiolipin assays. In 3-10% of APS patients, ß2 GP1 antibodies may be the only positive test. Positive results should be confirmed after 12 weeks to ensure persistency of antibodies. IgA ß2 GP1 antibodies are less common than IgG or IgM and can occur in isolation.
	Anti-Cardiolipin IgG, IgM & IgA (ELISA)	●			●	●		APS	97% [16]	Antibodies to cardiolipin are present in SLE patients (30-40%) and APS. Prevalence of cardiolipin in APS is high but specificity for APS is lower than other anti-phospholipid antibodies. Positive results should be confirmed after 12 weeks.
	Anti-Phosphatidylserine /Prothrombin (PS/PT) IgM & IgG (ELISA)				●	●		APS	22-37% [3]	Anti-PS/PT antibodies are markers for APS that have been found to significantly correlate with lupus anticoagulant (LAC) [16]. Unlike LAC, anti-PS/PT testing is unaffected by anti-coagulant therapy.
Thyroid Markers	**Marker (method)**							**Associated Disease**	**Sensitivity**	**Interpretation**
	Anti-Thyroglobulin IgG (ELISA)	●						Hashimoto's Thyroiditis & Graves' Disease	60-85% [17]	Anti-thyroglobulin antibodies are found in 60-85% of patients with Hashimoto's thyroiditis and 30-80% of patients with Graves' disease.
	Anti-Thyroid Peroxidase IgG (ELISA)	●						Hashimoto's Thyroiditis & Graves' Disease	71-97% [17]	Anti-thyroid peroxidase antibodies are found in > 90% of patients with Hashimoto's thyroiditis & 71-97% of patients with Graves' disease. Over 95% of thyroiditis patients have Thyroglobulin IgG and/or Thyroid peroxidase antibodies.
Vasculitis Markers	**Marker (method)**							**Associated Disease**	**Sensitivity**	**Interpretation**
	ANCA (IFA)						●	ANCA associated vasculitis	77% [19]	The c-ANCA pattern produces a granular cytoplasmic pattern with interlobular accentuation on ethanol fixed neutrophils. c-ANCA patterns are associated with necrotizing segmental glomerulonephritis and GPA [19, 20].
									85% [19]	The p-ANCA pattern produces perinuclear staining with or without nuclear extension. The p-ANCA pattern is commonly detected in patients with MPA and about 40% of patients with EGPA [20, 24].
	Anti-PR3 IgG (CIA)						●	Granulomatosis with Polyangiitis (GPA)	81% [19]	Anti-PR3 antibodies are primarily associated with GPA and to a lesser extent, found in MPA (10%) and EGPA [18, 19]. However, anti-PR3 antibodies can also be seen in connective tissue disease, IBD, some infections, malignancy, and as a reaction to drugs. Therefore, results should be interpreted with care in light of the clinical findings and workup [21].
	Anti-MPO IgG (CIA)						●	Microscopic Polyangiitis (MPA)	85% [19]	Anti-MPO antibodies are primarily associated with MPA and to a lesser extent, found in GPA (6%) and EGPA [18,19]. However, anti-MPO antibodies can also be seen in connective tissue disease, IBD, some infections, malignancy, and as a reaction to drugs. Therefore, results should be interpreted with care in light of the clinical findings and workup [21].
	Anti-GBM IgG (CIA)						●	Goodpasture's Syndrome (GPS)	96% [22]	Anti-GBM antibodies are often associated with Goodpasture's disease and anti-GBM nephritis [22]. A significant proportion of patients with anti-GBM disease are also positive for ANCA.

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AVISE HCQ

A test to aid in assessing adherence to HCQ and individual exposure to HCQ as measured in whole blood.

HCQ Level	Interpretation & Consideration
Therapeutic (>1000 ng/mL)	Level associated with clinical efficacy. HCQ is likely absorbed effectively.
Sub-therapeutic (200-1000 ng/mL)	Patient could be partially adherent to therapy. Patients with HCQ lower than 1000 ng/mL can be at greater risk for disease flare.
Underexposed (<200 ng/ml)	Patient is likely non-adherent to HCQ therapy.

AVISE MTX

A test to aid in assessing adherence to MTX and individual exposure to active MTX metabolites in red blood cells.

MTXPG Level	Interpretation & Consideration
Therapeutic (>60 nmol/L)	Patient is metabolizing MTX effectively. Level is consistent with clinical efficacy.
Intermediate (20-60 nmol/L)	Patient may need more exposure to MTX.
Sub-therapeutic (<20 nmol/L)	Patient may not be metabolizing MTX effectively or patient may be non-adherent with therapy.

Methodology Definitions:

FACS: Fluorescence-Activated Cell Sorting
IFA: Immunofluorescence
ELISA: Enzyme-Linked Immunosorbent Assay
CIA: Chemiluminescent Immunoassay
IT: Immunoturbidimetry

AVISE Interpretation Guide

A deeper look at the AVISE® Lupus two tier algorithm

TIER 1

Tier 1 criteria is highly specific for SLE

Any of the Following:
Anti-Sm >10 U/mL
EC4d >75 Net MFI
BC4d >200 Net MFI
Anti-dsDNA ≥ 302 U/ML

(Positive anti-dsDNA ELISA Confirmed by Crithidia)

Negative

Positive

If Tier 1 is Negative move to Tier 2

TIER 2

When levels are elevated	Impact on Index
ANA Component ELISA	+
CB-CAPs Component EC4d & BC4d	+
Specificity Component Anti-CCP Anti-SS-B/La Anti-CENP Anti-Scl-70 Anti-Jo-1	−

Negative **< -0.1** **Index Value** **>0.1** **Positive**

Tier 2 generates an index value based on the following components

- Level of ANA ELISA result (negative, positive, strong positive)
- Measurement of classical complement activation (CB-CAPs component) measured on a continuous scale
- Presence of auto-antibodies specific to other autoimmune CTDs

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AVISE® CTD and AVISE Lupus result report

The result for the AVISE Lupus algorithm is featured first, plotted along a gradient of increasing likelihood for presence of SLE

Analytes included in Tier 1 and Tier 2 along with respective assessments are reported in two distinct sections

AVISE CTD

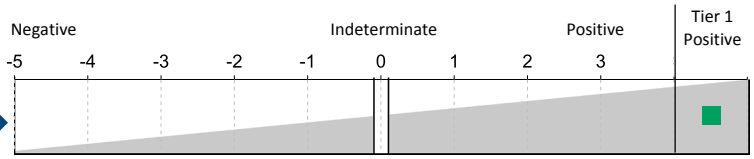
Order ID 739814
Provider Exagen Provider MD

Specimen
Collected 12/22/2022
Received 12/23/2022
Test Order
Created 12/23/2022
Reported 12/28/2022

Patient **Sample, Susan S.**
Gender - DOB Female - 01/01/1996
Identifier Received
Exagen ID 541163

AVISE CTD Test Report

AVISE Lupus Result: **Tier 1 Positive**



Tier 1 Analytes	Value	Interpretation	Reference Range	*Tier 1 Assessment*
Anti-dsDNA IgG	413.00 IU/mL	POSITIVE	<201 - Negative | 201-<302 - Equivocal |≥302 - Positive	Positive
Confirmation by Crithidia luciliae		Positive anti-dsDNA confirmed by Crithidia		
Anti-Smith IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	
CB-CAP: EC4d - Erythrocyte-bound C4d	8 Net MFI	Negative	<15 - Negative | 15 -75 - Positive | >75 - Strong Positive	
CB-CAP: BC4d - B-lymphocyte-bound C4d	125 Net MFI	POSITIVE	<61 - Negative | 61-200 - Positive | >200 - Strong Positive	

Note:

The Tier 1 result is associated with an increased likelihood of SLE and is the product of the following analyte values meeting the Tier 1 criteria: Anti-dsDNA, confirmed by Crithidia IFA

Tier 2 Analytes	Value	Interpretation	Reference Range	*Tier 2 Assessment*
ANA IgG	>150 Units	STRONG POSITIVE	<20 - Negative | 20-<60 - Positive | ≥60 - Strong Positive	Not assessed due to Tier 1 Positive
CB-CAP: EC4d - Erythrocyte-bound C4d	8 Net MFI	Negative	<15 - Negative | 15-75 - Positive | >75 - Strong Positive	
CB-CAP: BC4d - B-lymphocyte-bound C4d	125 Net MFI	POSITIVE	<61 - Negative | 61-200 - Positive | >200 - Strong Positive	
Anti-SS-B/La IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	
Anti-Scl-70 IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	
Anti-Centromere Protein B (CENP) IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	
Anti-Jo-1 IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	
Anti-CCP IgG	1.0 U/mL	Negative	<7 - Negative | 7-10 - Equivocal | >10 - Positive	

Note:

Approved by: Richard Safrin, MD Richard E Safrin MD Date: 12-28-2022

Results were obtained using flow cytometry for complement C4d fragment bound to erythrocytes (EC4d) and B-lymphocytes (BC4d). Autoantibodies were determined using solid phase immunoassays. ANA was determined by indirect immunofluorescence and solid phase assays. ANA by solid phase assay 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, confirmed using Crithidia, anti-Sm or elevated EC4d and BC4d) was associated with a sensitivity of 46% and a specificity of 97%. Among the 440 subjects negative in Tier 1, a positive index score composite of ANA (by ELISA), EC4d/BC4d and positivity for anti-citrullinated peptide antibodies, SS-B/La, CENP, Jo-1 or Scl-70 resulted in sensitivity of 62% for SLE and specificity of 89%. Two tier combination yielded 80% sensitivity for SLE and 86% specificity for other rheumatic diseases (98% specificity vs. healthy).

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AVISE® CTD and AVISE Lupus page 2

Details for ANA (HEp-2) are at the top of the page including any observed nuclear and cytoplasmic patterns

Far left column identifies positive(+) and strong positive(++) interpretations for each analyte

Notes provide clarification and summary statements

AVISE CTD

Order ID 739814
Provider Exagen Provider MD

Specimen
Collected 12/22/2022
Received 12/23/2022

Test Order
Created 12/23/2022
Reported 12/28/2022

Patient Sample, Susan S.
Gender - DOB Female - 01/01/1996
Identifier Received
Exagen ID 541163

	SLE-Associated Analytes		Value		Interpretation	Reference Range
++	ANA IgG		>150	Units	STRONG POSITIVE	ELISA: <20 - Negative | 20-<60 - Positive | ≥60 - Strong Positive
+	ANA by HEp-2	*Titer:*	1:320		POSITIVE	IFA: <1:80 - Negative | ≥1:80 - Positive
		Nuclear Pattern: Homogeneous				
		Cytoplasmic Pattern: Observed				
+	Anti-dsDNA IgG		413.00	IU/mL	POSITIVE	ELISA: <201 - Negative | 201-<302 - Equivocal |≥302 - Positive
+	Confirmation by Crithidia luciliae		POSITIVE			IFA: Negative
	Anti-Smith IgG		1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	CB-CAP: EC4d - Erythrocyte-bound C4d		8	Net MFI	Negative	FACS: <15 - Negative | 15-75 - Positive | >75 - Strong Positive
+	CB-CAP: BC4d - B-lymphocyte-bound C4d		125	Net MFI	POSITIVE	FACS: <61 - Negative | 61-200 - Positive | >200 - Strong Positive

	Other Autoimmune Disease Auto-Antibodies	Value		Interpretation	Reference Range
	Anti-U1RNP IgG	3.0	U/mL	Negative	ELFA: <5 - Negative | 5-10 - Equivocal | >10 - Positive
	Anti-RNP70 IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-Ro52 IgG	5.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-Ro60 IgG	3.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-SS-B/La IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-Scl-70 IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-RNA Pol III IgG	10.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-Centromere Protein B (CENP) IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-Jo-1 IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive

	Rheumatoid Arthritis Auto-Antibodies	Value		Interpretation	Reference Range
	Rheumatoid Factor IgM	2.0	IU/mL	Negative	ELFA: <3.5 - Negative | 3.5-5 - Equivocal | >5 - Positive
	Rheumatoid Factor IgA	1.0	IU/mL	Negative	ELFA: <14 - Negative | 14-20 - Equivocal | >20 - Positive
	Anti-CCP IgG	1.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive

	Antiphospholipid Syndrome Auto-Antibodies	Value		Interpretation	Reference Range
	Anti-Cardiolipin IgM	2.0	U/mL	Negative	ELFA: <10 - Negative | 10-40 - Weak Positive | >40 - Positive
	Anti-Cardiolipin IgG	4.0	U/mL	Negative	ELFA: <10 - Negative | 10-40 - Weak Positive | >40 - Positive
	Anti-β2 Glycoprotein 1 IgM	3.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive
	Anti-β2 Glycoprotein 1 IgG	2.0	U/mL	Negative	ELFA: <7 - Negative | 7-10 - Equivocal | >10 - Positive

	Thyroid Auto-Antibodies	Value		Interpretation	Reference Range
	Anti-Thyroglobulin IgG	<12	IU/mL	Negative	ELFA: <40 - Negative | 40-60 - Equivocal | >60 - Positive
	Anti-Thyroid Peroxidase IgG	<4	IU/mL	Negative	ELFA: <25 - Negative | 25-35 - Equivocal | >35 - Positive

Notes:

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Exagen
1261 Liberty Way, Vista CA 92081
CLIA# 05D1075048
CAP# 7201051 I NYSDOH PFI# 8369
Laboratory Directors:
Richard Safrin, MD
R. Harper Summers, MD
Provider Relations: 888.452.1522

SA1267 (12/22)

Provider Relations: 888.452.1522

Call Provider Relations with any questions or to arrange for a clinical consultation

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