2.04.123	Serum Biomarker Panel Testing for Systemic Lupus Erythematosus and Other Connective Tissue Diseases		
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Section:	2.0 Medicine	**Page:**	Page 1 of 15

Policy Statement

Serum biomarker panel testing with proprietary algorithms and/or index scores for the diagnosis of systemic lupus erythematosus and other connective tissue diseases is considered **investigational**.

Policy Guidelines

There is no specific CPT code for this panel of tests. There are codes that would likely be used for some of the component tests such as:

- **83520**: Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
- **86038**: Antinuclear antibodies (ANA)
- **86039**: Antinuclear antibodies (ANA); titer
- **86146**: Beta 2 Glycoprotein I antibody, each
- **86147:** Cardiolipin (phospholipid) antibody, each Ig class
- **86200:** Cyclic citrullinated peptide (CCP), antibody
- **86225:** Deoxyribonucleic acid (DNA) antibody; native or double stranded
- **0039U:** Deoxyribonucleic acid (DNA) antibody, double stranded, high avidity (PLA code effective 04/01/18)
- **86235:** Extractable nuclear antigen, antibody to, any method (e.g., nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody
- **86376:** Microsomal antibodies (e.g., thyroid or thyroid-kidney), each
- **86800:** Thyroglobulin antibody
- **88184:** Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only, first marker
- **88185**: Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
- **88187:** Flow cytometry, interpretation; 2 to 8 markers. 8
- **88188:** Flow cytometry, interpretation; 9 to 15 marker
- **88189:** Flow cytometry, interpretation; 16 or more markers

Some payers such as Medicare might instruct the use of the unlisted chemistry code for the whole panel:

- **84999:** Unlisted chemistry procedure

Due to the reporting of an index score for the entire panel, the test would more accurately be reported with the unlisted multianalyte assay with algorithmic analysis (MAAA) CPT code (81599).

Description

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease (CTD) that can be difficult to diagnose because patients often present with diverse, nonspecific symptoms that overlap with other CTDs; to further complicate matters, commonly used laboratory tests are not highly accurate. Moreover, similar symptoms may also present themselves in patients with fibromyalgia. Currently, differential diagnosis depends on a combination of clinical signs and symptoms and individual laboratory tests. More accurate laboratory tests for SLE and other CTDs could facilitate diagnosis of the disease. Recently, laboratory-developed, diagnostic panel tests

with proprietary algorithms and/or index scores for the diagnosis of SLE and other autoimmune CTDs have become commercially available.

Related Policies

- Multibiomarker Disease Activity Blood Test for Rheumatoid Arthritis

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. The Avise® tests (Exagen Diagnostics) are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Rationale

Background
Connective Tissue Diseases

Systemic Lupus Erythematosus
SLE is an autoimmune CTD. It is one of several types of lupus, the others being cutaneous and drug-induced lupus. About 90% of lupus patients are women between the ages of 15 and 44 years. SLE causes inflammation and can affect any part of the body, most commonly the skin, heart, joints, lungs, blood vessels, liver, kidneys, and nervous system. Although generally not fatal, SLE can increase mortality, most commonly from cardiovascular disease due to accelerated atherosclerosis. SLE can also lead to kidney failure, which may reduce survival. The survival rate in the U. S. is approximately 95% at 5 years and 78% at 20 years.[1,] The morbidity associated with SLE is substantial. Symptoms such as joint and muscle pain can impact the quality of life and functional status. SLE also increases patients' risk of infection, cancer, avascular necrosis (bone death), and pregnancy complications (e.g., preeclampsia, preterm birth). The course of the disease is variable, and patients generally experience flares of mild-to-severe illness and remission.

Other Connective Tissue Diseases
Several other CTDs may require a differential diagnosis from SLE (e.g., rheumatoid arthritis, Sjögren syndrome, antiphospholipid syndrome, and polymyositis).

Rheumatoid arthritis is a chronic inflammatory peripheral polyarthritis. Rheumatoid arthritis can lead to deformity through stretching of tendons and ligaments and destruction of joints through erosion of cartilage and bone. Rheumatoid arthritis can also affect the skin, eyes, lungs, heart, and blood vessels.

Graves disease is an autoimmune disorder that leads to overactivity of the thyroid gland. The disease arises from thyroid-stimulating hormone receptor antibodies. It is the most common cause of hyperthyroidism. Blood tests may show raised thyroid-stimulating immunoglobulin antibodies.

Hashimoto disease, also known as chronic lymphocytic thyroiditis, is an autoimmune disorder and is the most common cause of hypothyroidism second to iodine insufficiency. It is characterized by an underactive thyroid gland and gradual thyroid failure. Diagnosis is confirmed with blood tests for thyroid-stimulating hormone (T4) and antithyroid antibodies.

Sjögren syndrome is an autoimmune disorder characterized by dryness of the eyes and mouth due to diminished lacrimal and salivary gland function. Affected individuals may also have symptoms of fatigue, myalgia, and cognitive dysfunction, which may be difficult to distinguish clinically from fibromyalgia or medication side effects. Typical antibodies include antinuclear antibody (ANA), anti-Sjögren-syndrome-related antigen, anti-Sjögren syndrome type B, or rheumatoid factor.

Antiphospholipid syndrome is a systemic autoimmune disorder characterized by venous or arterial thrombosis and/or pregnancy morbidity. Antiphospholipid antibodies are directed against phospholipid-binding proteins.

Polymyositis and dermatomyositis are inflammatory myopathies characterized by muscle weakness and inflammation. Dermatomyositis may also have skin manifestations.

Diagnosis
Patients with SLE often present with nonspecific symptoms such as fever, fatigue, joint pain, and rash, which can make the disease difficult to diagnose. In some patients, the diagnosis of SLE can be made with certainty (e.g., when there are typical symptoms of rash and joint symptoms, and laboratory testing shows a high-titer abnormal ANA in a pattern specific for SLE). However, in many other patients, the symptom patterns of SLE are less clear, and ANA testing is equivocal; as a result, cascade testing with additional serologic tests may be ordered. In addition, ANA testing alone can result in false-positives due to low specificity.

Classifications
The diagnosis of SLE has been based on a combination of clinical symptoms and laboratory results. In 1997 the American College of Rheumatology (ACR) updated 1982 criteria for the classification of SLE.[2,3,]

The ACR classification criteria are as follows:
1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Mouth or nose ulcers (usually painless)
5. Arthritis (nonerosive) in two or more peripheral joints, along with tenderness, swelling, or effusion
6. Serositis: pleuritis or pericarditis
7. Renal disorder: excessive protein in the urine, or cellular casts in the urine
8. Neurologic disorder: seizures and/or psychosis, in the absence of offending drugs or known metabolic derangements
9. Hematologic disorders: hemolytic anemia, leukopenia, lymphopenia, or thrombocytopenia

10. Immunologic disorder: antibodies to double-stranded DNA (anti-dsDNA), antibodies to Smith antigen (anti-Sm), positive antiphospholipid antibody, or false-positive serologic test for syphilis known to be positive for at least six months
11. ANA test in the absence of drugs known to induce it.

These criteria were originally developed for research but they have been widely adopted in clinical care. Individuals who meet 4 or more of the 11 criteria are diagnosed with SLE. If a patient meets fewer than four of the criteria, lupus can still be diagnosed by clinical judgment; it is recommended that a rheumatologist confirm the diagnosis.[4,] ANA testing is usually performed for patients who present with signs and symptoms involving two or more organ systems, and individuals who test positive are recommended for additional laboratory testing.[5,] Assessments of ACRs 1982 criteria have reported sensitivities ranging from 78% to 95% and specificities ranging from 89% to 100%, with lower accuracy in patients with mild disease.[5,]

The Systemic Lupus International Collaborating Clinics (SLICC; 2012), an international research group, developed revised criteria for diagnosing SLE.[6,] These criteria include more laboratory tests than the earlier ACR criteria, including elements of the complement system. Patients are classified as having SLE if they satisfy 4 or more of the 18 criteria below, including at least 1 clinical criterion and 1 immunologic criterion, or they have biopsy-confirmed nephritis compatible with SLE and with ANA or anti-dsDNA antibodies. In a sample of 690 patients, the SLICC criteria had a sensitivity of 97% and a specificity of 84% for diagnosing SLE, whereas the ACR criteria applied to the same sample had a sensitivity of 83% and a specificity of 96%. It is not clear how well-accepted the SLICC recommendations are in the practice setting. Table 1 outlines the SLICC criteria.

Table 1. Clinical and Immunologic Criteria

Clinical Criteria
• Acute cutaneous lupus (including but not limited to lupus malar rash)
• Chronic cutaneous lupus (including but not limited to discoid rash)
• Oral ulcers
• Nonscarring alopecia in the absence of other causes
• Synovitis involving ≥2 joints, characterized by swelling or effusion or and ≥30 min of morning stiffness
• Serositis
• Renal: excessive protein in the urine or cellular casts in the urine
• Neurologic disorder: seizures, psychosis, mononeuritis complex, or peripheral, or cranial neuropathy
• Seizures
• Hemolytic anemia
• Leukopenia or lymphopenia
• Thrombocytopenia
Immunologic Criteria
• Antinuclear antibody above laboratory reference range
• Antibodies to double-stranded DNA above laboratory reference range
• Antibodies to Smith nuclear antigen
• Antiphospholipid antibody
• Low complement (low C3, low C4, or low CH150)
• Direct Coombs tests in the absence of hemolytic anemia

As noted, the SLICC classification system includes a wider range of laboratory tests than the ACR criteria. To date, the most common laboratory tests performed in the diagnosis of SLE are serum ANA, and, if positive, tests for anti-dsDNA and anti-Sm. ANA tests are highly sensitive (i.e., with a high negative predictive value) but have low specificity and relatively low positive predictive value, particularly when the ANA is positive at a low level. Specificity of testing can be increased by testing for specific antibodies against individual nuclear antigens (extractable nuclear antigens) to examine the "pattern" of ANA positivity. These include antigens against single- and dsDNA, histones, Sm, Ro, La, and RNP antibodies. The presence of anti-dsDNA or anti-Sm is highly specific for SLE because few patients without SLE test positive; however, neither test has high sensitivity.[7,] The presence of other antibody patterns may indicate the likelihood of other

diagnoses. For example, the presence of Ro and La antibodies suggests Sjögren syndrome, while the presence of antihistone antibodies suggests drug-induced lupus.

Better diagnostic tests for SLE and other CTDs would be useful in clinical practice. A variety of biomarkers, including markers associated with the complement system, are being explored to aid in the diagnosis of lupus. The complement system is part of the immune system and consists of 20 to 30 protein molecules that circulate in the blood in an inactive form until activated by a trigger (e.g., an infection), and when the protein molecules are activated, a sequence of events known as the complement cascade is initiated. This cascade involves the proteolysis of a complement protein into a smaller protein and a peptide. The smaller protein is able to bind to the complex one at the surface of the invading microorganism, and the peptide diffuses away. For example, in the first step, complement protein C3 is cleaved into C3b and C3a. C3b binds to the surface of the microorganism and activates the next step in the cascade, the proteolysis of C5, and the small peptide, C3a diffuses away. The precursors C3 and C4 and the complement activation products (e.g., C3a, C5a, C4d) have been considered as SLE biomarkers. More recently, cell-bound complement activation products, which live longer than circulating complement activation products, have been investigated as biomarkers of SLE.

In addition to the exploration of individual biomarkers with higher accuracy than accepted markers (e.g., ANA, anti-dsDNA), there is interest in identifying a panel of tests with high sensitivity and specificity for SLE diagnosis. At least onemultibiomarker test to aid diagnosis of SLE and other CTDs is commercially available. This panel, Avise CTD (Exagen Diagnostics), contains 22 different tests. It combines 2 smaller panels, a 10-marker panel that includes common SLE tests, as well as cell-bound complement activation products (known as Avise Lupus) and a 12-marker panel that focuses on CTDs other than SLE (known as Avise CTD). Avise CTD includes nuclear antigen antibodies markers to help distinguish CTD, a rheumatoid arthritis panel to rule-in or rule-out rheumatoid arthritis, an antiphospholipid syndrome panel to assess risk for thrombosis and cardiovascular events, and a thyroid panel to help rule-in or rule-out Graves disease and Hashimoto disease. Specific biomarkers in the panel are listed in Table 2.

Table 2. Avise Systemic Lupus Erythematosus Tests

Systemic Lupus Erythematosus Tests
10-marker Avise Lupus test
Auto-antibodies: ANA, anti-dsDNA, antimutated citrullinated vimentin, C4d erythrocyte-bound complement fragment, C4d lymphocyte-bound complement, anti-Sm, Jo-1, Sci-70, CENP, SS-B/La
Avise CTD test
Avise Lupus test plus the following:
Auto-antibodies: U1RNP, RNP70, SS-A/Ro
Rheumatoid arthritis auto-antibodies: rheumatoid factor IgM, rheumatoid factor IgA, anti-cyclic citrullinated peptide IgG
Anti-phospholipid syndrome auto-antibodies: cardiolipin IgM, cardiolipin IgG, β2-glycoprotein 1 IgG, β2-glycoprotein 1 IgM
Thyroid auto-antibodies: thyroglobulin IgG, thyroid, thyroid peroxidase

ANA: antinuclear antibody; anti-dsDNA: antibodies to double-stranded DNA; anti-Sm: antibodies to Smith nuclear antigen; CTD: connective tissue disease; Ig: immunoglobulin.

The Avise CTD test assesses all 22 markers. Avise CTD uses a three-step process.[8,] The 10-marker panel is done in 2 tiers, and the add-on 12-marker panel is done in a third step to further assist with the differential diagnosis of CTD. In addition, ANA testing is done by enzyme-linked immunosorbent assay and by indirect immunofluorescence. The 2-tiered testing approach to the 10-marker panel is described next.

> Tier 1: Tests for anti-Sm, EC4d, BC4d, and anti-dsDNA. If any tests are positive, the result is considered suggestive of SLE and no further testing is done. Cutoffs for positivity are greater than 10 U/mL for anti-Sm, greater than 75 U/mL for EC4d, greater than 200 U/mL for BC4d, and greater than 301 U/mL for anti-dsDNA. Positive findings for anti-dsDNA are confirmed with a *Crithidialuciliae* assay.

> Tier 2: If the tier 1 tests are negative, an index score is created, consisting of results of tests for ANA, EC4d and BC4d, antimutated citrullinated vimentin, anti-Jo-1, anti-Sci-70, anti-CENP, and anti-Ss-B/La. In other words, there are six additional markers and the ratio of EC4d to BC4d, both of which were measured in tier 1.

The index score (tier 2), calculated using a proprietary algorithm, rates how suggestive test results are of SLE. Although there is information on cutoffs used to indicate positivity for individual markers, information is not available on how precisely the index score is calculated. The score can range from -5 (highly nonsuggestive of SLE) to 5 (highly suggestive of SLE), and a score of -0.1 to 0.1 is considered indeterminate.

Exagen also offers the Avise Lupus Prognostic test, a 10-marker panel that can be ordered with the Avise Lupus and Avise CTD panels. The prognostic test focuses on patients' risk of lupus nephritis, neuropsychiatric SLE, thrombosis, and cardiovascular events. The test includes anti-C1q, anti-ribosomal P, anti-phosphatidylserine/prothrombin immunoglobulin (Ig) M and IgG, anti-cardiolipin IgM, IgG, and IgA and anti-β2-glycoprotein 1 IgM, IgG, and IgA. Four of the ten markers are included in both panel tests.

Treatment
Treatments for SLE can ameliorate symptoms, reduce disease activity, and slow progression of organ damage; however, there is no cure. Muscle and joint pain, fatigue, and rashes are generally treated initially with nonsteroidal anti-inflammatory drugs. Antimaltimes drugs such as hydroxychloroquine can relieve some symptoms of SLE including fatigue, rashes, and joint pain. Patients with more severe symptoms (e.g., heart, lung, or kidney involvement) can be treated with corticosteroids or immune suppressants. There are also biologic treatments (e.g., rituximab) approved by the U.S. Food and Drug Administration for the treatment of rheumatoid arthritis and are being evaluated for SLE.

Literature Review
Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Systemic Lupus Erythematosus And Other Connective Tissue Diseases
Clinical Context and Test Purpose
The purpose of serum biomarker panel testing is to provide a diagnostic option that is an alternative to or an improvement on existing tests, such as established SLE classification systems and individual serum biomarker tests, in patients with signs and/or symptoms of SLE.

The question addressed in this evidence review: Does the use of a serum biomarker panel improve the net health outcome in patients with signs and/or symptoms of SLE or other CTDs?

The following PICOTS were used to select literature to inform this review.

Patients
The population of interest are individuals with signs and/or symptoms of SLE.

Interventions
The test being considered is serum biomarker panel testing.

SLE is an autoimmune CTD that can be difficult to diagnose because patients often present with diverse, nonspecific symptoms that overlap with other CTDs; to further complicate matters, commonly used laboratory tests are not highly accurate. Moreover, similar symptoms may also present themselves in patients with fibromyalgia. Currently, differential diagnosis depends on a combination of clinical signs and symptoms and individual laboratory tests. More accurate laboratory tests for SLE and other CTDs could facilitate the diagnosis of the disease. Recently, laboratory-developed, diagnostic panel tests with proprietary algorithms and/or index scores for the diagnosis of SLE and other autoimmune CTDs have become commercially available.

Patients with signs and/or symptoms of SLE are actively managed by rheumatologists, cardiologists, pulmonologists, nephrologists, and primary care providers in an outpatient clinical setting.

Comparators
Comparators of interest include established SLE classification systems and individual serum biomarker tests.

Comparators are actively managed by rheumatologists, cardiologists, pulmonologists, nephrologists, and primary care providers in an outpatient clinical setting.

Outcomes
The general outcomes of interest are test accuracy, symptoms, and quality of life (QOL).

Table 3. Outcomes of Interest for Individuals With Signs and/or Symptoms of SLE

Outcomes	Details	Timing
Test accuracy	Sensitivity and specificity in detecting biomarkers for SLE	FU for several years to assess accuracy of diagnosis
Symptoms	Malar rash, discoid rash, photosensitivity, mouth or nose ulcers, arthritis (nonerosive), among others	≥ 2 weeks
Quality of life	Relief of symptoms Reduction in joint and organ damage	≥ 3 years

FU: follow-up; SLE: systemic lupus erythematosus.

Study Selection Criteria
Below are selection criteria for studies to assess whether a test is clinically valid.
   a. The study population represents the population of interest. Eligibility and selection are described.
   b. The test is compared with a credible reference standard.
   c. If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
   d. Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g. ,receiver operating characteristic[ROC], area under receiver operating characteristic [AUROC], c-statistic, likelihood ratios) may be included but are less informative.
   e. Studies should also report reclassification of diagnostic or risk category.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Novel Panel Components: Cell-Bound Complement Activation Products
As discussed, CB-CAPs are key components of a commercially available biomarker panel test for a lupus diagnosis. CB-CAPs include C4d levels on erythrocytes, platelets, and B cells.

A study by Liu et al (2009) evaluated lymphocyte-bound CAPs (LB-CAPS).[9] This cross-sectional study included 224 patients with SLE (according to American College of Rheumatology [ACR] classification criteria), 179 patients with other autoimmune or inflammatory diseases, and 114 healthy controls. Levels of LB-CAPs, T-cell bound C4d (TC4d) and C3d (TC3d), and B-cell bound C4d (BC4d), and C3d (BC3d) were measured in all participants. The diagnostic accuracy of these markers was accessed using ROC analysis. The area under the curve was 0.727 for TC4d and 0.770 for BC4d. TC4d was estimated to be 56% sensitive and 80% specific for differentiating SLE from other diseases. BC4d had 56% sensitivity and 80% specificity.

In addition, the authors compared CB-CAPs with other, conventionally used, SLE markers. The markers were evaluated as a confirmatory test in patients who tested positive for antinuclear antibody (ANA). This analysis only included the SLE patients, 223 (99.6%) of 224 of whom were positive for ANA. Of the 223 ANA-positive patients, 141 (63%) patients had elevated levels of TC4d and/or BC4d. In contrast, 59 (28%) of the 209 ANA-positive patients tested positive for double-stranded DNA (anti-dsDNA). Moreover, when the more commonly used complement activation products (serum C3, serum C4) were evaluated, 67 (30%) of 221 of ANA-positive patients tested positive for C3 and 82 (37%) of 221 patients tested positive for C4.

Previously, a cross-sectional study of platelet C4d by Navratil et al (2006) assessed 105 patients with SLE (according to ACR criteria), 115 patients with other autoimmune or inflammatory diseases, and 100 healthy controls.[10] Abnormal levels of platelet C4d were detected in 18% of SLE patients. False-negative rates and sensitivity rates were not reported. The authors reported that the marker was 100% specific for a diagnosis of SLE compared with healthy controls and 98% specific compared with patients who had other diseases.

Serum Biomarker Panel Tests
Putterman et al (2014) published data from a large cross-sectional, industry-sponsored study evaluating serum biomarkers for the diagnosis of SLE.[11] They analyzed the 10 markers in the Avise Lupus (plus ANA) using a 2-tier testing logic similar to that employed in the commercially available panel (see the Background section). The study evaluated 2 cohorts (total $n=794$ patients); 593 participants were enrolled between April and August 2010, and 201 participants enrolled between June 2011 and September 2013. Together, the 2 cohorts consisted of 304 patients who met ACR classification criteria for SLE, 161 patients diagnosed with other rheumatic diseases and 205 healthy volunteers. Results of serum testing were available for 764 (96%) of 794 participants.

The diagnostic accuracy of the CB-CAP EC4d and BC4d were compared with reduced complement (C3, C4) and anti-dsDNA. The AURO Characteristic curve was significantly higher for EC4d (0.82) and BC4d (0.84) than for C3 (0.73) and C4 (0.72) ($p<0.001$). The AUROC curve was significantly higher for BC4d than for anti-dsDNA (0.79; $p=0.009$) but the difference was not statistically significant between EC4d and anti-dsDNA.

A total of 140 (46%) patients with SLE, 9 (3%) patients with other diseases, and 1 healthy volunteer tested positive for at least 1 of the 4 tier 1 markers. Patients testing negative for tier 1 tests underwent tier 2 testing and an index score was calculated. A total of 102 (62%) of 164 patients with SLE analyzed in tier 2 had an index score greater than 0 (i.e., suggestive of SLE). Moreover,

245 of 276 patients with other rheumatic diseases had an index score of less than 0 (i.e., not suggestive of SLE). When the results of tier 1 and 2 testings were combined, the overall sensitivity for SLE was 80% (242/304) and the overall specificity for distinguishing SLE from other diseases was 86% (245/285). The specificity for distinguishing between SLE and healthy volunteers was 98% (201/205).

As shown in Table 4, the specificity and area under the curve were higher for models including CB-CAPs than in those without these markers; sensitivity was slightly lower.

Table 4. Diagnostic Accuracy of Various Combinations of Markers

Measures	dsDNA, Sm, and ANA	dsDNA, Sm, ANA, Plus Antibody Specificity Components But Not CB-CAPs	Two-Tiered Testing Using All Markers, Including CB-CAPs EC4d and BC4d
Sensitivity, %	89	83	80
Specificity, %	53	76	86
Area under the curve	0.78	0.80	0.91

ANA: antinuclear antibodies; CB-CAP: cell-bound complement activation product; dsDNA: double-stranded DNA; Sm: Smith nuclear antigen.

An earlier industry-sponsored study by Kalunian et al (2012)[12,] reported on the first cohort of 593 individuals included in the Putterman et al (2014) analysis. The sample consisted of 210 patients with SLE who met ACR classification criteria, 178 patients with other rheumatic diseases, and 205 healthy volunteers. Authors evaluated the performance of a 7-marker biomarker panel for the diagnosis of SLE; some markers are included in a commercially available panel test. The biomarkers included ANA, anti-dsDNA, antimutated citrullinated vimentin, and the CB-CAPs (EC4d, PC4d, BC4d).

A subsequent industry-sponsored study by Wallace et al (2016) analyzed serum biomarkers as well as an algorithm for diagnosing SLE.[13,] This study analyzed markers in the Avise Lupus (plus ANA) test using a 2-tier testing logic to evaluate SLE patients who met ACR criteria (n=75) and patients with primary fibromyalgia (n=75). High expression of CB-CAP EC4d or BC4d had 43% sensitivity and 96% specificity for the diagnosis of SLE. Use of a multianalyte assay with the algorithm, including CB-CAP levels, generated indeterminate results in 12 of the 150 subjects enrolled. For the remainder of patients, use of the algorithm to diagnosis SLE was 60% sensitive and 100% specific. Study limitations included as election of patients with well-established diagnosis and long duration of disease.

In multivariate logistic regression, SLE diagnosis was associated with a positive ANA test, a negative antimutated citrullinated vimentin test, and elevated EC4d and BC4d levels (area under the curve, 0.92; p<0.001). The weighted sum of these 4 markers correctly categorized 106 (71.6%) of 148 SLE patients who were anti-dsDNA-negative. (The investigators evaluated the 4-marker index score among individuals who tested negative for anti-dsDNA because of the low sensitivity of this test [29.5%], thus the high false-negative rate.) The specificity of the 4-marker index was 98.0% (200/204 healthy volunteers with test results were correctly classified). When anti-dsDNA was added to the 4-marker panel, the test had 80% sensitivity for SLE (168/210 SLE patients were correctly classified). Moreover, this 5-marker test had 97.6% specificity among healthy individuals (200/205 were correctly classified as not having SLE). The 5-marker test also had 87% specificity in patients with other rheumatic diseases; the most false-positives (n=9) were in patients with rheumatoid arthritis. The biomarkers in the 5-marker test are part of the 10-marker Avise 2.0 SLE test marketed by Exagen. It is not clear whether the index score reported along with the Avise 2.0 panel is the same as or different from the index score reported in the Kalunian et al (2012) study.

A limitation of the Putterman et al (2014) and Kalunian et al (2012) studies is that study sample populations included patients with SLE who met ACR classification criteria, but not patients with symptoms suggestive of SLE who failed to meet ACR criteria. It is not known how the diagnostic

accuracy of the panel test compares with the ACR classification criteria or with concurrent clinician diagnosis (in the Putterman et al [2014] study, the mean time since SLE diagnosis was 11 years). Furthermore, although they are included in the Systemic Lupus International Collaborating Clinics classification criteria, the complement factors C3 and C4 are not widely used in clinical practice to diagnose lupus and, therefore, the clinical significance of higher diagnostic accuracy for EC4d and BC4d is unclear.

Mossell et al (2016) reported on an industry-sponsored retrospective study of 23 patients who had a positive Avise Lupus test result and 23 patients who had a negative result.[14,] All patients were ANA-positive but negative for auto-antibodies specific for SLE, representing cases difficult to diagnosis. Each positive Avise test case was matched to a control (negative test) from the same clinic with the same ANA level. A chart review was performed by a nonblinded rheumatologist approximately one year after the test results were available. Of the cases with a positive Avise Lupus test, 20 (87%) were diagnosed with SLE during follow-up. This compared with 4 (17%) individuals who had a negative result on the Avise Lupus test, resulting in a sensitivity of 83.3% and specificity of 86.4%. Interpretation of this study is limited due to its retrospective design, relatively short follow-up to monitor the progression of the disease, and the lack of an independent reference standard, because the diagnosis was based in part on the results of that test. The authors noted that prospective studies would be performed.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No studies were identified that provided direct evidence on the impact of serum biomarker panel testing for SLE on patient outcomes.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A more accurate and timelier diagnosis of SLE (i.e., before multiorgan system involvement) and other CTDs could lead to better patient management (e.g., more appropriate medical treatment). This, in turn, could improve health outcomes (e.g. ,less joint or organ damage, improved survival).

Connective Tissue Diseases Other Than SLE
Clinical Context and Test Purpose
The purpose of serum biomarker panel testing is to provide a diagnostic option that is an alternative to or an improvement on existing tests, such as clinical diagnosis and individual serum biomarker tests, in patients with signs and/or symptoms of CTD(besides SLE).

The question addressed in this evidence review: Does the use of a serum biomarker panel improve the net health outcome in patients with signs and/or symptoms of SLE or other CTDs?

The following PICOTS were used to select literature to inform this review.

Patients
The population of interest are individuals with signs and/or symptoms of CTD (other than SLE).

Interventions
The test being considered is serum biomarker panel testing.

Comparators
Comparators of interest include clinical diagnosis and individual serum biomarker tests.

Comparators are actively managed by rheumatologists, cardiologists, pulmonologists, nephrologists, and primary care providers in an outpatient clinical setting.

Outcomes
The general outcomes of interest are test accuracy, symptoms, and QOL.

Table 5. Outcomes of Interest for Individuals With Signs and/or Symptoms of CTD (Besides SLE)

Outcomes	Details	Timing
Test accuracy	Sensitivity and specificity in detecting biomarkers for CTDs other than SLE	FU for several years to assess accuracy of diagnosis
Symptoms	Dry eyes and mouth, fatigue, cognitive dysfunction, muscle weakness and inflammation	≥ 2 weeks
Quality of life	Symptom relief Reduction in joint and organ damage	≥ 3 years

CTD: connective tissue disease; FU: follow-up; SLE: systemic lupus erythematosus.

Study Selection Criteria
Below are selection criteria for studies to assess whether a test is clinically valid.
   a. The study population represents the population of interest. Eligibility and selection are described.
   b. The test is compared with a credible reference standard.
   c. If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
   d. Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g., ROC, AUROC, c-statistic, likelihood ratios) may be included but are less informative.
   e. Studies should also report reclassification of diagnostic or risk category.

Novel Panel Components: CB-CAPs and CTDs
As discussed above, the study by Liu et al (2009) evaluated LB-CAPS.[9] Of the 517 participants, 179 patients had autoimmune or inflammatory diseases other than SLE. Not all of these diseases were CTDs but several CTDs were included in the study. Levels of LB-CAPs,TC4d andTC3d, andBC4d, andBC3dwere measured in all participants. The diagnostic accuracy of these markers was assessed using ROC analysis. The area under the curve was 0.727 for TC4d and 0.770 for BC4d. TC4d was estimated to be 56% sensitive and 80% specific for differentiating SLE from other diseases. BC4d had 56% sensitivity and 80% specificity.

Also discussed above, the cross-sectional study of platelet C4d by Navratil et al (2006) included 420 total participants, 115 of whom had rheumatic inflammatory/autoimmune or hematologic diseases other than SLE, several of which were CTDs.[10] The authors reported that the marker was 98% specific for a diagnosis of SLE compared to the patients with other diseases.

Serum Biomarker Panel Tests for CTDs Other Than SLE
As previously discussed, Putterman et al (2014) published data from a large cross-sectional, industry-sponsored study evaluating serum biomarkers for the diagnosis of SLE.[11] They analyzed the ten markers in the Avise Lupus (plus ANA) using a 2-tier testing logic similar to that employed in the commercially available panel (see the Background section). Of the 794 patients in the study, 161 were diagnosed with rheumatic diseases other than SLE.

A total of 140 (46%) patients with SLE, 9 (3%) patients with other diseases, and 1 healthy volunteer tested positive for at least 1 of the 4 tier 1 markers. Patients testing negative for tier 1 underwent tests tier 2 testing and an index score was calculated. A total of 245 of 276 patients with other rheumatic diseases had an index score of less than 0 (i.e., not suggestive of SLE). When the results of tier 1 and tier 2 testings were combined, the overall specificity for distinguishing SLE from other diseases was 86% (245/285).

In the earlier study by Kalunian et al (2012)[12] out of 593 participants, 178 patients had rheumatic diseases, 210 had SLE, and 205 were healthy volunteers. Authors evaluated the performance of a 7-marker biomarker panel for the diagnosis of SLE; some markers are included in a commercially available panel test. The biomarkers included ANA, anti- dsDNA, antimutated citrullinated vimentin, and the CB-CAPs (EC4d, PC4d, BC4d). In relation to SLE, the combination of anti-dsDNA and the multivariate logistic regression analysis index score yielded 87% specificity against other rheumatic diseases.

Summary of Evidence for Diagnosing CTDs Other Than SLE
All studies found centered around diagnosing SLE with other CTDs as comparators and did not assess the sensitivity of the biomarker tests to detect CTDs other than SLE. For individuals with signs and/or symptoms of CTD (besides SLE) who receive serum biomarker panel testing, more studies are needed. The relevant outcomes are test accuracy, symptoms, and QOL. The evidence is insufficient to determine the effects of the technology on health outcomes.

Summary of Evidence
For individuals with signs and/or symptoms of SLE who receive serum biomarker panel testing, the evidence includes several diagnostic accuracy studies. The relevant outcomes are test accuracy, symptoms, and QOL. One study evaluated a panel similar to a commercially available test; it found that the panel test had somewhat higher specificity and lower sensitivity than the most common currently used biomarkers. The clinical significance of this degree of difference in diagnostic accuracy is unclear. One case-control study found high sensitivity and specificity for a commercially available test for diagnosing SLE, but this retrospective analysis has several limitations, and prospective studies are therefore needed. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with signs and/or symptoms of CTD (besides SLE) who receive serum biomarker panel testing, more studies are needed. The relevant outcomes are test accuracy, symptoms, and QOL. The evidence is insufficient to determine the effects of the technology on health outcomes.

Supplemental Information
Practice Guidelines and Position Statements

No guidelines or statements were identified.

U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in April 2019 did not identify any ongoing or unpublished trials that would likely influence this review.

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Documentation for Clinical Review

- No records required

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

IE
The following services may be considered investigational.

Type	Code	Description
CPT®	0039U	Deoxyribonucleic acid (DNA) antibody, double stranded, high avidity *(Code effective 4/1/2018)*
	0062U	Autoimmune (systemic lupus erythematosus), IgG and IgM analysis of 80 biomarkers, utilizing serum, algorithm reported with a risk score *(Code effective 10/1/2018)*
	81599	Unlisted multianalyte assay with algorithmic analysis
	83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
	84999	Unlisted chemistry procedure
	86038	Antinuclear antibodies (ANA);
	86039	Antinuclear antibodies (ANA); titer
	86146	Beta 2 Glycoprotein I antibody, each
	86147	Cardiolipin (phospholipid) antibody, each Ig class
	86200	Cyclic citrullinated peptide (CCP), antibody
	86225	Deoxyribonucleic acid (DNA) antibody; native or double stranded
	86235	Extractable nuclear antigen, antibody to, any method (e.g., nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody
	86376	Microsomal antibodies (e.g., thyroid or liver-kidney), each
	86800	Thyroglobulin antibody
	88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
	88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
	88187	Flow cytometry, interpretation; 2 to 8 markers
	88188	Flow cytometry, interpretation; 9 to 15 marker
	88189	Flow cytometry, interpretation; 16 or more markers
HCPCS	None	
ICD-10 Procedure	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action	Reason
10/31/2014	BCBSA Medical Policy adoption	Medical Policy Committee
08/01/2016	Policy revision without position change	Medical Policy Committee
09/01/2017	Policy title change from Serum Biomarker Panel Testing for Systemic Lupus Erythematosus Policy revision without position change	Medical Policy Committee
05/01/2018	Coding update	Administrative Review
08/01/2018	Policy revision without position change	Medical Policy Committee
10/01/2018	Coding update	Administrative Review
10/01/2018	Coding update	Administrative Review

Effective Date	Action	Reason
09/01/2019	Policy revision without position change	Medical Policy Committee

Definitions of Decision Determinations

Medically Necessary: A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.