ANA/ENA Testing

Policy Number: AHS – G2022 – ANA/ENA Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 06/01/2021 Revision Date: N/A	

I. Policy Description

The antinuclear antibody (ANA) assay is used to detect autoantibodies (AAB) against intracellular antigens, originally known as antinuclear antibodies (Tan, 1989). The name antinuclear for the ANA test, maintained for historical and laboratory coding purposes, does not convey that autoantibodies to cell compartments other than the nucleus are also detected (Chan et al., 2015). The term "extractable nuclear antigens" (ENA) is an artefact from when the antigens were extracted from the cell into saline solution prior to testing, and analysis of ENA assists in autoimmune disease diagnosis and progression. ENAs include Sm, U1 RNP, Ro, and La antigens (Bloch, 2020). Commonly used as part of the initial diagnostic workup to screen for evidence of systemic autoimmunity (Satoh et al., 2007), detection and identification of AABs are important in the diagnosis of systemic autoimmune rheumatic diseases (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).

II. Related Policies

Policy Number	Policy Title
AHS-G2098	Immune Cell Function Assay
AHS-G2127	Vectra DA Blood Test for Rheumatoid Arthritis
AHS-G2155	General Inflammation Testing

III. 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. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx?from2=search1.asp& or the manual website

1. Testing for antinuclear antibodies (ANA) MEETS COVERAGE CRITERIA for individuals in whom the clinical suspicion of autoimmune diseases is high based on signs, symptoms and other factors.

2. ENA panel testing of specific autoantibodies such as nRNP, SS-A, SS-B, Sm, RNP, Sc170, or Jo1 MEETS COVERAGE CRITERIA in patients with abnormal, raised antibody titer or abnormal immunological findings in serum and clinical correlation with the appropriate autoimmune disorder.

3. Testing of dsDNA MEETS COVERAGE CRITERIA up to four (4) times per year after an initial positive ANA test, and clinical correlation.

4. Testing of specific antibodies when ANA test is negative or low positive MEETS COVERAGE CRITERIA only in the following situations:

    a. Testing for Anti-Jo-1 in unique clinical subset of myositis

    b. Testing for Anti-SSA in the setting of lupus or Sjörgren's syndrome

5. Monitoring of disease with ANA testing or ANA titers DOES NOT MEET COVERAGE CRITERIA.

6. ANA and/or ENA testing of individuals with nonspecific symptoms including, but not limited to, fatigue and musculoskeletal pain if not present with other symptoms suggestive of SLE, DOES NOT MEET COVERAGE CRITERIA.

7. Testing of ANA and/or ENA DOES NOT MEET COVERAGE CRITERIA in individuals during wellness visits or general encounters without abnormal findings.

8. Testing of specific antibodies in the absence of a positive ANA test DOES NOT MEET COVERAGE CRITERIA in all other situations.

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. The use of cell-bound complement activation products (e.g. AVISE Lupus) DOES NOT MEET COVERAGE CRITERIA for the diagnosis of systemic lupus erythematosus (SLE).

10. Any other serum biomarker panel testing with proprietary algorithms and/or index scores for the diagnosis of systemic lupus erythematosus or connective tissue diseases (e.g. Avise CTD) DOES NOT MEET COVERAGE CRITERIA for all applications.

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, Doherty, Grainge, Lanyon, & Zhang, 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, O'Dwyer, & Petri, 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, Schaffer, Rizzi, & Voll, 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-staged 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, 2019).

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, Andrade, Fritzler, & Shoenfeld, 2015) and has predictive value (Satoh et al., 2007); 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., 2007). 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-Ro (SS-a)	Ro-RNP complex	SLE, Sjögren's syndrome	TLR7
	Anti-La (SS-B)	La antigen	SLE, Sjögren's syndrome	TLR7
	Anti-Sm	Small nuclear RNP	SLE	TLR7
	Anti-dsDNA	dsDNA	SLE	TLR9
	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	
	ACA	Cardiolipin	Antiphospholipid syndrome, SLE	NLRP3
Modified proteins	ACPA	Citrullinated proteins	RA	TLR4
	Anti-Carp	Carbamylated proteins	RA	
Extracellular	RF	RF (IgG)	RA	
	Lupus anticoagulant	Phospholipids	Antiphospholipid syndrome	TLR4?
	α3 Chain of basement membrane collagen (type IV collagen)	α3 Chain of basement membrane collagen (type IV collagen)	Goodpasture's syndrome	

ACA, 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 LE cell (Hargraves, Richmond, & Morton, 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, 2019). 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., 2007); 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, 2019; 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, Oh, Cha, Koh, & Kang, 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, 2019). 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, 2019). 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 (D. J. Wallace & Gladman, 2019).

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 twothirds 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, 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 intracellular specific antigens (ENA) and to dsDNA/nucleosomes
Nuclear speckled ≥1:160	Anti-dsDNA and antibodies to intracellular specific antigens (ENA), possibly including anti-RNA polymerase III
Nuclear Scl70-like ≥1:160	Antibodies to intracellular specific antigens (ENA) (possibly including anti-PM/Scl)
Cytoplasmic speckled ≥1:160	Antibodies to intracellular specific antigens (ENA), including anti-tRNA synthetases and anti-P ribosomal
Pleomorphic PCNA-like (any titre)	Anti-PCNA
Centromere	No confirmation necessary if high titres. Execute specific test for anti-CENP B only in dubious cases (low titre or centromeric pattern not clearly recognizable)

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

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, 2020).

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, 2020). The 10 SLE relevant markers in this test include anti-dsDNA, antiSmith (anti-Sm) antibodies, erythrocyte-bound C4d or B-lymphocyte-bound C4d (BC4d), ANA, CB-
CAPs, and autoantibody specificity components (Exagen, 2020). As noted on their website, "The AVISE Lupus test is an ideal test for ANA positive patients with a clinical suspicion of lupus" (Exagen, 2020).

Clinical Utility and Validity

A variety of manual or automated single or multiplex immunoassays have been introduced to make the process of detecting autoantibodies more efficient, including enzyme-linked immunoabsorbant assays (ELISA), fluorescent microsphere assays, and chemiluminescence immunoassays—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, 2019). The advantages of these alternative approaches to ANA IIF testing include their suitability for highthroughput 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, 2019; 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, 2019). In a recent study which evaluated the performance of an automated chemiluminescence immunoassay (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, Bakker-Jonges, Kuijpers, & Schreurs, 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, 2019). 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. 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 antiENA 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. 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 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)."

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 allcause 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)."

Daniel J. 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) (Daniel J. 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, Goldman, Barken, and Alexander (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 criterial 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, Taylor, and McMahon (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 AVISEnegative 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)."

A study by Clarke et al. (2020) demonstrates the cost-effective management of systemic lupus erythematosus (SLE) using a multivariate assay panel (MAP) rather than standard diagnostic laboratory tests (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 falsepositive 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).

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).

V. Guidelines and Recommendations

American College of Rheumatology (ACR, 1997, 2015; Rouster-Stevens et al., 2014; Yazdany et al., 2013)

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, 2018). 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, 2018):

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 American College of Rheumatology 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-RNP, anti-Ro/SSA, anti La/SS-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 CAP, ACR, 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 tests for pediatric rheumatology, two 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.” They also state, “Do not repeat a confirmed positive ANA in patients with established JIA or SLE (Rouster-Stevens et al., 2014).”

Canadian Rheumatology Association (CRA) (Keeling et al., 2018)

In the 2018 CRA guidelines and recommendations for assessing and monitoring SLE, they state, “Best clinical practice includes a complete history and physical examination at baseline, with laboratory monitoring possibly including but not limited to complete blood count (CBC), liver enzymes, creatine kinase, creatinine and estimated glomerular filtration rate (eGFR), urine routine/microscopic (urinalysis), urine protein-creatinine ratio, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complements (C3, C4), anti-dsDNA, antinuclear antibodies, antibodies to extractable nuclear antigens, antiphospholipid antibodies (aPL), lupus anticoagulant (LAC), anticardiolipin (aCL), antiβ2glycoprotein I (anti-β2-GPI), and lipid profile. Follow-up (sic) laboratory monitoring will depend on the patient’s clinical status and may include CBC, eGFR, urinalysis, urine protein-creatinine ratio, CRP, and/or ESR, C3, C4, and anti-dsDNA antibodies.” The CRA goes on to note that “There is no current evidence that compares outcomes when specific tests are performed or not performed at baseline or at followup [sic]. This best-practice statement is therefore based on the utility of results to inform subsequent care of the patient with SLE” (Keeling et al., 2018).

Choosing Wisely Canada

The CRA also made a recommendation regarding ANA through Choosing Wisely Canada. In it, they note “Don’t order ANA as a screening test in patients without specific signs or symptoms of systemic lupus erythematosus (SLE) or another connective tissue disease (CTD)” (CRA, 2019).

British Society for Rheumatology (BSR) (Gordon et al., 2018)

In 2018, the BSR released their guidelines concerning the management of SLE in adults. With a Grade B recommendation, they state that the diagnosis of SLE requires at least one immunological abnormality alongside clinical features of the disease. “If there is a clinical suspicion of lupus, blood tests (including serological marker tests) should be checked.” Also, with a Grade B recommendation they state that a positive ANA test in the absence of clinical features of an autoimmune rheumatic disease is of poor value since approximately 5% of all adults will test positive; moreover, a negative ANA test result indicates low probability of SLE since 95% of SLE patients will test positive. “The presence of anti-dsDNA antibodies [Grade B], low complement levels [Grade C] or anti-Smith (Sm) antibodies [Grade C] are highly predictive of a diagnosis of SLE in patients with relevant clinical features. Anti-Ro/La and anti-RNP antibodies are less-specific markers of SLE [Grade C] as they are found in other autoimmune rheumatic disorders as well as SLE [Grade C].” They do state the following: “All lupus patients should be tested for aPLs because their presence indicates a group at increased risk of arterial/venous thrombotic events and adverse pregnancy outcomes.” Regarding the

use of antibodies in monitoring the disease, they state, "Serial anti-dsDNA antibodies and C3 and C4 levels are useful because rising, high anti-dsDNA antibodies and falling, low complement levels are associated with flare, particularly in patients with LN. In general, concomitantly rising anti-dsDNA titres and decreasing C3 and/or C4 levels are more important predictors of current or impending flares than the absolute levels, and levels of anti-dsDNA antibodies may actually fall at the time of flare (Gordon et al., 2018)." They specifically state that ANA, anti-Sm, and anti-RNP antibodies do not require repeat testing, but anti-dsDNA and aPL (LA, aCL, anti-beta2-glycoptroteinI) can be reviewed every few months (every 1-3 months for assessment, every 6-12 months for monitoring). (Gordon et al., 2018)

The BSR also makes the following recommendation through ChoosingWisely UK: "Testing ANA and ENAs should be reserved for patients suspected to have a diagnosis of a connective tissue disease, e.g. lupus. Testing ANA and ENAs should be avoided in the investigation of widespread pain or fatigue alone. Repeat testing is not normally indicated unless the clinical picture changes significantly" (BSR, 2018).

European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) (Aringer et al., 2019)

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 HEp2 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, 2019)

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" (AAP, 2019).

European Dermatology Forum S1 (Knobler et al., 2017)

This guideline addresses sclerosing diseases of the skin, such as localized scleroderma, systemic sclerosis and overlap syndromes.

The guideline recommends against routine screening for antinuclear antibodies. Screening for extractable nuclear antigens is also only recommended to "confirm or exclude" systemic sclerosis. The Forum also mentions that both rheumatoid factor and anti-cyclic citrullinated peptide antibodies may be detected in systemic sclerosis, but are associated with arthritis (Knobler et al., 2017).

European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN, 2019)

The ESPGHAN notes that positivity for circulating autoantibodies is "key" for diagnosis of autoimmune hepatitis (AIH). They also state that identifying certain autoantibodies may differentiate

between the two types of AIH ("ANA and SMA characterize AIH-1; anti-LKM1 and anti-LC-1 define AIH-2") (MieliVergani et al., 2018).

Systemic Lupus International Collaborating Clinics (SLICC) (Petri et al., 2012)
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. State and Federal Regulations, as applicable

A search for "antinuclear" on the FDA website on February 8, 2021 yielded 26 results. 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). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Billing applicable codes is not a guarantee of payment; see Section III for indications and limitations of coverage that may affect payment

Code Number	Code Description
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
81599	Unlisted multianalyte assay with algorithmic analysis
0039U	Deoxyribonucleic acid (DNA) antibody; double stranded, high avidity
0062U	Autoimmune (systemic lupus erythematosus), IgG and IgM analysis of 80 biomarkers, utilizing serum, algorithm reported with a risk score

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
06-01-2021	Initial presentation