Biomarker Testing for Autoimmune Rheumatic Disease

Policy Number: AHS – G2022 – Biomarker Testing for Autoimmune Rheumatic Disease	Prior Policy Name and Number, as applicable: AHS-G2022-ANA/ENA Testing
Effective Date: 10/01/2023	

I. Policy Description

Systemic autoimmune rheumatic diseases (SARDs) are a diverse group of conditions that primarily affect the joints, bones, muscle, and connective tissue (AAFP, 2019). SARDs are characterized by dysregulated immunity and inflammatory responses, resulting in damage and destruction to joints, connective tissues, skin, blood elements, and other target organs; however, considerable diversity in clinical presentation, disease course, and treatment response exists (Guthridge et al., 2022).

The diagnostic workup for SARDs may involve the antinuclear antibody (ANA) assay, which is used to detect autoantibodies (AAB) against intracellular antigens, originally known as antinuclear antibodies (Tan, 1989). Commonly used as part of the initial diagnostic workup to screen for evidence of systemic autoimmunity (Satoh et al., 2014), detection and identification of AABs are important in the diagnosis of SARDs, such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), mixed connective tissue disease (MCTD), systemic sclerosis (SSc), and idiopathic inflammatory myopathies (IIMs) (Tebo, 2017). Extractable nuclear antigens or ENAs (a historical term from when the antigens were extracted from the cell into saline solution prior to testing) include Sm, U1 ribonucleoprotein (RNP), Ro, and La antigens, and are also useful for evaluating individuals with suspected connective tissue disease (Bloch, 2022a).

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request.

1) For individuals with a high clinical suspicion of autoimmune disease, testing for antinuclear antibodies (ANA) **MEETS COVERAGE CRITERIA**.
   a) Once per lifetime in individuals with stable symptoms.
   b) Repeat testing only if a significant change in symptoms occurs.
2) For individuals with an abnormal, raised ANA titer or with abnormal immunological findings in the serum and a clinical correlation with the appropriate autoimmune disorder, extractable nuclear antigens (ENA) panel testing of specific autoantibodies **MEETS COVERAGE CRITERIA**.
3) For individuals with an initial positive ANA test and a diagnosis of systemic autoimmune rheumatic disease, testing of dsDNA up to four (4) times per year **MEETS COVERAGE CRITERIA**.
4) For individuals with a negative or low positive ANA test, the following condition specific antibody testing **MEETS COVERAGE CRITERIA**:
   a) Testing for anti-Jo-1 in a unique clinical subset of myositis.
   b) Testing for anti-SSA in the setting of lupus or Sjögren's syndrome.
5) Monitoring of disease with ANA testing or ANA titers **DOES NOT MEET COVERAGE CRITERIA**.
6) For individuals with nonspecific symptoms, ANA and/or ENA testing **DOES NOT MEET COVERAGE CRITERIA**.
7) For all other situations not described above, testing of specific antibodies in the absence of a positive ANA test **DOES NOT MEET COVERAGE CRITERIA**.
8) For asymptomatic individuals, testing of ANA and/or ENA during a wellness visit or a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

9) For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA score, PrismRA) **DOES NOT MEET COVERAGE CRITERIA.**
10) For the diagnosis of systemic lupus erythematosus (SLE), the use of cell-bound complement activation products (e.g., AVISE Lupus) **DOES NOT MEET COVERAGE CRITERIA.**
11) For the diagnosis, prognosis, or monitoring of SLE or connective tissue diseases, serum biomarker panel testing with proprietary algorithms and/or index scores (e.g., AVISE CTD, AVISE SLE Monitor, AVISE SLE Prognostic) **DOES NOT MEET COVERAGE CRITERIA**.

III. Table of Terminology

Term	Definition
AAB	Autoantibodies
AAP	American Academy of Pediatrics
ACL	Anticardiolipin
ACP	American College of Pathologists
ACR	American College of Rheumatology
AIH	Autoimmune hepatitis
AIIF	Automated indirect immunofluorescence
ANA	Antinuclear antibody
Anti La/SS-	Anti La/Sjogren Syndrome-B
Anti-C1q	Autoantibodies against C1q
Anti-dsDNA	Anti-double-stranded DNA
Anti-RNP	Antinuclear ribonucleoprotein
Anti-Ro/SS-	Anti-Ro/Sjogren Syndrome related antigen A autoantibodies
Anti-Sm	Anti-Smith antibodies
APL	Antiphospholipid antibodies
BC4d	B-lymphocyte-bound C4d
BSR	British Society for Rheumatology
CBC	Complete blood count
CB-CAPs	Cell-bound complement activation products
CCP	Cyclic citrullinated peptides
CDC	Centers for Disease Control and Prevention
CENP	Centromere protein B
CIA	Chemiluminescence immunoassay
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRP	C-reactive protein
CTD	Connective tissue diseases
CV	Coefficient of variation
ds	Double-stranded
dsDNA	Double-stranded DNA
EC4d	C4d bound to erythrocytes
eGFR	Estimated glomerular filtration rate
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ENA	Extractable nuclear antigens
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FEIA	Fluorescence enzyme immunoassay
HEp-2	Human epithelial type 2
ICAP	International Consensus on ANA staining Patterns

IFA	Immunofluorescence assay
IIF	Indirect immunofluorescence
IIMs	Idiopathic inflammatory myopathies
IQ	Interquartile
ISLM	Italian Society of Laboratory Medicine
JIA	Juvenile idiopathic arthritis
Jo-1	Histidyl t-RNA synthetase
LAC	Lupus anticoagulant
LDT	Laboratory developed test
LE cell	Lupus erythematosus cell
LFA	Lupus Foundation of America
MAP	Multianalyte assay panel
MCTD	Mixed connective tissue disease
MIA	Multiplex immunoassay
MIIF	Manual indirect immunofluorescence
PC	Positive concordance
PMPM	Per member per month
PPPM	Per patient per month
RA	Rheumatoid arthritis
RNP	Ribonucleoprotein
SARDs	Systemic autoimmune rheumatic diseases
SDI	SLICC damage index
SDLT	Standard diagnosis laboratory testing
SELENA	Safety of Estrogens in Lupus National Assessment
SjS	Sjögren's syndrome
SLE	Systemic lupus erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
SRDs	Systemic rheumatic diseases
SS-B/La	Sjogren's syndrome Type-B
SSc	Systemic sclerosis

IV. Scientific Background

Autoimmune diseases occur when an individual's immune system mistakenly attacks his or her own tissue. This can lead to a variety of conditions and diseases which vary in severity. Autoimmune diseases are estimated to affect 5% of the US population (Sirotti et al., 2017), are associated with increased morbidity and mortality, and are among the leading causes of death (under 65 years) and disability for women in the US (Simon et al., 2017).

Systemic lupus erythematosus (SLE) is one of more than 80 known autoimmune disorders, affecting approximately 23.2/100,000 people in the United States (Rees et al., 2017). The Lupus Foundation in America recently reported that lupus affects approximately 1.5 million people in the United States (LFA, 2019). SLE can present with a wide range of clinical manifestations, typically related to connective-tissue disorders, and often mimics other illnesses (Zucchi et al., 2019). This autoimmune disorder leads to inflammation and irreversible damage in one or more

organs, including the joints, skin, nervous system, and kidneys (Durcan et al., 2019). The cause of SLE is not entirely understood, but it is predicted to manifest due to a combination of genetic and environmental factors, such as vitamin D deficiency, sunburn, and/or viral infections (Finzel et al., 2018). SLE affects women more than men and is a challenging disease to diagnose because of a broad assortment of signs, symptoms, and serological abnormalities (Durcan et al., 2019). SLE morbidity can be attributed to both tissue damage, toxic treatments, and complications associated with treatments, such as immunosuppression, long-term organ damage due to corticosteroid therapy, and accelerated coronary artery disease (Durcan et al., 2019; Fava & Petri, 2019). An early SLE diagnosis is particularly challenging as early-stage tests lack specificity; further, clinical signs and symptoms often only appear after organ damage has occurred, indicating later stages of the disease (Thong & Olsen, 2017). SLE diagnoses are made based on lab findings, clinical manifestations, serology, and histology of impacted organs (Thong & Olsen, 2017). However, current SLE screening tests are notoriously unreliable (Bhana, 2022).

Rheumatoid arthritis (RA) affects more than one million adults in the United States. RA is characterized by chronic inflammation of the synovial tissue of joints, cartilage, and bone (Cohen et al., 2021; Johnson, Sanchez, & Schoenbrunner, 2019; Luan et al., 2021; Pappas et al., 2021; Scherer, Haupl, & Burmester, 2020). Pathological abnormalities in patients with RA includes chronic synovitis, which results in joint devastation (Johnson et al., 2019; Luan et al., 2021; Scherer et al., 2020). Cellular and humoral response aberrations result in autoimmunity; antibodies and rheumatoid factors against post-translational modified proteins (including modifications such as citrullination). As such, synthetic cyclic citrullinated peptides (CCP) have been developed for diagnostic use (Scherer et al., 2020). To date, the etiology of RA has not been fully elucidated, though recent studies have suggested that genetic, epigenetic, and environmental factors contribute to RA presentation (Johnson et al., 2019; Scherer et al., 2020). Due to the complexity of RA pathogenesis, there is no model drug to cure RA.

Biologic markers or “biomarkers” can provide objective measurements that reflect underlying pathophysiological processes, pathogenic processes, or responses to treatment. Most measures of monitoring disease and treatment progress rely on subjective measurements, such as joint evaluation, so biomarkers may be a useful complement in patient management (Taylor & Maini, 2022). Joint damage at the molecular level may be occurring before any clinical signs appear so identifying any indications of disease activity could allow clinical interventions to be taken earlier(Mc Ardle et al., 2015). Markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are part of clinical measures such as the DAS. However, these two biomarkers are nonspecific; abnormal amounts of these markers may be due to other reasons apart from RA and may be completely normal in patients with RA (Centola et al., 2013; Curtis et al., 2012). This non-specificity is not limited to ESR and CRP. For example, antibodies (usually called rheumatoid factors or RF) produced against immunoglobulin G (IgG) are often tested to diagnose RA, but these antibodies may be produced in response to another rheumatic condition or a separate chronic infection (Shmerling, 2021). Autoantibodies to citrullinated protein epitopes, such anti-cyclic citrullinated peptide (anti-CCP2), has also been a focus of biomarker research in RA. Both RF and anti-CCP2 have similar sensitivities for the diagnosis of RA, but anti-CCP2 is positive in 20%-30% of RA patients who are negative for RF (Shapiro, 2021). RA is a heterogenous condition, and no single biomarker is a reliable predictor of RA disease activity (Mc Ardle et al., 2015).

Currently, conventional synthetic disease modifying anti-rheumatic drugs (cDMARDs) are the first line of RA therapy. Unfortunately, some RA patients do not respond to cDMARDs and clinical guidelines suggest use of alternative therapies such as TNFi. TNFi is the most common targeted treatment for RA patients who fail to respond to cDMARDs (Bergman et al., 2020; Johnson et al., 2019; Luan et al., 2021; Pappas et al., 2021). TNFi treatment, however, is not without limitations. Unfortunately, the majority of patients fail to respond to TNFi treatment (measured by American College of Rheumatology (ACR)50-indicates 50% disease improvement) and only 10-25% achieve remission (Cohen et al., 2021; Curtis et al., 2014; Johnson et al., 2019; Pappas et al., 2021). Currently, there is no way to predict whether RA patients will respond to TNFi therapy, and approximately three months is needed to determine whether a patient is responding (Johnson et al., 2019; Pappas et al., 2021). Accordingly, there has been a push to create a personalized medicine approach to identify non-responders to enhance clinical outcomes (Johnson et al., 2019; Pappas et al., 2021).

The systems by which the immune system maintains tolerance to an individual's own antigens can be overcome by release of intracellular antigens following excessive cell death, ineffective clearance of apoptotic debris, inflammation-induced modification of self-antigens, or molecular mimicry, leading to the production of antibodies against self-antigens or autoantibodies (AAB) (Suurmond & Diamond, 2015). Autoantibodies mediate both systemic inflammation and tissue injury and may play a role in the pathogenesis of many autoimmune diseases (Suurmond & Diamond, 2015). Generally, AAB development precedes the clinical onset of autoimmune disease (Damoiseaux et al., 2015) and has predictive value (Satoh et al., 2014); thus, AABs serve as good serological markers to screen for evidence of autoimmunity (Aggarwal, 2014). Autoantibodies can target a variety of molecules (including nucleic acids, lipids, and proteins) from many cellular localizations—nucleus, cytoplasm, cell surface, extracellular organelles (Suurmond & Diamond, 2015), and different specific AABs are associated with particular diagnoses, symptoms, unique syndromes, subsets of disease, and clinical activity (Satoh et al., 2014). See Table 1 from Suurmond and Diamond (2015), below:

Table 1. Autoantibody recognition in systemic autoimmune disease

Antigen location	Antibody	Antigen	Disease	PRR recognition
Nuclear	Anti-dsDNA/dsDNA-nucleosome	dsDNA/nucleosome complex	SLE, lupus nephritis	TLR9
	Anti-ssDNA/ssDNA-nucleosome	ssDNA/nucleosome complex	SLE, lupus nephritis	TLR9
	Anti-Sm	Small nuclear RNP	SLE	TLR7
	Anti-RNA	RNA	SLE	TLR8
	Anti-histone	Histones	SLE (drug induced)	TLR2 and TLR4
	Anti-Scl-70	Topoisomerase I	Systemic sclerosis	
	Anti-centromere	Centromere	Systemic sclerosis/CREST syndrome	
Cytoplasmic/mitochondrial	ANCA	Myeloperoxidase (p-ANCA) and proteinase 3 (c-ANCA)	Vasculitis: Wegener's granulomatosis	
	ASA	Cardiolipin	Antiphospholipid syndrome, SLE	TLR4/9
Membrane proteins	AChR	Acetylcholine receptor	MG	TLR4
	Anti-Ro/La	Extracellular proteins	SLE	
Extracellular	RF	IgG (Fc)	RA	
	Lupus anticoagulant	Phospholipids	Antiphospholipid syndrome	TLR4/2
	α3 Chain of basement membrane collagen (type IV collagen)	α3 Chain of basement membrane collagen (type IV collagen)	Goodpasture's syndrome	

ASA, anti-cardiolipin antibody.

However, serum AAB are present in 18.1% of the general population, and titers are higher in females and increase with age (Selmi et al., 2016). Additionally, only in a few cases does the antibody titer correlates with the severity of clinical manifestations or the response to treatment

(Damoiseaux et al., 2015). The use of ANA detection as a diagnostic test originated with the observation of the lupus erythematosus (LE) cell (Hargraves et al., 1948). Since then, several tests have been developed to detect these antibodies.

The indirect immunofluorescence (IIF) test is the most widely used assay for the detection of AAB and remains the reference method of choice (ACR, 2015). Detection of ANAs by the IIF technique demonstrates binding to specific intracellular structures within the cells, resulting in staining patterns reported using the consensus nomenclature and representative patterns defined by The International Consensus on ANA staining Patterns (ICAP) initiative (Chan et al., 2016) and the degree of binding reflected by the fluorescence intensity or titer (Tebo, 2017). The test takes advantage of a HEp-2 cell line, which have large, easy to visualize, nuclei and contain nearly all of the clinically important autoantigens, making these cells ideal for the detection of the corresponding AABs (Bloch, 2022b). The ANA IIF assay using HEp-2 slide has a high sensitivity for screening of SARDs and efforts to harmonize the nomenclatures for testing and reporting (Chan et al., 2015) have made this a powerful screening tool (Tebo, 2017). The frequency of ANA in SLE and SSc is 95–100%, 50–70% in SJS and 30–50% in rheumatoid arthritis (RA) (Satoh et al., 2014); however, their isolated finding in an otherwise healthy individual has a low positive predictive value which needs to be integrated with other laboratory parameters and patient risk factors (Selmi et al., 2016). Disadvantages of the indirect immunofluorescence test include its labor-intensiveness, significant training requirements for competence, and subjectivity in titer and pattern recognition; moreover, because the staining pattern usually does not identify the responsible autoantibody, additional testing may be required (Bloch, 2022b; Tebo, 2017). Automated image analysis provides a viable option for distinguishing between positive and negative results although the ability to assign specific patterns is insufficient to replace manual microscopic interpretation (Yoo et al., 2017).

The antinuclear antibody (ANA) test is commonly used in the evaluation of autoimmune disorders, as these antibodies are responsible for attacking healthy or normal cells. More than 95% of individuals with SLE will have a positive ANA test (Bhana, 2022). However, ANAs are present in “a significant proportion of normal individuals and lacks specificity or prognostic value” (Thong & Olsen, 2017). In particular, approximately only 11-13% of individuals with a positive ANA test will actually have SLE, and approximately 15% will be completely healthy (Bhana, 2022). Other SLE diagnostic methods include the monitoring of anti-double-stranded DNA (anti-dsDNA), C3 and C4 complement levels, CH50 complement levels, erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) levels, antiphospholipid antibodies, and urine protein-to-creatinine ratios (Wallace & Gladman, 2022).

If SLE is suspected based on the clinical picture following a positive ANA screen, the sera should be tested for antibodies to double-stranded DNA (dsDNA). Anti-dsDNA antibodies are present in two-thirds of patients with SLE, and they have a good association with disease activity and lupus nephritis. Serial monitoring of anti-dsDNA antibodies has modest correlation with disease activity (Aggarwal, 2014).

A positive ANA screen should also be followed by identification of sub-specificities by screening for antibodies to extractable nuclear antigens (ENAs). ENAs were identified by using saline extract of nuclei as the antigen. Antibodies to ENA can be determined using double immunodiffusion, immunoblotting, enzyme-linked immunosorbent assays (ELISA), or bead-

based assay using recombinant or affinity-purified antigens. Different ENAs have an association with different connective tissue diseases (Aggarwal, 2014).

Reflex tests for positive ANA screens have been proposed to improve appropriateness in diagnosis of SARDs and avoid unnecessary second level testing. For specific autoantibodies responsible for certain fluorescent ANA patterns, such as homogeneous, speckled, fine grainy (Scl70-like), nucleolar, centromeric or speckled cytoplasmic, the identification of precise autoantibody markers is considered essential while for others it is not deemed to be necessary (Tonutti et al., 2016). See Table 1 from Tonutti et al, 2016, below.

Table 1

ANA reflex test procedure with titres ≥1:160 and typical patterns

ANA IIF pattern on HEp-2 cells	Reflex test(s)
Nuclear homogeneous ≥1:160	Antibodies to extractable specific antigens (ENA) and to dsDNA/nucleosome
Nuclear speckled ≥1:160	Anti-dsDNA and antibodies to extractable specific antigens (ENA), possibly including anti-RNA polymerase III
Nuclear Scl70-like ≥1:160	Antibodies to extractable specific antigens (ENA), possibly including anti-PM/Scl
Cytoplasmic speckled ≥1:160	Antibodies to extractable specific antigens (ENA), including anti-tRNA synthetases and anti-P ribosomal
Pericentriolar PCNA-like dots only	Anti-PCNA
Centromere	No confirmation necessary if high titres. Execute specific test for anti-CENP B only in doubtful cases (low titre or centromeric pattern not clearly recognizable)

ENA: including SS-A/Ro52 and Ro60, SS-B/La, Sm, RNP, Jo-1, and Scl70

Proprietary Testing

Proprietary tests exist for the assessment of SLE. For example, the "SLE-key" by ImmunArray is a molecular diagnostic test that is intended to help rule out an SLE diagnosis. This test determines the pattern of circulating antibodies and compares it to the proprietary pattern of antigens, "iCHIP". The pattern is compared to both SLE-affected and healthy control patterns, and an algorithm is used to assess the patient's likelihood of being affected with SLE. iCHIP was developed based on 250 affected and 250 healthy patients, and out of a 163 patient sample, the key was validated to "rule out" SLE at 94% sensitivity, 75% specificity, and 93% negative predictive value (ImmunArray, 2016, 2017). Another set of proprietary tests offered are from Exagen, under the "AVISE" line. Their line of tests utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. This includes tests for prognosis (10 biomarkers including various autoantibodies such as anti-C1q and antiribosomal P), diagnosis (10 biomarkers, includes ENA panel), and monitoring (6 biomarkers, includes anti-dsDNA and anti-C1q). AVISE CTD (standing for connective tissue disease) is intended to assist with the differential diagnosis of several autoimmune diseases and includes several ANA biomarkers, as well as an ENA panel. Other tests offered, such as AVISE Anti-CarP (evaluates autoantibodies to carbamylated proteins for rheumatoid patients) still include ANA components (AVISE, 2022).

AVISE Lupus by Exagen is a laboratory developed test (LDT) designed to assist in SLE diagnoses. This LDT utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. The AVISE Lupus test also uses cell-bound complement activation products (CB-CAPs) to measure complement system activation (Exagen, 2022). The 10 SLE relevant markers in this test include anti-dsDNA, anti-Smith (anti-Sm) antibodies, erythrocyte-bound C4d or B-lymphocyte-bound C4d (BC4d), ANA, CB-CAPs, and autoantibody specificity components

(Exagen, 2022). As noted on their website, "The AVISE Lupus test is an ideal test for ANA positive patients with a clinical suspicion of lupus" (Exagen, 2022).

PrismRA is a molecular signature test that predicts TNFi non-response prior to treatment initiation. PrismRA utilizes a 23-feature blood-based molecular signature response classifier (MSRC) which integrates next generation RNA sequencing data and clinical features (clinical metrics, demographic variables, C reactive protein (CRP) and anti-CCP antibodies) to predict patients' response to TNFi treatment (Cohen et al., 2021). A high score is indicative of decreased likelihood of the RA patient to respond to TNFi therapies.

Vectra DA is a multi-biomarker disease activity (MBDA) blood test which combines the levels of 12 serum biomarkers into a single score from 1 to 100 to provide an objective measure of RA disease activity. It is intended for use with existing symptom-based disease activity measures to improve long-term outcomes for RA patients (van der Helm-van Mil et al., 2013). While multi-biomarker panels are emerging as a potentially useful tool in the management of RA, there is not yet a consensus as to their clinical utility (Taylor & Maini, 2022).

Analytical Validity

A variety of manual or automated single or multiplex immunoassays have been introduced to make the process of detecting autoantibodies more efficient, including ELISA, fluorescent microsphere assays, and chemiluminescence immunoassays (CIA)—each with different performance characteristics (Tebo, 2017). In these assays, a panel of purified native or recombinant autoantigens is prepared, and each antigen is immobilized on a solid surface (microtiter plate, fluorescent microsphere, or membrane) and incubated with diluted human serum (Bloch, 2022b). The advantages of these alternative approaches to ANA IIF testing include their suitability for high-throughput testing, semi-quantification of test results, the lack of subjectivity, and the consolidation of ANA-related tests in a single platform as a positive test also provides identification of the responsible autoantibody (Bloch, 2022b; Tebo, 2017). It has been estimated that solid phase assays may decrease the labor cost of ANA testing by as much as 95 percent (Bloch, 2022b). In a recent study which evaluated the performance of an automated CIA and fluorescence enzyme immunoassay (FEIA) and compared their performance to that of IIFA, both FEIA and CIA screen significantly outperformed IIF, with a higher specificity for FEIA and higher sensitivity for CIA (van der Pol et al., 2018). The use of solid phase assays as the initial test for the detection of ANA is concerning because the number of autoantigens that are included in solid phase assays is limited compared with the number that are present in the HEp-2 cell substrate, thus limiting sensitivity (Bloch, 2022b). Consequently, IIF remains the gold standard, and in cases of strong clinical suspicion of SARD and a negative screen from a solid phase assay, IIF should be performed (van der Pol et al., 2018).

Tipu et al. (2018) investigated the specificity and pattern for ANA in systemic rheumatic disease patients. 4347 samples were sent, and 397 were positive for ANA. Of these 397, 96 were positive on the anti-ENA screen and tested for anti-ENA reactivity. Anti-SSA antibodies were found in 59 of these samples. The most common ANA patterns were "coarse" and "fine-speckled" (43 and 22 of 81 respectively). However, no specific ANA pattern was associated with anti-ENA reactivity (Tipu & Bashir, 2018).

Kim et al. (2019) performed a meta-analysis comparing ANA measurement by automated indirect immunofluorescence (AIIF) and manual indirect immunofluorescence (MIIF). 22 studies including 6913 positive and 1818 negative samples of manual indirect immunofluorescence (MIIF) were included. Among this cohort, 524 samples with combined systemic rheumatic diseases (SRDs), 132 systemic lupus erythematosus (SLE) samples, and 104 systemic sclerosis (SSc) samples, and 520 controls were available. Positive concordance (PC) between AIIF and MIIF was 93.7%, although PC of total pattern and titer were lower. Clinical sensitivities of AIIF vs MIIF were 84.7% vs 78.2% for combined SRDs, 95.5% vs 93.9% for SLE, and 86.5% vs 83.7% for SSc. Clinical specificities of AIIF vs MIIF were 75.6% vs 79.6% for combined SRDs, 74.2% vs 83.3% for SLE, and 74.2% vs 83.3% for SSc. The authors concluded that the sensitivities did not differ between methods, but the specificities of SLE and SSc were statistically significant changes (Kim et al., 2019).

Dervieux et al. (2017) performed the analytical validation of Exagen's multianalyte panel test for SLE. This assay uses quantitative flow cytometry to assess the levels of the complement split product C4d bound to erythrocytes (EC4d) and B-lymphocytes (BC4d), in units of mean fluorescence intensity (MFI), and immunoassays to assay for antinuclear and anti-double stranded DNA antibodies (e.g. autoantibodies). The results were reported on a two-tiered index score as either positive or negative. The authors included specimens from both patients with SLE as well as individuals without SLE. Controls consisting of three-level C4 coated positive beads were run daily. The authors note that at ambient temperature both EC4d and BC4d are stable for 2 days and for 4 days if the samples are stored at 4˚C. "Median intra-day and inter-day CV [coefficient of variation] range from 2.9% to 7.8% (n=30) and 7.3% to 12.4% (n=66), respectively. The 2-tiered index score is reproducible over 4 consecutive days upon storage of blood at 4°C. A total of 2,888 three-level quality control data were collected from 6 flow cytometers with an overall failure rate below 3%. Median EC4d level is 6 net MFI (Interquartile [IQ] range 4-9 net MFI) and median BC4d is 18 net MFI (IQ range 13-27 net MFI) among 86,852 specimens submitted for testing. The incidence of 2-tiered positive test results is 13.4%" (Dervieux et al., 2017).

Putterman et al. (2014) compared the performance of C4d CB-CAPs on erythrocyte and B cells with antibodies to dsDNA, C3, and C4 in patients with SLE. A total of 794 individuals participated in this study, which included 205 healthy controls, 304 patients with SLE, and 285 patients with other rheumatic diseases. Both erythrocytes and B cells were measured with flow cytometry, and antibodies, including anti-dsDNA, were measured with solid-phase immunoassays. SLE activity was determined using the SLE Disease Activity Index Safety of Estrogens in Lupus National Assessment (SELENA) Modification, and the two-tiered AVISE Lupus test was developed. Results showed that "The combination of EC4d and BC4d in multivariate testing methodology with anti-dsDNA and autoantibodies to cellular and citrullinated antigens yielded 80% sensitivity for SLE and specificity ranging from 70% (Sjogren's syndrome) to 92% (rheumatoid arthritis) (98%vs. normal)" (Putterman et al., 2014). Overall, the measurement of CB-CAPs was more sensitive for SLE diagnostic purposes than complement or anti-dsDNA measurements.

Ramsey-Goldman et al. (2020) evaluated the use of CB-CAPs, using flow cytometry, or a multianalyte assay panel (MAP) that includes CB-CAPs (e.g., AVISE Lupus) on patients with suspected SLE (n = 92) who fulfilled three classification criteria of the American College of

Rheumatology (ACR). They also compared the data with individuals with established SLE (n = 53). At the initial visit, the individuals with suspected SLE had statistically higher positive CB-CAP (28%) or MAP results (40%) than individuals with established SLE. "In probable SLE, MAP scores of >0.8 at enrollment predicted fulfillment of a fourth ACR criterion within 18 months (hazard ratio 3.11, P<0.01)." The authors, who did acknowledge compensation from Exagen, conclude that "[a] MAP score above 0.8 predicts transition to classifiable SLE according to ACR criteria" (Ramsey-Goldman et al., 2020).

Clinical Utility and Validity

ANA, ENA, and SDLT

Oglesby et al. (2014) performed a cost-savings impact analysis on when the diagnosis of SLE is made and how it affects the clinical and economic outcomes. Using a claims database of claims made between January 2000 and June 2010, the authors separated individuals into two groups (n = 4166 per group) —early diagnosis (within 6 months of onset of symptoms) and late diagnosis (6 or more months after the onset of symptoms)—based upon an algorithm using a patient's ICD-9 diagnosis code(s) on the claim(s) and when SLE medications were dispensed. Additional propensity scores were matched using data based on "age, gender, diagnosis year, region, health plan type, and comorbidities". Results show that the early diagnosis group had lower rates of mild, moderate, and severe flares as well as lower rates of hospitalization as compared to the late diagnosis group. Moreover, "[c]ompared with the late diagnosis patients, mean all-cause inpatient costs PPPM [per patient per month] were lower for the early diagnosis patients (US$406 vs. US$486; p = 0.016). Corresponding SLE-related hospitalization costs were also lower for early compared with late diagnosis patients (US$71 vs US$95; p = 0.013)." The values are adjusted to 2010 US dollars. The authors note that the other resource use and cost categories were consistent, concluding "[p]atients diagnosed with SLE sooner may experience lower flare rates, less healthcare utilization, and lower costs from a commercially insured population perspective" (Oglesby et al., 2014).

A study by Yeo et al. (2020) demonstrates that there is little benefit to repeat ANA testing if the initial test was negative by evaluating the cost of repeat ANA testing. From 2011 to 2018, 36,715 ANA tests were performed for 28,840 patients at a total cost of $675,029. Of these tests, 21.4% were repeats in which 54.9% of the patients initially tested negative. Of those who tested negative and repeated ANA testing, only 19% of the patients had a positive result when the test was repeated once in under two years, and this positive test did not lead to a change in diagnosis. Therefore, the authors conclude that "repeat ANA testing after a negative result has low utility and results in high cost" (Yeo et al., 2020).

Deng et al. (2016) investigated the clinical utility of ANA testing through different assays to see which one was most appropriate for evaluating patients with CTD. With 1000 samples collected, they compared an enzyme immunoassay (EIA), immunofluorescence assay (IFA), and multiplex immunoassay (MIA) in terms of specificity and sensitivity of testing. The researchers found that through using weights to define a patient sample that reflected the intended testing population and a normalized specificity of 90% to standardize the comparison between tests, the MIA, EIA, and IFA had sensitivities of 67%, 67%, and 56%, respectively. However, with a varying clinical cutoff, the IFA could obtain a sensitivity of 94% and a corresponding specificity of only 43%.

This demonstrated that the sensitivity and specificity could easily vary with predetermined cutoffs; but, there were "no statistically significant differences in the clinical utility of the IFA, EIA, or MIA" (Deng et al., 2016).

Alsaed et al. (2021) compared the performance of ANA testing via ELISA vs IIF for CTDs. From a sample of 1457 patients and 12,439 tests ordered in 2016, they found that with "cut-off ratio ≥ 1.0 for ANA-ELISA and a dilutional titre ≥ 1:80 for ANA-IIF, the sensitivity of ANA-IIF and ANA-ELISA for all CTDs were 63.3% vs 74.8% respectively. For the SLE it was 64.3% vs 76.9%, Sjogren's Syndrome was 50% vs 76.9% respectively. The overall specificity of ANA-ELISA was 89.05%, which was slightly better than ANA-IIF 86.72%". This communicated the ELISA was slightly better than IIF in sensitivity and specificity, which could influence the convention of using IIF going forward if these findings are reflected in other cohort studies.

Biomarker analysis

Wallace et al. (2019) performed a randomized prospective trial to assess the clinical utility of the AVISE lupus MAP test (MAP/CB-CAP) as compared to standard diagnosis laboratory testing (SDLT). 145 patients with a history of positive antinuclear antibody status were randomly assigned to either an SDLT arm (n = 73) or the MAP/CB-CAP arm (n = 72) of the study. Treatment changes were recorded based on either the SDLT or MAP/CB-CAP results. Even though the demographics between the two arms of the study were similar, the results were different. "Post-test likelihood of SLE resulting from randomisation in the MAP/CB-CAPs testing arm was significantly lower than that resulting from randomisation to SDLT arm on review of test results (−0.44±0.10 points vs −0.19±0.07 points) and at the 12-week follow-up visit (−0.61±0.10 points vs −0.31±0.10 points) ($p<0.05$). Among patients randomised to the MAP/CB-CAPs testing arm, two-tiered positive test results associated significantly with initiation of prednisone ($p=0.034$)" (Wallace et al., 2019). The authors conclude that testing such as the AVISE Lupus test has clinical utility and does affect treatment decisions.

A longitudinal, retrospective study by Mossell et al. (2016) of 46 patients who were anti-nuclear antibodies (ANA) positive but SLE-specific autoantibodies negative was conducted to evaluate the clinical utility of the AVISE Lupus test. 23 of the patients were in the "case" group (i.e. positive result based on the AVISE Lupus test), and 23 patients were in the "control" or negative results group. The charts of each individual were reviewed at two different times: T0 (or the initial time) and T1 (or approximately 1 year later). The case group was diagnosed with SLE at a higher rate than the control group (87% vs. 17%, respectively); moreover, the case group fulfilled 4 of the ACR classification criteria of SLE at a higher rate than the control group (43% vs 17%, respectively). The authors found that the sensitivity of the AVISE Lupus test (83%) is statistically significantly higher than the ACR score (42%, $p = 0.006$). Even at the initial baseline, individuals in the case group were prescribed anti-rheumatic medications more frequently (83% vs. 35%, $p = 0.002$) than the control group, indicating that a positive AVISE Lupus test may result in a more aggressive early treatment therapy (Mossell et al., 2016).

Liang et al. (2020) assayed the utility of the AVISE test in predicting lupus diagnosis and progression in 117 patients who previously did not have a diagnosis of SLE. The study assessed the patients at the time of the initial AVISE test ($t = 0$) and two years later ($t = 2$) using the SLE diagnosis criteria of the Systemic Lupus International Collaborating Clinics (SLICC) and ACR

and the SLICC Damage Index (SDI) to measure SLE damage. After two years, patients who tested positive developed SLE at a significantly higher rate than those who tested negative using the AVISE test (65% vs 10.3%, $p < 0.0001$). AVISE-positive patients have more SLE damage after two years than AVISE-negative patients (1.9±1.3 vs 1.03±1.3, p=0.01). In particular, the authors note that the levels of BC4d "correlated with the number of SLICC criteria at t=0 (r=0.33, p< 0.0001) and t=2 (r=0.34, p<0.0001), as well as SDI at t=0 (r=0.25, p=0.003) and t=2 (r=0.26, p=0.002)" (Liang et al., 2020).

Alexander et al. (2021) further validated the clinical utility of the AVISE lupus test via a systematic review of medical records of ANA-positive patients with positive (>0.1) or negative (<-0.1) MAP scores. They found that the "odds of higher confidence in SLE diagnosis increased by 1.74-fold for every unit increase of the MAP score" with statistical significance, demonstrating that the test still further solidifies a diagnosis of SLE and can help inform "appropriate treatment decisions" (Alexander et al., 2021).

A study by Clarke et al. (2020) demonstrates the cost-effective management of systemic lupus erythematosus (SLE) using a MAP rather than SDLTs. The higher specificity of MAP allows for an earlier SLE diagnosis, prompt initiation of the appropriate therapy, and fewer unnecessary and costly hospitalizations or investigations. Current SDLTS, such as ANA tests, have a high diagnostic sensitivity, but a high false-positive rate. MAP combines complement C4d activation products on erythrocytes and B cells with SDLTs, with antibodies to nuclear antigens, dsDNA IgG (with Crithidia confirmation), Smith, Sjogren's syndrome type-B (SS-B/La), topoisomerase I (Scl-70), centromere protein B (CENP), histidyl t-RNA synthetase (Jo-1), and cyclic citrullinated peptites (CCP) to improve SLE diagnosis. MAP "yields improved overall diagnostic performance with a sensitivity and specificity of 80% and 86%, respectively, compared with a sensitivity and specificity of 83% and 76%, respectively, for SDLTs. Despite the lower sensitivity, the superior specificity of MAP (86%) over SDLTs (76%) results in a higher positive predictive value associated with MAP (36.75%) compared with SDLTs (26.02%)" (Clarke et al., 2020). The improved specificity of MAP resulted in a cost savings of $1,991,152 to a US commercial plan over a 4-year time horizon, which translates to $0.04 in per member per month (PMPM) savings (Clarke et al., 2020).

Clinical validation of PrismRA was conducted in the Comparative Effectiveness Registry to Study Therapies for Arthritis and Inflammatory Conditions (CERTAIN) study (Bergman et al., 2020; Mellors et al., 2020). The CERTAIN trial was conducted by the Consortium of Rheumatology Researchers of North America which consisted of 43 sites and 117 rheumatologists (Mellors et al., 2020). This prospective study analyzed baseline RNA sequencing and clinical assessments to determine the effectiveness of PrismRA to predict TNFi non-response. Evaluation of the clinical response to TNFi was performed at six months and was determined by ACR50. The CERTAIN study built and validated the biomarker panel used for MSRC analyses. The study found that PrismRA demonstrated a positive predictive value of 89.7%, a specificity of 86.8%, and a sensitivity of 50% (Mellors et al., 2020; Pappas et al., 2021).

Inadequate TNFi response predictions were further validated on integrated blood samples from CERTAIN and NETWORK-004 studies. NETWORK-004 was a 24-week blinded prospective study conducted at 73 sites to evaluate the ability of MSRC to identify TNFi non-responders at three and six months by ACR50 (evaluations were also conducted using other scales such as

Disease Activity Score (DAS28)-CRP, and Clinical Disease Activity Index). CERTAIN samples were used for transcript biomarker feature selection (n=100) and cross validation of MSRC (n=245). In the NETWORK-004 cohort, MSRC validation was performed in samples from naïve (n=146) and TNFi exposed (n=113) patients. ACR50 of patients stratified by MSRC at six months according to prediction of an inadequate response to TNFi therapy had an odds ratio of 4.1 (95% CI 2.0–8.3; p value=0.0001). Patients with a non-response MSCR were 26 times less likely to achieve remission evaluated three months after TNFi therapy (Cohen et al., 2021). Both studies found that PrismRA was able to accurately predict TNFi non-responders according to multiple clinically validated measurement scales (Cohen et al., 2021; Mellors et al., 2020).

Bergman et al. (2020) preformed modeling of the projected improvements from PrismRA and determined that ACR50 improved in the stratified cohort (40%) compared to the unstratified patient cohort (30%) and decreased costs of ineffective treatment by 19%. Further, PrismRA was shown to be a better predictor of inadequate response to TNFi treatment than clinical metrics alone (Bergman et al., 2020). Pappas et al. (2021) conducted a 32-question decision-impact survey involving 248 rheumatologists to determine whether predictive tests such as PrismRA appear to have clinical utility in RA patients' ability to respond to TNFi therapy. The study demonstrated that rheumatologists overwhelmingly supported the clinical need of predictive technologies to determine whether RA patients would respond to TNFi therapies and that payers should provide coverage of predictive technology (Pappas et al., 2021).

According to Curtis et al. (2012), the MBDA algorithm (Vectra DA) was developed by screening 396 candidate biomarkers. An algorithm was then created to generate a composite score based on the 12 biomarkers most correlated to RA clinical disease activity which are as follows:

- Interleukin-6 [IL-6]
- Tumor necrosis factor receptor type I [TNFRI]
- Vascular cell adhesion molecule 1 [VCAM-1]
- Epidermal growth factor [EGF]
- Vascular endothelial growth factor A [VEGF-A]
- YKL-40
- Matrix metalloproteinase 1 [MMP-1]
- MMP-3
- CRP
- Serum amyloid A [SAA]
- Leptin
- Resistin

These biomarkers represent several processes related to RA, such as cartilage remodeling and cytokine signaling pathways. A score of ≤29 is considered "low" activity, between 29 and 44 is "moderate" activity, and >44 is "high" activity. The MBDA is intended to provide separate information from a clinical evaluation of joints and should be used as a complement, not as a replacement (Curtis et al., 2012).

This MBDA has been shown to correlate significantly ($r=0.72$; $p<0.001$) with a disease activity score based on the 28-joint Disease Activity Score based on CRP (DAS28-CRP) and has been validated for clinical use as a disease activity marker in RA (Curtis et al., 2012). Both Hirata et

al. (2013) and Bakker et al. (2012) found the MBDA score to correlate well with disease activity and could complement other existing measures of RA assessment. Remission based on the MBDA score was a significant predictor of radiographic non-progression, whereas both remission-defined DAS28-CRP and American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria was not. The MBDA test was also useful in assessing the risk of radiographic progression among patients who met clinical remission criteria. MBDA results may provide an important addition to clinical assessment, however, further studies are needed to confirm its clinical utility in the management of RA (van der Helm-van Mil et al., 2013).

Li et al. (2013) evaluated the impact of an MBDA blood test for rheumatoid arthritis (RA) on treatment decisions made by six health care providers (HCPs) in 101 patients. HCPs completed surveys before and after viewing the MBDA test result, recording dosage and frequency for all RA medications and assessment of disease activity. Frequency and changes in treatment plan that resulted from viewing the MBDA test result were determined. The MBDA test results were found to have changed 38% of patients' treatment plans. Furthermore, treatment plans were changed 63% of the time the MBDA test results were found to be "not consistent" or "somewhat consistent" with the clinical assessment of disease activity. However, any improvement in clinical outcomes caused was not reported, and the overall amount of drug use was not affected (Li et al., 2013).

Another study by Li et al. (2016) assessed the correlation between MBDA score and disease progression in 163 RA patients. The study found that low radiographic progression was associated with low MBDA scores, and higher scores were associated with more frequent and severe progression. Notably, MBDA scores correlated with progression even when a conventional measure such as the DAS28 indicated otherwise. For example, low risk of progression was associated with a low MBDA score, even when a concurrent DAS28 score was high. The authors concluded that MBDA may be a good complement for conventional measures, as well as provide information on changing treatment plans (Li et al., 2016).

Curtis et al. (2018) initially studied the influence of age, obesity and other comorbidities on the MBDA test. A cross-sectional analysis of RA patients who have participated in an MBDA test was used (n=357). "Of 357 eligible patients, 76% (n = 273) had normal CRP (<10mg/L) with high (33%), moderate (45%), and low (22%) disease activity by MBDA. The MBDA score was significantly associated with BMI, age, CDAI [clinical disease activity index], and SJC [swollen joint count] (Curtis et al., 2018)." Almost one third of participants had normal CRP scores but high MBDA scores. "In this real-world analysis, the MBDA score was associated with RA disease activity, obesity, and age, and was negligibly affected by common comorbidities (Curtis et al., 2018)."The authors conclude by suggesting that an adjusted MBDA score may require development to account for BMI and age. Such a study was then published the following year. Curtis et al. (2019) developed an MBDA test that will include additional factors such as sex, age and obesity in RA patients. Obesity, or adiposity, was measured using either BMI or serum leptin concentration. Two cohorts were studied, totaling 1736 patients. Overall, the authors have developed "a leptin-adjusted MBDA score that has significantly improved [the] ability to predict clinical disease activity and radiographic progression (Curtis et al., 2019)." It was suggested that this leptin-adjusted MBDA score "significantly adds information to DAS28-CRP and the original MBDA score in predicting radiographic progression. It may offer improved clinical utility for

personalized management of RA" (Curtis et al., 2019).

A recent study analyzed the measurement of serum biomarkers at early RA disease onset in hopes to better predict disease progression (Brahe et al., 2019). MBDA score and changes in this score were evaluated to predict DAS28-CRP remission. A total of 180 patients participated in this study and were treated with either methotrexate and adalimumab (n = 89) or methotrexate and placebo (n = 91) in addition to a glucocorticoid injection into swollen joints; results showed that "Early changes in MBDA score were associated with clinical remission based on DAS28-CRP at 6 months" (Brahe et al., 2019).

In a study by Ma et al. (2020), the MBDA test was used to explore the role of biomarkers in predicting remission of RA. Serum samples for 148 patients were assessed for MBDA score at three months, six months, and at one year. RA patients on greater than six months stable therapy in stable low disease activity were assessed every three months for one year. Patients not fulfilling any remission criteria at baseline were classified as 'low disease activity state' (LDAS). Patients not fulfilling any remission criteria over 1 year were classified as 'persistent disease activity' (PDA). Of the 148 patients, 27% were in the LDAS group and over 1 year and 9% of patients were classified as PDA. Baseline MBDA score and concentrations of IL-6, leptin, SAA and CRP were significantly lower in all baseline remission criteria groups in comparison to LDAS groups. The individual MBDA biomarkers (IL-6, leptin, SAA, CRP) and initial MBDA score was able to differentiate between remission at baseline and LDAS. The authors state that these findings highlight the potential value of repeated measurements of MBDA score to evaluate the stability of clinical disease activity over time (Ma et al., 2020).

In a combined analysis of the OPERA, SWEFOT, and BRASS studies in which a newer version of the MBDA score was validated, Curtis analyzed the prognostic value of the adjusted MBDA score for radiographic progression in RA. The new MBDA score, used in these three studies, adjusts for age, sex, and adiposity. Curtis evaluated associations of radiographic progression (ΔTSS) per year with the adjusted MBDA score, seropositivity, and clinical measures using linear and logistic regression. The adjusted MBDA score was validated in SWEFOT, compared with the other two cohorts, and used to generate curves for predicting risk of radiographic progression. The adjusted MBDA score was found to be the "strongest, independent predicator of radiographic progression (ΔTSS > 5) compared with seropositivity (rheumatoid factor and/or anti-CCP), baseline TSS, DAS28-CRP, CRP SJC, or CDAI. Its prognostic ability is not significantly improved by the addition of DAS28-CRP, CRP, SJC, or CDAI" (Curtis et al., 2021).

Fleischmann et al. (2022) engaged in a multicenter, randomized, placebo-controlled trial of repository corticotropin injection (RCI) in patients with active RA. The utility of an MBDA score was measured against the utility of the Disease Activity Score to assess disease activity in RA. Study participants received 80 units of RCI twice weekly, and those who had low disease activity at week 12 were given either 80 units of RIC or a placebo twice weekly. The changes in disease activity (measured by DAS28-ESR, CDAI, and MBDA scores) were analyzed, including correlations between MBDA scores and both DAS28-ESR and CDAI scores. Results showed "changes from baseline in DASw8-ESR and CDAI scores suggested the RCI therapy led to clinically meaningful improvements in disease activity, but improvements from baseline in MBDA scores were below the minimally important difference threshold." The authors

concluded that MBDA scores were not "sufficiently responsive" in the assessment of RA disease activity. The authors also said that MBDA should not be used as a preferred disease activity measure for RA patients (Fleischmann et al., 2022).

V. Guidelines and Recommendations

American College of Rheumatology

In 1997, the Diagnostic and Therapeutic Criteria Committee of the ACR revised the 1982 criteria for SLE. Often referred to as the 1997 ACR criteria, these revisions included the addition of "[p]ositive finding of antiphospholipid antibodies based on 1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2) a positive test result for lupus anticoagulant using a standard method, or 3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test (Hochberg, 1997)." The 1997 ACR criteria consists of 11 possible different criterion and each criterion may have more than one definition. A minimum score of 4 out of 11 is indicative of SLE. According to the Centers for Disease Control and Prevention (CDC), rheumatologists can use these criteria "to classify SLE for research purposes"(CDC, 2022). The 1997 ACR criteria in a study by Mosca et al. (2019), using a cohort of 616 patients, has a reported accuracy of 75.5%, sensitivity of 66.1%, and specificity of 91.6%. The criteria are as follows (ACR, 1997; CDC, 2022):

1. Malar Rash
2. Discoid Rash
3. Photosensitivity
4. Oral Ulcers
5. Nonerosive Arthritis
6. Pleuritis or Pericarditis
7. Renal Disorder
8. Neurologic Disorder
9. Hematologic Disorder
10. Immunologic Disorder
11. Positive Antinuclear Antibody

The ACR published a statement on the Methodology of Testing for Antinuclear Antibodies (ACR, 2015) which states:

1. The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing.
2. Hospital and commercial laboratories using alternative bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering healthcare providers on request that the alternative assay has the same or improved sensitivity compared to IF ANA.
3. In-house assays for detecting ANA as well as anti-DNA, anti-Sm (anti-Smith antidbodies), anti-RNP (antinuclear ribonucleoprotein), anti-Ro/SS-A (anti-Ro/Sjogren Syndrome-A), anti La/SS-B (anti-La/Sjogren Syndrome-B), etc., should be standardized according to national (e.g, CDC) and/or international (e.g., WHO, IUIS) standards.

4. Laboratories should specify the methods utilized for detecting ANAs when reporting their results.

The above positions were reaffirmed in 2019 (ACR, 2019).
The ACR also have developed a list of 5 tests, treatments or services that are commonly used in rheumatology practice, but their value should be questioned. The ANA testing was the first on the final top 5 items list with level of evidence Grade 1C. In their review, the Task Force considered recommendations currently published by American College of Pathologists (ACP), ACR, and Italian Society of Laboratory Medicine (ISLM). They have issued the following recommendation: "Do not test antinuclear antibody (ANA) subserologies without a positive ANA and clinical suspicion of immune-mediated disease (Yazdany et al., 2013)." For their list of 5 things to question for pediatric rheumatology, two points pertain to ANA testing (Rouster-Stevens et al., 2014). "Do not order autoantibody panels unless positive ANAs and evidence of rheumatic disease. There is no evidence that autoantibody testing (including ANA and autoantibody panels) enhances the diagnosis of children with musculoskeletal pain in the absence of evidence of rheumatic disease as determined by a careful history and physical examination." The latter recommendation also stated, "Do not repeat a confirmed positive ANA in patients with established JIA [juvenile idiopathic arthritis] or SLE (Rouster-Stevens et al., 2014)." These guidelines were reviewed and reaffirmed in 2021.

European League Against Rheumatism/American College of Rheumatology (EULAR/ACR)

The EULAR/ACR published a joint guideline to develop new classification criteria for systemic lupus erythematosus (SLE). In it, they stated that antinuclear antibodies (ANA) "at a titer of ≥1:80 on HEp-2 cells or an equivalent positive test" was to be an "entry criterion": if absent, the condition is not SLE; if present, apply additive criteria such as leukopenia or oral ulcers. Antiphospholipid antibodies, complement proteins, and SLE-specific antibodies (anti-dsDNA antibodies, Anti-Smith antibodies) are all included as additive criteria for SLE diagnosis (Aringer et al., 2019).

American Academy of Pediatrics (AAP)

The AAP released guidelines through ChoosingWisely. In it, they state "Do not order antinuclear antibody (ANA) and other autoantibody testing on a child unless there is strong suspicion or specific signs of autoimmune disease. A positive ANA may occur secondary to polyclonal activation of the immune system following an infection, or it may be positive without any identifiable reason/disease in up to 32% of the population. Limiting patients on which to order ANA would reduce unnecessary physician visits and laboratory expenses as well as parental anxiety. "Lupus panels" and other similar panels should also not be ordered without concerns for specific autoimmune disease" (AAP, 2019).

Systemic Lupus International Collaborating Clinics (SLICC)

The 2012 SLICC Classification Criteria for SLE splits the 17 criteria into two divisions—either clinical or immunologic. An individual scoring at least a 4, including at least one clinical criterion and one immunologic criterion, is classified as having SLE. The criteria are cumulative and do not need to be concurrently expressed or present (Petri et al., 2012). Mosca et al. (2019) also

analyzed the accuracy and validity of the SLICC classification criteria, using a cohort of 616 patients, reporting an accuracy of 83.1%, sensitivity of 83.5%, and specificity of 82.4%. The criteria include the following (Petri et al., 2012):

A. Clinical Criteria

1. Acute cutaneous lupus, such as lupus malar rash or subacute cutaneous lupus
2. Chronic cutaneous lupus, such as classic discoid rash or discoid lupus/lichen planus overlap
3. Nonscarring alopecia
4. Oral or nasal ulcers
5. Joint disease
6. Serositis
7. Renal criteria, such as urine protein-to-creatinine ratio representing 500 mg protein/24 hours or red blood cell casts
8. Neurologic criteria, such seizures, psychosis, myelitis, and so on
9. Hemolytic anemia
10. Leukopenia or lymphopenia
11. Thrombocytopenia

B. Immunologic Criteria

1. ANA
2. Anti-dsDNA
3. Anti-Sm
4. Antiphospholipid antibodies
5. Low complement (Low C3, Low C4, or Low CH50)
6. Direct Coombs test in the absence of hemolytic anemia

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
81490	Autoimmune (rheumatoid arthritis), analysis of 12 biomarkers using immunoassays, utilizing serum, prognostic algorithm reported as a disease activity score

	Proprietary test: Vectra®DA Lab/Manufacturer: Crescendo Bioscience, Inc.
86038	Antinuclear antibodies (ANA)
86039	Antinuclear antibodies (ANA); titer
86225	Deoxyribonucleic acid (DNA) antibody; native or double stranded
86235	Extractable nuclear antigen, antibody to, any method (eg, nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody
0039U	Deoxyribonucleic acid (DNA) antibody, double stranded, high avidity Proprietary test: Anti-dsDNA, High Salt/Avidity Lab/Manufacturer: University of Washington, Department of Laboratory Medicine/Bio-Rad
0062U	Autoimmune (systemic lupus erythematosus), IgG and IgM analysis of 80 biomarkers, utilizing serum, algorithm reported with a risk score Proprietary test: SLE-key® Rule Out Lab/Manufacturer: Veracis Inc
0312U	Autoimmune diseases (eg, systemic lupus erythematosus [SLE]), analysis of 8 IgG autoantibodies and 2 cell-bound complement activation products using enzyme-linked immunosorbent immunoassay (ELISA), flow cytometry and indirect immunofluorescence, serum, or plasma and whole blood, individual components reported along with an algorithmic SLE-likelihood assessment Proprietary test: Avise® Lupus Lab/Manufacturer: Exagen Inc

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
01/01/2022	Initial Effective Date
07/19/2022	Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria. Added 0312U mapped to CC9, removal of code 81599 Revised code disclaimer statement
06/12/2023	Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Title changed to “Biomarker Testing for Autoimmune Rheumatic Disease” Addition of “a) Once per lifetime in individuals with stable symptoms. b) Repeat testing only if a significant change in symptoms occurs.” To CC1, now reads: “1) For individuals with a high clinical suspicion of autoimmune

	disease, testing for antinuclear antibodies (ANA) MEETS COVERAGE CRITERIA. a) Once per lifetime in individuals with stable symptoms. b) Repeat testing only if a significant change in symptoms occurs. **Information from G2127 wrapped into this policy, resulting in a new CC9: "9) For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA score, PrismRA) DOES NOT MEET COVERAGE CRITERIA." **Added CPT code 81490 Committee approved 6/12/2023