Quest Diagnostics™

Autoimmune Skin Diseases

Laboratory Support of Diagnosis and Management

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CLINICAL BACKGROUND

Autoimmune skin diseases are disorders in which the immune system attacks the skin, causing rashes, blisters, discoloration, ulcers, or patches of dead skin ("scales"). These conditions vary in prevalence and presentation, as discussed below and in **Tables 1** and **2**.

Some autoimmune skin diseases primarily manifest as skin disease. These include psoriasis, chronic urticaria, blistering diseases, and vitiligo. Autoimmune *rheumatic skin* diseases involve the skin but are often also accompanied by joint pain and diminished joint mobility. Examples include systemic lupus erythematosus (SLE), systemic sclerosis (SSc), dermatomyositis, mixed connective tissue disease (MCTD), spondyloarthropathies (SpA; including psoriatic arthritis), juvenile idiopathic arthritis (JIA), and several vasculitides.

Accurate diagnosis is important, because many of these diseases are systemic: they may affect the heart, lungs, gastrointestinal tract, nervous system, and kidneys, leading to serious health conditions. However, clinical diagnosis can be challenging because of potentially overlapping symptoms. In addition to the cutaneous manifestations described above, many autoimmune skin diseases are also characterized by more general symptoms, such as fever, malaise, fatigue, and weight loss. Furthermore, many diseases considered systemic autoimmune diseases have cutaneous manifestations. Therefore evaluation requires careful assessment of the entire patient.

This Clinical Focus provides background on the available laboratory tests and their use in diagnosing and classifying 1) autoimmune diseases primarily manifesting as skin disease and 2) autoimmune rheumatic diseases that involve the skin. Where applicable, laboratory tests that may aid in selecting and monitoring therapy are also discussed, citing and following guidelines when available.

This material is provided for educational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on their education, clinical expertise and assessment of the patient. The treating healthcare professional should refer to the manufacturer's approved labeling for prescribing, warnings, side effects and other important information.

Table 1. Common Signs and Symptoms of Autoimmune Skin Diseases[1-7,a]

Sign or Symptom	Ps	CU	Vitiligo	Autoimmune Blistering Disease					
				BP	MMP	IgA	EBA	DH	PV
Skin-/hair-/nail-related									
Alopecia									
Rash/discoloration		X	X	X		X		X	
Raynaud phenomenon									
Skin lesions or plaques	X	X		X	O	X	X	X	X
Nail involvement	X						X		O
Joint-/muscle-related									
Joint pain, stiffness, or inflammation									
Muscle weakness									
Myalgia									
General									
Cough									
Ear involvement									
Eye involvement					X				
Fatigue									
Fever									
GI involvement								X	
Kidney involvement									
Malaise									
Nasal or oral symptoms					X	X	X		X
Nervous system involvement				O					
Respiratory involvement							X		
Weight loss									X
Other									
Adenopathy									
Anemia									
Dysphagia									X
Swelling of hands									

X indicates common; O indicates less common but not rare.
CU, chronic urticaria; BP, bullous pemphigoid; MMP, mucous membrane pemphigoid; IgA, linear IgA disease; EBA, epidermolysis bullosa acquisita; DH, dermatitis herpetifomis; PV, pemphigus vulgaris; Ps, psoriasis.
[a] This is not a complete list of signs and symptoms; some conditions have more signs and symptoms than could be presented here.

Table 2. Common Signs and Symptoms of Autoimmune Rheumatic Disease Involving the Skin[8-12,a]

Sign or Symptom	SLE	SSc	DM	MCTD	SpA			Small Vessel Vasculitides					
								ANCA			Immune Complex		
					PsA	ReA	JIA	GPA	EGPA	MPA	CV	HUV	IgA
Skin-/hair-/nail-related													
Alopecia	X												
Rash/discoloration	X		X	X			X		X	X	X	X	X
Raynaud phenomenon	X	X	X	X							X		
Skin lesions or plaques	O		O	O	O	X	X	O		O	X	X	X
Nail involvement					O	X				O			
Joint-/muscle-related													
Joint pain, stiffness, or inflammation	X	X	X	X	X	X	X	X	X	X	X	X	X
Muscle weakness	O		X	X									
Myalgia	O			X				X	X	X			
General													
Cough								X	X				
Ear involvement								X					
Eye involvement	X				X		O	X		X		X	
Fatigue	X		X	X	X				X	X			
Fever	X		X	X	X		X	X	X	X			
GI involvement		X		X		X			X	X		X	X
Kidney involvement	X	X		O				X	O	X	X	X	X
Malaise	X								X	X	X		
Nasal or oral symptoms				O				X	X				
Nervous system involvement	X			O				X	X	X	X		
Respiratory involvement			O	X			X	X	X				
Weight loss			X		X				X	X			
Other													
Adenopathy	X						X						
Anemia	X			X									
Dysphagia		X	X										
Swelling of hands		X	O	X		X							

X indicates common; O indicates less common but not rare.

ANCA, antineutrophil cytoplasmic antibodies; GPA, granulomatosis with polyangiitis; HUV, hypocomplementemic urticarial vasculitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; DM, dermatomyositis; MCTD, mixed connective tissue disease; PsA, psoriatic arthritis; ReA, reative arthritis; SpA, spondyloarthropathies; JIA, juvenile idiopathic arthritis; CV, cryoglobulinemic vasculitis.

[a] This is not a complete list of signs and symptoms; some conditions have more signs and symptoms than could be presented here.

Autoimmune Skin Diseases

Psoriasis

Psoriasis is the most common chronic inflammatory condition of the skin, most often characterized by patches comprising round or oval plaques with a scaly appearance.[1] Prevalence estimates are 700 to 3,200 per 100,000 persons in the United States.[13,14] Men and women are equally affected.[14] The onset of psoriasis is most likely to occur between the ages of 15 and 30 years, but may occur at any age.[1] Prevalence increases with age until about 60 years.[15] Triggers for psoriasis include stress, direct skin trauma (Koebner phenomenon), and streptococcal throat infection.[1]

Most (90%) affected patients have plaque psoriasis with scaly patches localized to the scalp, buttocks, trunk, and extensor surfaces of the arms and legs.[1] Nail pitting, resembling fungal infection, is observed in about half of patients.[10]

Other types of psoriasis vary in severity. Symptoms range from rash-like inverse psoriasis in skin folds to wide-spread erythema and systemic symptoms in erythrodermic psoriasis. Guttate psoriasis, more common in younger patients (<30 years), manifests as scattered erythematous papules resembling "drops," usually on the trunk. Pustular psoriasis lacks plaques and is usually localized to palms and soles; however, a rare generalized form exists that may be life-threatening.[1]

Individuals with psoriasis are at risk for developing psoriatic arthritis (see **Spondyloarthropathies**), especially patients with severe psoriasis and nail pitting.[16] Patients are also at increased risk for cardiovascular disease,[17] which contributes to an approximately 6-year lower life expectancy compared to the general population.[18]

Chronic Urticaria

Chronic urticaria refers to hives lasting at least 6 weeks. In the United States, about 130 persons per 100,000 are currently treated for chronic urticaria, and 530 persons per 100,000 have the disease at some point in their lifetime. About twice as many women as men are affected.[19] Peak incidence of chronic urticaria is between the ages of 20 and 40 years.[19] The onset of urticaria may be induced by specific physical factors (eg, heat, cold, sunlight, pressure) or be spontaneous with no clear trigger.[20] Most cases of chronic urticaria spontaneously self-resolve, although moderate and severe cases may persist up to 5 years.[19]

Vitiligo

Vitiligo is characterized by patches of pigmentation loss in the skin, without inflammation. Prevalence estimates for vitiligo range from 100 to 2,400 persons per 100,000 in the United States.[21] Conflicting results have been reported as to whether more males or females are affected.[21] Age of onset is usually about 20 years.[4] Progression is poorly understood, but prevalence may peak at ≥60 years of age.[4] Psychological stress is a possible trigger in genetically susceptible individuals.[22] Loss of pigmentation leads to sensitivity to sun exposure and can cause low self-esteem, stigmatization, and depression.[4] Otherwise, vitiligo is usually benign. Autoimmune thyroid disease is the most common comorbidity, present in 14% of patients.[23]

Autoimmune Blistering Disease

Autoimmune blistering diseases are caused by autoantibodies that target specific proteins in skin and mucous membranes resulting in separation of epidermal and dermal layers. These diseases are rare, and few prevalence estimates exist. The largest study to date found that bullous pemphigoid (BP) was the most common (26 persons per 100,000), followed by pemphigus vulgaris (9 persons per 100,000), linear IgA disease (LAD, 2 patients per 100,000), and mucus membrane (also called cicatricial) pemphigoid (2 patients per 100,000).[24] BP, pemphigus vulgaris, and LAD affect men and women equally, whereas mucus membrane pemphigoid affects about twice as many women as men.[6] A childhood form of LAD, chronic bullous disease of childhood (CBDC), affects about twice as many girls as boys.[6]

These diseases mainly affect the middle-aged to elderly. For example, the age of onset is 60 to 80 years for BP and 40 to 60 years for pemphigus vulgaris and mucus membrane pemphigoid. [6] LAD is an exception: the adult form usually first appears at around 30 years, and the childhood form (CBDC) at <5 years.[6]

Triggers for autoimmune blistering diseases may include neurological disease (eg, multiple sclerosis for BP), pregnancy (for gestational pemphigus), gluten (for dermatitis herpetiformis), malignancy (for paraneoplastic pemphigus), drugs, operations, radiation, and physical trauma.[3,5,7]

Autoimmune blistering diseases are generally classified as pemphigoid or pemphigus diseases:

- Pemphigoid diseases are subepidermal autoimmune blistering diseases. In BP, autoantibodies target

hemidesmosomes anchoring epidermal cells to the basal lamina. Deep, tense blisters form on the skin; mucous membranes are not usually involved. In contrast, mucous membrane pemphigoid involves blistering of mucous membranes in contact with the skin, eyes, mouth, nose, or genitals.[25] LAD may involve blistering on the skin or mucous membranes; about 50% of CBDC patients develop oral ulcers.[6]

- In pemphigus diseases, autoantibodies target desmosomes and form flaccid blisters in the suprabasal ("prickle cell") layer in the epidermis, contrasting with subepidermal pemphigoid. Pemphigus diseases include pemphigus vulgaris, pemphigus foliaceus (including pemphigus erythematosus, which overlaps with SLE[26]), paraneoplastic pemphigus, and IgA pemphigus.

The pemphigoid diseases may be self-limiting and spontaneously resolve,[6] although patients with BP have an elevated mortality risk compared to the age-matched general population.[3] The major cause of death is infection.[3] Mucus membrane pemphigoid may lead to serious ocular and oral complications, and LAD has a variable course in adults; symptoms may persist for years or reoccur.[6] Pemphigus diseases are the most serious of the autoimmune blistering diseases and may be fatal if left untreated.[6]

Autoimmune Rheumatic Diseases Involving the Skin

Systemic Lupus Erythematosus

SLE is a severe autoimmune rheumatic disease. The prevalence of SLE in the United States is about 42 to 150 persons per 100,000, with >90% of patients being female.[27] Disease onset can occur at any age. Incidence peaks at above age 40 for most populations. For African American women, however, the peak incidence is during the reproductive years.[27]

Cutaneous lesions occur in 72% to 85% of patients with SLE[28]; disease-specific features include malar erythema (a butterfly-shaped rash on the face); lesions caused, or exacerbated, by sun exposure; and disk-shaped round lesions, usually on the face or scalp where they may cause scarring alopecia. Bullous SLE is a rare form of lupus that presents with blisters as an initial or concurrent manifestation of the disease.[29,30]

Mortality is high if SLE is left untreated, especially in older patients (>50 years).[27]

Systemic Sclerosis

SSc is a chronic, multisystem, heterogeneous autoimmune disease characterized by hardening of the skin caused by overproduction of collagen. The disease affects about 28 persons per 100,000 in the United States, with >4 times as many women affected as men.[31] Age at diagnosis is typically >40 years.[31]

The 2 most common types of SSc are:

- Diffuse cutaneous SSc (dcSSc), which affects internal organs such as the heart and lung, as well as the skin
- Limited cutaneous SSc (lcSSc), also called CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome.

SSc accounts for an estimated 22 years of life lost for women and 26 years of life lost for men, with the dcSSc form having higher mortality.[32]

Dermatomyositis

Dermatomyositis is an inflammatory disorder of the skin and muscles. It affects about 6 to 21 persons per 100,000 in the United States, with about 4 times as many women as men being affected.[33,34] Dermatomyositis may appear in childhood (juvenile dermatomyositis [JDM]) or adulthood, with prevalence peaking in the elderly (>65 years).[33]

This rheumatic skin disease is characterized by heliotrope rash on the eyelids and violet to red inflammation of joints, especially in the hand with papules ("Göttron's papules") or without papules ("Göttron's sign").[35] Muscle symptoms usually appear within 6 months of onset of the skin disease and include progressive weakness of the upper and lower proximal extremities, as well as the neck flexors. One form of dermatomyositis, amyopathic dermatomyositis (ADM), does not affect the muscles.

Juvenile DM may be associated with calcinosis,[36] and adult DM with cardiovascular disease, cancer, or interstitial lung disease.[37,38] These manifestations represent the major causes of mortality, which is estimated to be <3% for JDM and between 5% and 48% for adult dermatomyositis.[36-38]

Mixed Connective Tissue Disease

MCTD is a rheumatic disease with a wide range of signs and symptoms, which usually overlap with other autoimmune rheumatic diseases.[39] The only reported prevalence estimate for MCTD in the United States is in American Indian/Alaskan Native people,[40] for whom it is about 6 persons per 100,000. Studies indicate that females are predominantly affected (5 times more than males), with incidence highest in the 60-69 year age group.[39]

Raynaud phenomena (80% of patients), arthritis/arthralgia (86%), and swollen hands (64%) are the most common manifestations of MCTD.[39]

Compared to other autoimmune rheumatic diseases, MCTD is considered relatively benign with an overall mortality not greater than the general population.[39]

Spondyloarthropathies

The term "spondyloarthropathies" (SpA) encompasses a group of inflammatory rheumatic diseases that cause arthritis. SpA diseases can be subdivided based upon rheumatological symptoms. Axial SpA involves the spine and sacroiliac joints, and peripheral SpA involves peripheral arthritis, enthesitis, and dactylitis.[41,42] Psoriatic arthritis and reactive arthritis are SpAs characterized by skin-related symptoms, with overlapping presentations involving both axial and peripheral joints.[12,43]

The estimated prevalence of psoriatic arthritis is about 160 per 100,000 persons in the general population.[13] Nearly twice as many men are affected as women.[44] Individuals with psoriasis may develop psoriatic arthritis, on average about 12 years after the onset of the skin condition,[1] although for a minority of patients arthritis may precede psoriasis.[43] Untreated psoriatic arthritis may become disabling.

The prevalence of reactive arthritis in the United States is unknown.[45] The disease is predominantly associated with enteric or sexually transmitted infection (eg, *Clostridium difficile* infections and HIV).[12,45] Reactive arthritis affects 3 times as many men as women.[46] Peak incidence is younger than 40 years,[46] although this estimate may be skewed by early case reports predominantly involving younger people with sexually transmitted infections.[47] Skin-related symptoms include circinate balanitis (erosion/crusting of the penis), keratoderma blennorrhagica (pustules on the palms and soles of the feet), and psoriasis-like scales.[10] Reactive arthritis usually self-resolves in 6 to 12 months, although 25% of patients may go on to develop signs and symptoms of chronic conditions such as ankylosing spondylitis or inflammatory bowel disease.[12]

Juvenile Idiopathic Arthritis

Juvenile idiopathic arthritis (JIA) is defined as arthritis of unknown cause that appear before age 16 and persists for at least 6 weeks; psoriasis is the most common skin manifestation.[48] Prevalence estimates in the United States[49,50] are 45 to 58 persons per 100,000. JIA affects more than twice as many girls as boys.[49,50] Age at diagnosis is typically around 7 to 9 years,[49] with prevalence peaking at around 11 to 15 years in children.[50]

JIA can be a debilitating disease that persists into adulthood.[50]

Systemic Vasculitis

Autoimmune systemic vasculitis involving the skin includes antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides and immune complex vasculitides. Most of these diseases are characterized by purpura. The exception is an immune complex disease called hypocomplementemic urticarial vasculitis (HUV, anti-C1q vasculitis), in which lesions are urticarial rather than purpuric.[10]

The ANCA-associated vasculitides are rare. Prevalence estimates for the most common form of ANCA-associated vasculitides in the United States[51] range from 3 to 9 persons per 100,000. Men and women are equally affected; these diseases predominantly affect the middle-aged and elderly.[51]

Vasculitis may be a primary or secondary manifestation of disease and can be caused by certain infections, malignancy, rheumatic disease, medications, and a wide variety of autoimmune disorders. Untreated, the ANCA-associated vasculitides may rapidly progress to organ failure and death.[52]

The immune complex vasculitides are even rarer. The most common form is cryoglobulinemic vasculitis (also called mixed cryoglobulinemia), with an estimated prevalence of 1 person per 100,000 (female-to-male ratio of 3:1).[53] This prevalence may be an underestimate, however. Cryoglobulinemic vasculitis is highly associated with hepatitis C virus (HCV) infection; the prevalence is as high as 5% in HCV-positive patients in the United States.[54] Based on current prevalence estimates for chronic HCV[55] (1,000 persons per 100,000), HCV-associated cryoglobulinemic vasculitis prevalence could be as high as 10 to 50 persons per 100,000. Untreated HCV-associated cryoglobulinemic vasculitis can lead to chronic cutaneous ulcers, gangrene, and glomerulonephritis.[56]

INDIVIDUALS SUITABLE FOR TESTING

- Individuals who have signs and symptoms consistent with autoimmune skin disease (**Tables 1** and **2**)
- Individuals receiving pharmacotherapy for autoimmune skin disease

TEST AVAILABILITY

Quest Diagnostics offers many tests and panels that may be useful for classifying or diagnosing autoimmune skin disease and monitoring response to therapy (**Appendix**).

TEST SELECTION AND INTERPRETATION

Autoimmune Skin Diseases

Psoriasis

To diagnose psoriasis, clinical assessment by a dermatologist is generally considered the gold standard; laboratory testing is generally not required.[57,58] However, laboratory testing is important for guiding treatment and monitoring patient response to therapies.

Methotrexate

Methotrexate is one of the most commonly used systemic treatments for severe psoriasis. Guidelines suggest that the following be performed before or during methotrexate therapy[59]:

- Screening for HBV, HCV, and HIV infection:
  - Active HBV contraindicates methotrexate treatment; past HBV infection indicates a low risk (<1%) of reactivation of the virus by methotrexate treatment.
  - Chronic HCV infection indicates increased risk for fibrosis and patients should be closely monitored during methotrexate treatment.
  - Chronic HIV infection indicates increased risk for opportunistic infection and leucopenia during methotrexate treatment.
- Liver function tests at baseline and at least every 3 months during treatment; methotrexate is associated with hepatotoxicity.
- Complete blood cell (CBC) count at baseline; methotrexate suppresses production of red and white blood cells.
- Renal function evaluation at baseline. Methotrexate is excreted predominantly by the kidneys. It should be avoided in patients with a creatine clearance <20 mL/min, and dosage should be halved in those with creatinine clearance of 20 to 50 mL/min.
- Methotrexate monitoring during folinic acid rescue after methotrexate overdose, until levels are <0.05 µmol/L.

Tumor Necrosis Factor Blockers

Tumor necrosis factor (TNF) blockers, such as adalimumab (Humira®) and infliximab (Remicade®), are used to treat plaque psoriasis. TNF blockers have had a major impact on the therapy, but response rates vary. Laboratory testing for drug levels can indicate bioavailability, whereas testing for antidrug antibodies (ADAs) can help differentiate causes of insufficient bioavailability.**Table 3** contains result interpretation and management strategies when both drug and ADA levels are tested.

Table 3. Interpretation of Results in Patients With TNF Blocker Treatment Failure[60]

	ADA Not Detected (absent) (<10 AU)	ADA Detected (present) (≥10 AU)
Drug Levels Subtherapeutic[a] (<4 µg/mL for adalimumab and ≤0.1 µg/mL for infliximab)	Suggests insufficient bioavailability caused by nonimmune PK or patient adherence issues Consider increasing therapeutic dose or addressing potential adherence issues	Suggests insufficient bioavailability caused by immunogenicity Consider switching to different TNF blocker
Drug Levels Therapeutic[a] (4-7 µg/mL for adalimumab and >0.1 µg/mL for infliximab)	Suggests PD issue caused by TNF-independent disease Consider switching to a non-TNF treatment	Rare situation that may be caused by a false-positive result or nonfunctional ADAs Consider retest or testing for neutralizing antibody by cell-based assay

ADA, anti-drug antibody; PD, pharmacodynamic; PK, pharmacokinetic; TNF, tumor necrosis factor.
[a] Trough concentrations for psoriasis[61,62]

Chronic Urticaria

Diagnosis of chronic urticaria is based on clinical assessment and patient history.[20,63] For spontaneous chronic urticaria, routine laboratory testing may include measuring[63]

- Inflammatory markers, including CBC with differential blood count and either erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP); elevated levels may indicate that urticaria is associated with infection, malignancy, or other autoimmune disease (eg, vasculitis or lupus)[64]
- Liver enzymes; elevated levels may indicate that urticaria is associated with liver disease (eg, viral hepatitis infection)[65]
- Thyroid stimulating hormone; low or elevated levels may indicate that urticaria is associated with hyper- or hypothyroidism, respectively.

European guidelines recommend that differential blood count and ESR or CRP measurement be routinely performed, whereas US guidelines recommend that testing be offered only if indicated by physical examination and patient history.[20,63]

When indicated by the patient's history, guidelines recommend targeted testing for differential diagnosis. For example, targeted testing can rule out infection, allergies, and other autoimmune diseases (eg, HUV, see **Autoimmune Rheumatic Diseases Involving the Skin**).[20,63]

To identify underlying causes of chronic urticaria, US and European guidelines suggest that extended diagnostic testing may be appropriate:

- Both guidelines recommend measuring thyroid autoantibodies, which are frequently identified in patients with chronic urticaria (≥10% of patients).[66]
- European guidelines recommend testing for functional IgG antibodies against high-affinity IgE receptor (Fc epsilon RI alpha),[20] which are observed in 30% to 50% of patients.[63]

Quest offers tests and panels for chronic urticaria **(Appendix)** that include thyroid autoantibodies, functional IgG antibodies against Fc epsilon RI alpha (measured as histamine release), and immunoassays for IgG antibodies against IgE, which are observed in 5% to 10% of patients.[63]

Vitiligo

Vitiligo is diagnosed based on the patient's clinical history and presentation. Neither biopsy nor laboratory testing is usually necessary to confirm a diagnosis. However, laboratory testing is useful to assess the risk of autoimmune thyroid disease. Patients are at increased risk (>5 fold) of developing autoimmune thyroid disease compared with patients without vitiligo.[23] This risk increases with age.[23]

Sera from about one-fifth of patients with vitiligo test positive for either anti-thyroglobulin (Tg), anti-thyroid peroxidase, or anti-thyrotropin receptor antibodies.[23] Consequently, guidelines recommend screening on a periodic basis for abnormal thyroid function or autoantibodies in patients with vitiligo.[4,67]

Autoimmune Blistering Disease

In addition to clinical presentation and histopathology, diagnosis of autoimmune blistering diseases relies on immunofluorescence (IF) microscopy studies and immunoserological assays. Cutaneous direct IF identifies the distribution of autoantibodies in situ in perilesional skin biopsies. Indirect IF of serum specimens on monkey esophagus and murine bladder substrates can confirm distribution and provide titers for circulating IgG antibodies that reflect disease activity. Salt-split skin is often used to further differentiate many of these diseases by indicating whether antibodies are on the dermal or subdermal side of the basement membrane. Dermpath and Quest offer these and other tests **(Appendix)** to help diagnose and differentiate autoimmune blistering diseases **(Figures 1** and **2)**.[68,69]

Bullous pemphigoid

Diagnosis of BP requires 1 of the following 3 findings[70]:

- A linear distribution of C3 and IgG antibodies in the basement membrane observed on direct IF of perilesional skin biopsies from individuals meeting 3 of 4 criteria:
  - Age >70 years
  - Absence of atrophic scars
  - Absence of head and neck involvement
  - Absence of mucosal involvement
- Indirect IF of serum specimens on salt-split skin reveals that the IgG antibodies are on the epidermal ("roof") side[3,68,69]
- Detection of BP180 IgG, BP230 IgG, or both by ELISA

While combined, direct and indirect IF can establish antibody distribution consistent with BP; ELISA assays are useful for detecting circulating antibodies to antigens of interest. A meta-analysis of the BP180 ELISA assays estimated a pooled sensitivity of 87% and specificity of 98% for BP.[71] When BP180 is combined with BP230 ELISA, sensitivity estimates have ranged from 66% to 100%.[3] Thus, while ELISA may confirm a diagnosis of BP, patients who test negative for both BP180 and BP230 may still have BP especially if the diagnosis is supported by IF results.[3,70-72]

Serum concentrations of BP180 are useful for monitoring disease activity, and European guidelines recommend measuring titers at baseline and 60 and 150 days after initiation of treatment.[70] Small decreases (≤20%) in BP180 predict disease relapses within the first year of therapy.[70] Undetectable or low BP180 (<30 U/mL) at day 150 indicates a 90% probability of long-lasting remission.[70]

Rarer pemphigoid diseases

These include dermatitis herpetiformis, epidermolysis bullosa acquisita (EBA), mucous membrane pemphigoid (including laminin 332 mucous membrane pemphigoid [Lam 332MMP]), anti-laminin- γ 1 (P200) pemphigoid, anti-P105 pemphigoid, linear IgA disease, gestational pemphigoid, and lichen planus pemphigoides.

Differential diagnosis these diseases involves clinical assessment and laboratory testing **(Figures 1, 2,** and **Appendix)**. Notably:

- Gluten sensitivity distinguishes dermatitis herpetiformis. Positive ELISA results for tissue transglutaminase (sensitivity 47% to 95%; specificity >90%) or endomysium (sensitivity 52% to 100%; specificity ~100%) autoantibodies confirm diagnosis.[5]
- Clinical features of EBA subtypes overlap with those of both BP and mucous membrane pemphigoid. Differential diagnosis is aided by direct and indirect IF findings.[3,25] EBA has a distinctive "u-serrated" pattern on direct IF. The u-shaped appearance corresponds to antibody binding to type VII collagen distributed as upstanding arms between the rootlets of the basal keratinocytes.[73] Definitive diagnosis of EBA requires localization of target antigens to the dermal layer ("floor") by either (a) direct IF on unblistered salt-split skin from the original biopsy (if available) **(Figure 1)**, or (b) indirect IF on salt-split monkey skin **(Figure 2)**. However, because serum antibodies are detected in only 50% to 60% of EBA patients, absence of serum antibodies on indirect IF does not rule out EBA.[3]

Pemphigus diseases

On IF of perilesional biopsy specimens, pemphigus diseases have a "chicken wire" pattern of autoantibodies in the suprabasal layer of the epidermis. This pattern distinguishes pemphigus from pemphigoid diseases. Pemphigus autoantibodies primarily have specificity for the proteins desmoglein-1 and -3. A meta-analysis of desmoglein-3 ELISAs estimated a sensitivity of 97% and specificity of 98% for pemphigus vulgaris.[71] The autoantibodies involved can help differentiate the different types of pemphigus disease **(Figures 1** and **2)**. One form, pemphigus erythematosus, has overlapping pathology with SLE and is distinguished from other pemphigus by the presence of antinuclear antibodies (ANAs).[26]

Serum concentrations of desmoglein-1 and -3 autoantibodies correlate with disease activity. As an aid in therapeutic decision-making, European guidelines recommend measuring titers of 1 or both at baseline, 3 months after initiating therapy (eg, corticosteroids or immunosuppressive agents) and then every 3 to 6 months.[7] Titers >20 U/mL indicate active disease, titers of 9 U/mL to 20 U/mL are equivocal for disease activity, and titers <9 U/mL indicate inactive disease.

Screening before therapy

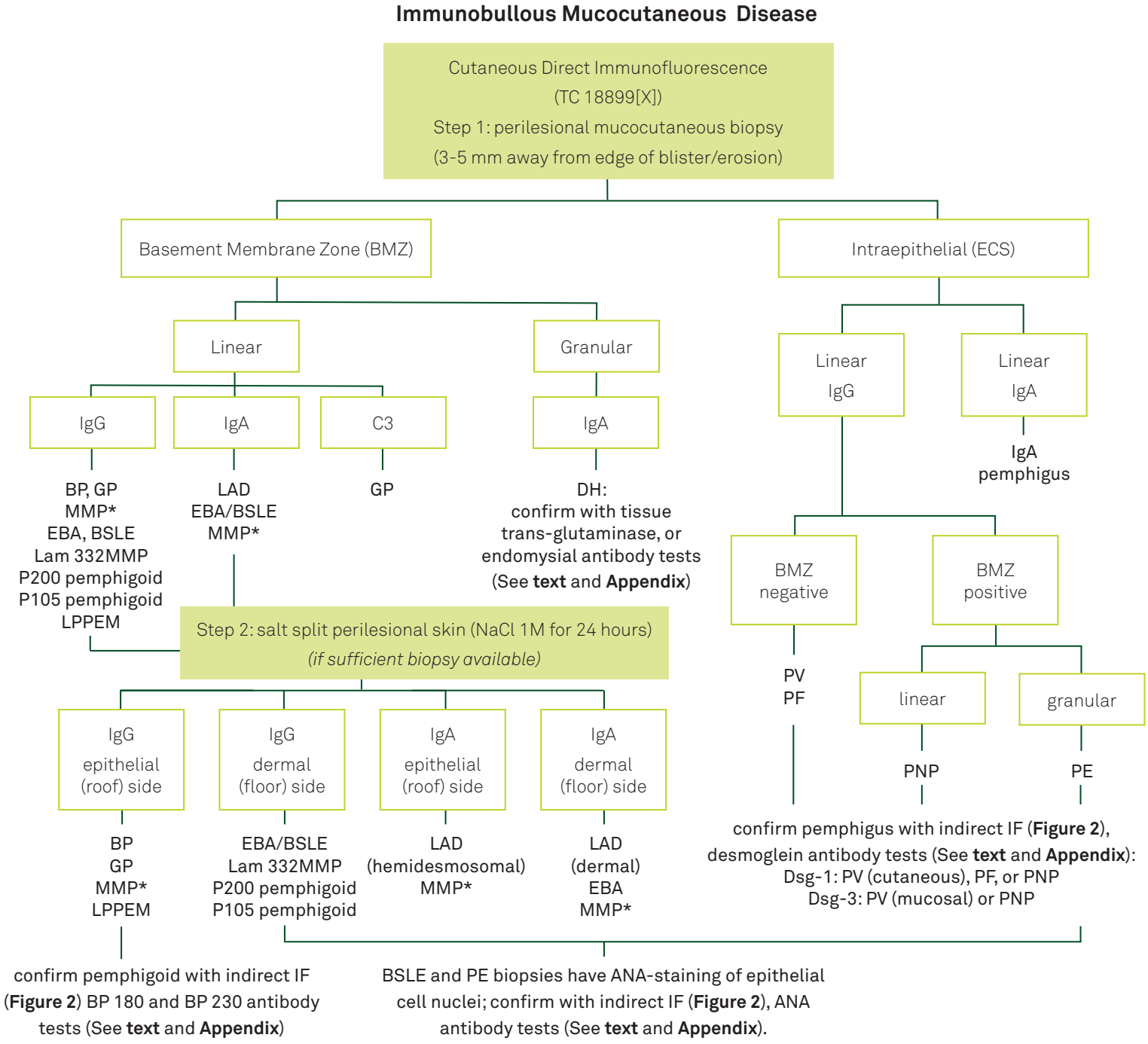
Prior to corticosteroid therapy and immunosuppressive therapy, the following testing is recommended both for BP and pemphigus diseases[7,70]:

- CBC
- Creatinine, blood electrolytes
- Transaminases, gamma glutamyltransferase, alkaline phosphatase
- Total serum protein, albumin
- Fasting serum glucose
- Hepatitis B, C and HIV

In addition, the following testing is recommended if indicated[7,70]:

- Thiopurine methyltransferase activity testing, when azathioprine treatment is considered
- Glucose 6-phosphate dehydrogenase, if dapsone treatment is considered
- Beta-human chorionic gonadotropin (HCG) to exclude pregnancy in females of childbearing age
- Serum IgA to exclude IgA deficiency if intravenous immunoglobulins are considered

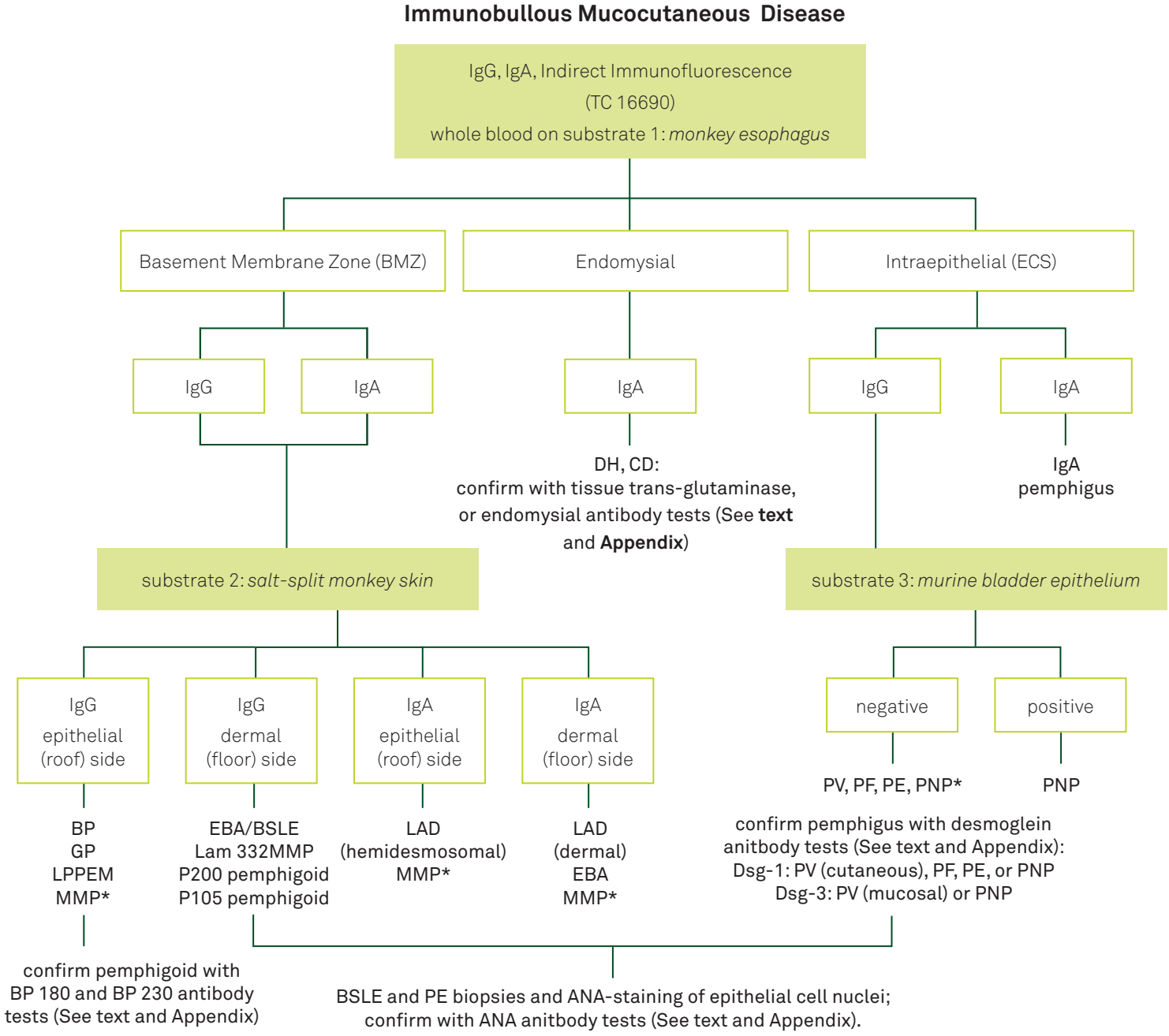
Figure 1. Direct Immunofluorescence in the Differential Diagnosis of Autoimmune Blistering Diseases



*, some cases; ANA, antinuclear antibodies; BP, bullous pemphigoid; BLSE, bullous systemic lupus erythematosus; BMZ, basement membrane zone; Dsg, desmoglein; EBA, epidermolysis bullosa acquisita; ECS, epithelial cell surface; GP, gestational pemphigoid; LAD, linear IgA disease; Lam 332MMP, laminin-332 MMP; LPPEM, lichen planus pemphigoides; MMP, mucous membrane pemphigoid; P200 pemphigoid, anti-laminin-γ 1 P200 pemphigoid; PE, pemphigus erythematosus; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris; SLE, systemic lupus erythematosus; TC, test code.

This figure was developed by the Institute for Immunofluorescence at Dermpath Diagnostics® and Quest Diagnostics based on expert opinion and, in part, on references 68 and 69. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education,clinical expertise, and assessment of the patient.

Figure 2. Indirect Immunofluorescence in the Differential Diagnosis of Autoimmune Blistering Diseases



*, some cases (for PNP, negative results are associated with a lichenoid phenotype); ANA, antinuclear antibodies; BP, bullous pemphigoid; BLSE, bullous systemic lupus erythematosus; BMZ, basement membrane zone; CD, celiac disease; Dsg, desmoglein; EBA, epidermolysis bullosa acquisita; ECS, epithelial cell surface; GP, gestational pemphigoid; LAD, linear IgA disease; Lam 332MMP, laminin-332 MMP; LPPEM, lichen planus pemphigoides; MMP, mucous membrane pemphigoid; P200 pemphigoid, anti-laminin-γ 1 P200 pemphigoid; PE, pemphigus erythematosus; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris; TC, test code.

Positive results reflex to titer at an additional charge. This figure was developed by the Institute for Immunofluorescence at Dermpath Diagnostics® and Quest Diagnostics based on expert opinion and, in part, on references 68 and 69. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

Autoimmune Rheumatic Diseases Involving the Skin

Systemic Lupus Erythematosus

Classification of SLE according to the Systemic Lupus International Collaborating Clinics (SLICC) criteria requires a combination of clinical and immunologic evaluation **(Table 4)**.[74] Laboratory testing can help assess some clinical and immunologic criteria including the presence or absence of proteinuria, anemia, leukopenia, lymphopenia, thrombocytopenia, and antibodies to DNA, Sm, antiphospholipids, and ANA. A patient is classified as having SLE if:

- Four criteria are met, including ≥1 clinical and ≥1 immunologic criterion **(Table 4)** *or*
- Biopsy-proven nephritis compatible with SLE and ANA or dsDNA antibodies are present

When considered individually, most of the laboratory tests included in the SLICC classification criteria provide high specificity (86% to 99%) and low-to-medium sensitivity for SLE (7% to 59%). The exception is ANA testing, which has moderate specificity (45%) and high sensitivity (96%)[74]; a negative ANA result can help rule out SLE, because ANA-negative SLE is rare.[75]

Though not included in the SLICC classification criteria, chromatin antibodies have relatively high sensitivity (64% to 69%) and specificity (92% to 99%)[76,77] for SLE, and may provide value when diagnosing SLE. RNP antibodies are also present in SLE patients, but are not specific to SLE; RNP antibodies are more useful for identifying MCTD.[78,79]

In bullous SLE, a polymorphic (linear, granular, or both) distribution of multiple antibodies (IgG, IgM, and/or IgA) is observed at the basement zone on cutaneous direct IF.[29,30]

Table 4. SLE Classification Criteria[74]

Clinical Criteria
1. Acute cutaneous lupus in the absence of dermatomyositis *or* subacute cutaneous lupus
2. Chronic cutaneous lupus
3. Oral ulcers[a] *or* nasal ulcers[a]
4. Nonscarring alopecia[a]
5. Synovitis of ≥2 joints *or* tenderness of ≥2 joints and >30 minutes morning stiffness
6. Pleurisy (>1 day), pleural effusion, or pleural rub[a] *or* pericardial pain (>1 day), pericardial effusion, pericardial rub, or pericarditis by ECG[a]
7. Urine protein-to-creatinine ratio indicates 500 mg protein/24 hours *or* red blood cell casts
8. Seizures, psychosis, myelitis, mononeuritis multiplex,[a] peripheral or cranial neuropathy,[a] or acute confusional state[a]
9. Hemolytic anemia
10. Leukopenia (<4,000/mm^3)[a] *or* lymphopenia (<1,000/mm^3)[a]
11. Thrombocytopenia (<100,000/mm^3)[a]
Immunologic Criteria
1. ANA level above reference range
2. dsDNA antibody level above reference range (or > twice reference range if tested by ELISA)
3. Sm antibody positive
4. Antiphospholipid antibody positive[b]
5. Low C3, C4, or CH50
6. Direct Coombs test if hemolytic anemia is absent

[a] If no other cause is present.

[b] As determined by positive result for lupus anticoagulant; false-positive result for rapid plasma reagin; medium-to-high titer of cardiolipin antibody; or positive results for β2-glycoprotein I antibody.

The staining pattern is "u-serrated" and may appear similar to that of EBA[73], but bullous SLE is distinguished by the presence of ANA and by satisfying SLE classification criteria **(Table 4)**.

Other rarer forms of lupus are differentiated from each other, and other photosensitive connective tissue diseases, by direct IF on lesional cutaneous biopsy **(Figure 3)**.[80,81]

Systemic Sclerosis

The European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for systemic sclerosis (SSc, **Table 5**) comprise mainly clinical criteria, but also include laboratory testing for autoantibodies.[82] A positive result for centromere, Scl-70 (topoisomerase I), or RNA polymerase III antibodies, is consistent with SSc but is not diagnostic. A positive ANA result suggests the need for testing for specific autoantibodies if clinical symptoms are consistent with SSc; 85% to 97% of patients with SSc are ANA-positive.[83,84]

The type of SSc can affect prognosis and treatment. Differentiation may be possible based on location of skin fibrosis (proximal vs distal extremities) and clinical manifestations; supported by laboratory test results. Scl-70 antibody is found in approximately 40% of patients with dcSSc, whereas centromere antibody is found in up to 90% of patients with lcSSc.[85] When detected by indirect IF, immunoprecipitation, or immunodiffusion, Scl-70 and centromere antibodies tend to be mutually exclusive in SSc patients.[85] Thus, a positive test result for Scl-70 antibody is consistent with dcSSc if clinical symptoms are present, and a positive test result for centromere antibody is consistent with lcSSc if clinical symptoms are present.

Dermatomyositis

The EULAR/ACR classification criteria for dermatomyositis include biopsy (if available), clinical, and laboratory evaluation. The criteria are validated for the idiopathic inflammatory myopathies (IIMs), including those with skin involvement: dermatomyositis, JDM, and ADM **(Table 6)**.[35]

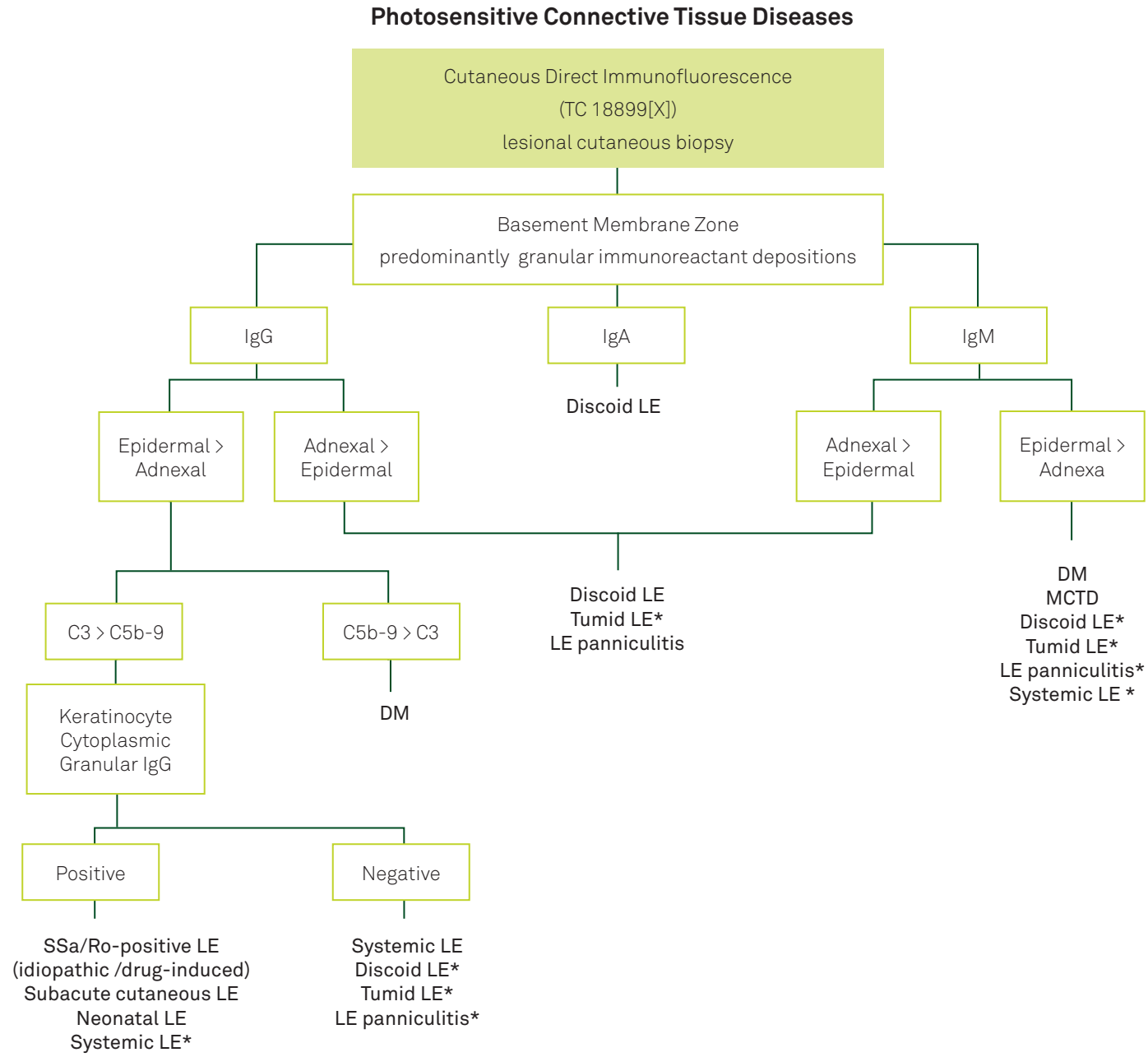
Table 5. Systemic Sclerosis Classification Criteria[82]

Classify a patient as having systemic sclerosis if sum of points is ≥9

Criteria	Points
Skin thickened on fingers of both hands, extending proximal to the metacarpophalangeal joints	9
Skin on fingers thickened (only count highest score)	
Puffy fingers	2
Sclerodactyly[a]	4
Lesions on fingertips (only count highest score)	
Ulcers on tip of digits	2
Pitting scars on fingertips	3
Telangiectasia	2
Abnormal nailfold capillaries	2
Pulmonary arterial hypertension and/or interstitial lung disease (max score is 2)	
Pulmonary arterial hypertension	2
Interstitial lung disease	2
Raynaud phenomenon	3
Presence of any SSc-related autoantibodies[b]	3

[a] Distal to metacarpophalangeal joints but proximal to proximal interphalangeal joints.
[b] 3 points for 1 or more of the following antibodies: centromere, Scl-70, or RNA polymerase III antibody; maximum score is 3.

Figure 3. Direct Immunofluorescence in the Differential Diagnosis of Lupus and Other Photosensitive Connective Tissue Diseases



*, some cases; DM, dermatomyositis; LE, lupus erythematosus; MCTD, mixed connective tissue disease; TC, test code.

This figure was developed by the Institute for Immunofluorescence at Dermpath Diagnostics® and Quest Diagnostics based on expert opinion and, in part, on references 80 and 81. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education,clinical expertise, and assessment of the patient.

Table 6. Dermatomyositis Diagnostic Criteria[35]

If no other cause is present and sum of points is ≥5.5 (≥6.7 with biopsy), then classify patient as having:

DM if rash[a] is present with muscle weakness[b] and age of onset is ≥18 years.

JDM if rash[a] is present with muscle weakness[b] and age of onset is <18 years.

ADM if rash[a] is present without muscle weakness[b] and age of onset is ≥18 years.

Criteria	Points without biopsy	Points with biopsy
Biopsy		
Endomysial infiltration of mononuclear cells surrounding, but not invading, myofibers		1.7
Perimysial or perivascular infiltration or both of mononuclear cells		1.2
Perifascicular atrophy		1.9
Rimmed vacuoles		3.1
Clinical		
Age of onset of first symptom assumed to be related to the disease:		
≥18 years and <40 years	1.3	1.5
≥40 years	2.1	2.2
Objective symmetric weakness, usually progressive, of the:		
proximal upper extremities	0.7	0.7
proximal lower extremities	0.8	0.5
Neck flexors are relatively weaker than neck extensors	1.9	1.6
Proximal muscles are relatively weaker than distal muscles in the legs	0.9	1.2
Heliotrope rash	3.1	3.2
Göttron papules	2.1	2.7
Göttron sign	3.3	3.7
Dysphagia or oesophageal dysmotility	0.7	0.6
Laboratory		
Jo-1 (anti-histidyl-tRNA synthetase) autoantibody present	3.9	3.8
Elevated serum levels of creatine kinase (CK)[c] or lactate dehydrogenase (LD)[c] or aspartate aminotransferase (ASAT/AST/SGOT)[c] or alanine aminotransferase (ALAT/ALT/SGPT)[c]	1.3	1.4

ADM, amyopathic; DM, dermatomyositis; JDM, juvenile DM.

[a] Heliotrope rash, Göttron sign, or Göttron papules.

[b] Objective symmetric weakness, usually progressive of the upper or lower extremities; neck flexors weaker than extensors; or proximal leg muscles weaker than distal.

[c] Serum levels above the upper limit of normal.

Although Jo-1 antibody is the only myositis-specific antibody currently included in the criteria, EULAR/ACR anticipate that others will be included in future updates.[35] These include the other antisynthetase antibodies—EJ, OJ, PL-7, and PL-12—as well as Mi-2 and SRP antibodies. Together with Jo-1 antibodies, these antibodies are found in about 50% of dermatomyositis patients and are often mutually exclusive.[86] Additional myositis-specific antibodies that have been more recently discovered include TIF1-γ (p155) and NXP-2 (p140) antibodies, which are prevalent in adults with cancer-associated dermatomyositis and children with JDM.[87,88] MDA-5 antibody, another myositis-specific antibody, is prevalent in patients with ADM and is associated with interstitial lung disease.[89]

Myositis-associated antibodies (Ku, PM/Scl, Sjögren's antibody [SS-A], Smith [Sm]/U1-RNP, antibody, and U1-RNP antibody) are less specific and are found in 1% to 13% of IIM patients.[86] A positive test result for a myositis-associated antibody in a symptomatic patient suggests the presence of dermatomyositis (with rash), or MCTD (discussed below), especially when a markedly elevated isolated SM/U1-RNP or U1-RNP antibody is present.

Mixed Connective Tissue Disease

MCTD should be considered when overlapping presentations of rheumatic diseases are observed, usually Raynaud phenomenon, coupled with edema in hands, and mixed SLE, SSc, or myositis-like symptoms. Four sets of MCTD classification criteria exist: Sharp, Alarcón-Segovia, Kasukawa, and Kahn.[90] The different sets require a variety of clinical and serological criteria be met, but all 4 require either a positive result or a high titer for RNP antibody. For example, the Kasukawa criteria require a positive anti-RNP test result, whereas the Alarcón-Segovia criteria require a high RNP antibody titer **(Table 7)**. The other sets of criteria factor in different laboratory test results, including a negative result for Sm and high titers of RNP or extractable nuclear antigen antibodies.[90]

In addition to overlapping symptoms of rheumatic disease, the first indication of MCTD is often a high ANA titer, which occurs in 94% to 97% of MCTD patients.[92] This test result should be followed by testing for antibodies to RNP, Sm, SS-A, SS-B, histone, and dsDNA.[93] Over 90% of MCTD patients are positive for antibodies to RNP, while the other antibodies occur less frequently (<20% of patients).[92,94] Antibodies to dsDNA, Sm, and SS-A may be present transiently in MCTD, but consistent presence of these antibodies may indicate SLE.[93]

Spondyloarthropathies

Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial SpA and peripheral SpA[41,42] were developed to diagnose nonradiographic and early stage SpA and thus can aid in diagnosing psoriatic and reactive arthritis. These criteria include laboratory testing for CRP and HLA-B27.[42]

CRP levels assist with classification of axial SpA. Elevated levels are consistent with axial SpA in the presence of other criteria. However, patients with negative results can still meet the criteria for SpA.

Testing for human leukocyte antigen (HLA)-B27 can help identify individuals with SpA.[41,42] A positive HLA-B27 result is consistent with any type of SpA (including reactive arthritis and psoriatic arthritis) or JIA.

In some populations, almost 75% of patients with nonradiographic axial SpA (lacking in radiographically defined sacroiliitis) have HLA-B27.[95] However, not every HLA-B27-positive person develops SpA; one study found that less than 14% of people with HLA-B27 have the disorder.[76] Thus, HLA-B27 testing is not diagnostic by itself and must be used in combination with other clinical and radiographic criteria.

HLA-B27 testing is included in the radiographic (imaging) and clinical arms of the axial SpA criteria **(Table 8)**; these criteria have a sensitivity of 82.9% and a specificity of 84.4%.[42] HLA-B27 testing is also included in peripheral SpA classification criteria, which have a sensitivity of 77.8% and a specificity of 82.2%.[41]

HLA-B27 status can also be determined by molecular methods. ASAS guidelines do not differentiate between the methods, possibly because results are almost always concordant. In a study of 300 Polish patients with suspected spondyloarthropathy, results for 297 patients (99%; 73 positive, 224 negative) were concordant between assays.[96] Discordant results may have been caused by cross-reactivity with other HLA-B antigens (eg, HLA-B07 or HLA-B40), which has been observed in other studies.[97] Thus, a molecular test may be slightly more reliable. Quest Diagnostics offers an antigen test and a molecular test for the detection of HLA-B27 **(Appendix)**.

Juvenile Idiopathic Arthritis

JIA should be considered when arthritis begins at <16 years of age, persists for ≥6 weeks, and has unknown etiology.[48] Although diagnosis of JIA is primarily clinical, laboratory testing to distinguish between the forms of JIA is recommended **(Table 9)**.[48]

Table 7. Mixed Connective Tissue Disease Diagnostic Criteria[90,a]

Kasukawa Criteria	Alarcón-Segovia Criteria
Diagnose MCTD if: 1. RNP antibody test is positive *and* 2. ≥1 common symptom is present *and* 3. ≥1 mixed symptom in ≥2 disease categories *Common Symptoms* 1. Raynaud phenomenon 2. Swollen fingers or hands *Mixed Symptoms* 1. SLE-like symptoms (polyarthritis, lymphadenopathy, facial erythema, pericarditis or pleuritis, leukothrombocytopenia) 2. SSc-like findings (sclerodatyly, pulmonary fibrosis, restrictive changes of lung, reduced diffusion capacity, hypomotility or dilation of esophagus) 3. PM-like findings (muscle weakness, elevated serum levels of muscle enzymes [creatinine phosphokinase], myogenic pattern on electromyogram)	**Diagnose MCTD if:** 1. RNP antibody titer >1:1,600 *and* 2. ≥3 clinical criteria present, including synovitis or myositis *Clinical Criteria* 1. Edema in hands 2. Synovitis 3. Myositis 4. Raynaud phenomenon 5. Acrosclerosis

MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SSc, systemic scleroderma; PM, polymyositis.
[a] Two of 4 existing MCTD criteria are shown here; they were selected because of their higher reported sensitivity and specificity (though the Kahn criteria perform similarly to Alarcón-Segovia criteria).[91,92]

Table 8. ASAS Classification Criteria for Spondyloarthritis

Axial SpA[42]		Peripheral SpA[41]
Patients with back pain for ≥3 months who are <45 years at onset and meet criteria in clinical or imaging arm		Patients with peripheral manifestations only
Clinical Arm HLA-B27 *and* ≥2 other SpA features from footnote a	**Imaging Arm** Sacroiliitis on imaging *and* ≥1 SpA feature from footnote a	Arthritis, enthesitis, or dactylitis *and* ≥1 SpA feature from footnote b *or* ≥2 other SpA features from footnote c

[a] HLA-B27, inflammatory back pain, arthritis, enthesitis, uveitis, dactylitis, psoriasis, Crohn's or ulcerative colitis, response to NSAIDs, family history of SpA, elevated CRP levels.
[b] HLA-B27, uveitis, psoriasis, Crohn's or ulcerative colitis, preceding infection, sacroiliitis on imaging.
[c] Arthritis, enthesitis, dactylitis, inflammatory back pain, family history of SpA.

Table 9. JIA Classification Criteria[48]

General		
1. Arthritis begins before 16 years of age		
2. Arthritis persists ≥6 weeks		
3. Other potential causes of arthritis are excluded		
JIA Form	**Inclusion Criteria**	**Exclusion Criteria**
Systemic arthritis	1. Arthritis in ≥1 joint *and* 2. Fever for ≥2 weeks that is daily for ≥3 days *and* 3. ≥1 of the following: 1. Evanescent erythematous rash 2. Generalized adenopathy 3. Hepatomegaly, splenomegaly, or both 4. Serositis	See footnotes a, b, c, and d.
Oligoarthritis	*Persistent form:* Arthritis in 1 to 4 joints during first 6 months of disease *and* in ≤4 joints during disease course *Extended form:* Arthritis in 1 to 4 joints during first 6 months of disease *and* in >4 joints after first 6 months	See footnotes a, b, c, d, and e.
Polyarthritis (RF negative)	Arthritis in ≥5 joints during first 6 months of disease *and* negative results in RF test	See footnotes a, b, c, d, and e.
Polyarthritis (RF positive)	Arthritis in ≥5 joints during first 6 months of disease *and* positive results in ≥2 RF tests run ≥3 months apart during first 6 months	See footnotes a, b, c, and e.
Psoriatic arthritis	1. Arthritis and psoriasis or 2. Arthritis *and* ≥2 of the following: 1. Dactylitis 2. Nail pitting or onycholysis 3. 1st degree relative with psoriasis	See footnotes b, c, d, and e.
Enthesitis-related arthritis	1. Arthritis and enthesitis *or* 2. Arthritis or enthesitis *and* ≥2 of the following: 1. Sacroiliac joint tenderness and/or inflammatory lumbosacral pain 2. Positive for HLA-B27 antigen 3. Arthritis onset in male >6 years of age 4. Acute anterior uveitis 5. 1st degree relative with ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with IBD, reactive arthritis, or acute anterior uveitis	See footnotes a, d, and e.
Undifferentiated arthritis	Arthritis that fulfills criteria for none of the above or ≥2 of the above types	NA

IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; NA, not applicable; RF, rheumatoid factor.
[a] Psoriasis in patient or 1st degree relative.
[b] Arthritis beginning after 6th birthday in HLA-B27-positive male.
[c] 1st degree relative with ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with IBD, reactive arthritis syndrome, or acute anterior uveitis.
[d] Positive results in ≥2 RF tests run ≥3 months apart.
[e] Presence of systemic JIA.

Systemic Vasculitis

Laboratory testing aids in diagnosis of autoimmune systemic vasculitis with skin involvement, including the ANCA-associated vasculitides and immune complex vasculitides.

ANCA-associated Vasculitides

Classification criteria for 2 ANCA-associated vasculitis disorders granulomatosis with polyangiitis (GPA, formerly Wegener granulomatosis) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome) include the results of physical, radiographic, and/or biopsy examinations and laboratory testing **(Table 10)**.[98,99] If GPA is suspected, testing for microhematuria can assist with classification. If EGPA is suspected, testing for eosinophilia can assist with classification. In addition, routine laboratory testing can detect anemia, leukocytosis, thrombocytosis, and elevated ESR and CRP levels, which are consistent with an acute-phase response in systemic vasculitis.[100]

Diagnosis should be confirmed via biopsy of the affected tissue when possible; this is true for GPA, EGPA, and another ANCA-associated vasculitis disorder, microscopic polyangiitis (MPA). Classification criteria for MPA are not published, but clinical and histological findings in patients with MPA include fibrinoid necrotizing vasculitis of predominantly small vessels without immune deposits, focal segmental necrotizing glomerulonephritis, pulmonary capillaritis, and neutrophilic infiltration of the alveolar wall.[100]

Differential diagnosis of GPA, EGPA, and MPA can be aided by testing for ANCA.[101] Each disorder is associated with predominance of a specific ANCA type.[102] The ANCA types are revealed by fluorescent patterns obtained in an indirect IF ANCA screen. For example, the cytoplasmic pattern (C-ANCA) is very common in GPA, but not MPA or EGPA. The perinuclear pattern (P-ANCA), on the other hand, is rare in GPA, common in MPA, and moderately common in EGPA cases. The atypical P-ANCA pattern is rare in all 3 of these; it is usually associated with nonvasculitic conditions such as inflammatory bowel disease.[103] The sensitivity and specificity of these markers for the various disorders are summarized in **Table 11**.[104-106]

An international consensus group recommends improving the diagnostic accuracy of the ANCA screen by combining it with immunoassays specific for myeloperoxidase (MPO) and proteinase-3 (PR3) antibodies **(Table 11)**.[102] The P-ANCA pattern predominantly reflects MPO specificity. Similarly, the C-ANCA pattern typically reflects specificity to PR3; however, concordance between C-ANCA and PR3 antibody is not 100% because C-ANCA has multiple targets.

Alternatively, a more recent international consensus statement recommends that high quality MPO- and PR3-immunoassays be used as a primary screening method for GPA and MPA, based on the improved performance of these assays **(Table 11)**.[107] Positive PR3 assay results are highly suggestive of GPA and positive MPO results are highly suggestive of MPA. The group proposes that indirect IF only be used in patients with negative MPO- and PR3-assay results and a high clinical suspicion of disease.[107]

Quest offers ANCA screens, as well as high-quality MPO- and PR3-immunoassays to support both diagnostic approaches (See **Appendix**).

Table 10. Granulomatosis With Polyangiitis and Eosinophilic Granulomatosis With Polyangiitis Classification Criteria

Classify patient as having granulomatosis with polyangiitis if ≥2 of the following are present[98]:	**Classify patient as having eosinophilic granulomatosis with polyangiitis if ≥4 of the following are present[99]:**
1. Oral ulcers or bloody or purulent nasal discharge	1. Asthma
2. Nodules, fixed infiltrates, or cavities on chest radiograph	2. Eosinophilia >10%
3. Microhematuria or red cell casts in urine sediment	3. Mono- or polyneuropathy
4. Granulomatous inflammation in wall of artery or peri- or extra-vascular area	4. Nonfixed pulmonary infiltrates
	5. Paranasal sinus abnormality
	6. Extravascular eosinophils

Table 11. Diagnostic Accuracy of Antibodies for Systemic Vasculitis

Marker(s)	% Sensitivity (Specificity)		
	GPA[106,107,a]	MPA[106,107,a]	EGPA[104]
ANCA	85 (93)	68 (87)	31
C-ANCA	81 (100)	3 (93)	5
C-ANCA+/PR3+	69 (100)	0	1
PR3+	77-81 (98-99)	9-12 (96-99)	
P-ANCA	4 (94)	65 (94)	21
P-ANCA+/MPO+	2 (99)	48 (100)	20
MPO+	5-9 (98-99)	71-88 (96-99)	

ANCA, antineutrophil cytoplasmic antibodies; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.
[a] Sensitivity and specificity based on patients tested for ANCA in a rheumatology clinic.

A positive ANCA screen supports a diagnosis of autoimmune-related systemic vasculitis in a symptomatic patient **(Table 11)**. Positive results are also seen in inflammatory bowel disease (ulcerative colitis) and occasionally in other autoimmune diseases (SLE, RA, autoimmune hepatitis). Exposure to certain drugs (eg, propylthiouracil, hydralazine, methimazole) and infectious agents (eg, HCV) can result in secondary vasculitis and an ANCA-positive screen result.[108,109] A negative ANCA, MPO antibody, and/or PR3 antibody result does not rule out systemic vasculitis.

Owing to limitations in sensitivity and specificity, ANCA, MPO antibody, and PR3 antibody test results should be interpreted carefully in light of clinical and other laboratory data.

Immune Complex Vasculitides

Immune complex vasculitides involving the skin include cryoglobulinemic vasculitis, HUV, anti-C1q vasculitis, and IgA vasculitis (Henoch-Schönlein).[101]

Diagnostic criteria for cryoglobulinemic vasculitis include laboratory testing for cryoglobulins, serum C4, RF, and IgM components **(Table 12)**.[8,110] Because cryoglobulinemic vasculitis is one of the most common HCV-related extrahepatic disorders, testing is included in first-line laboratory screening for patients with long-lasting HCV infections.[111]

Validated diagnostic criteria for HUV do not exist. Schwarz et al have proposed criteria that include laboratory testing for C1q antibodies **(Table 12)**.[112] This disease is very rare, and in the largest study to date (retrospective on 57 patients over 20 years), only half had C1q antibodies.[9] Low C1q levels were measured in 90% of the patients tested, suggesting that the latter is a more sensitive marker of the disease.[9]

Diagnosis of IgA vasculitis relies primarily on clinical presentation with laboratory testing only being used to assess kidney function **(Table 12)**.[11] IgA vasculitis with renal involvement is indicated by proteinuria[11],which is defined by (a) >300 mg urine albumin per 24 hours; or (b) >30 mg urine albumin per mmol creatinine (>300 µg urine albumin per mg creatinine by microalbumin testing) on a random morning urine sample.[113]

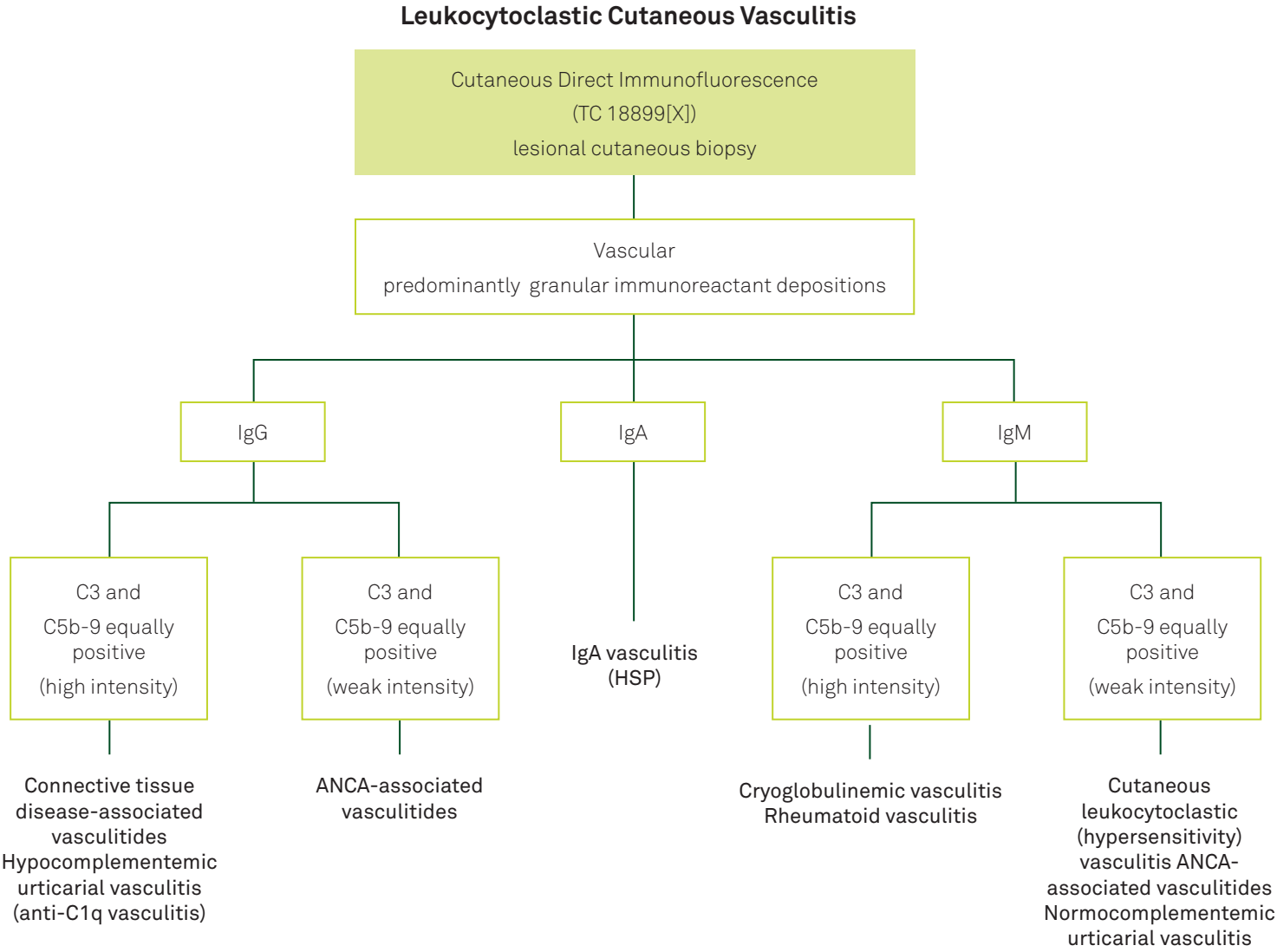
In addition, direct IF on lesional cutaneous biopsy specimens may help differentiate various forms of systemic vasculitis–from each other and from predominantly nonsystemic forms, such as cutaneous leukocytoclastic (hypersensitivity) vasculitis **(Figure 4)**. [114-117]

Table 12. Cryoglobulinemic, HUV, and IgA Vasculitis Classification Criteria[8,110]

Cryoglobulinemic vasculitis	HUV, anti-C1q vasculitis	IgA vasculitis
• Serum cryoglobulins • ≥2 of the following[8]: – History of purpura, particularly on lower limbs – History of purpura, leaving brown spots after disappearing – A diagnosis of viral hepatitis • ≥3 of the following: – Purpura – Fatigue or fever – Joint pain – Neuropathy • ≥2 of the following: – Low serum C4 – Positive RF – Presence of serum IgM components	• Chronic urticaria • Low complement levels • ≥2 of the following[112]: – Leukocytoclastic vasculitis – Evidence of systemic disease (eg, joint pain, ocular inflammation, glomerulonephritis, abdominal pain) – C1q antibody positivity	• Purpura or petechiae with lower limb predominance • ≥1 of the following[11]: – Leukocytoclastic vasculitis (with predominant IgA deposit) – Joint pain – Abdominal pain – Kidney involvement

HUV, Hypocomplementemic urticarial vasculitis; RF, rheumatoid factor.

Figure 4. Direct Immunofluorescence in the Differential Diagnosis of Vasculitis



ANCA, antineutrophil cytoplasmic antibody; HSP, Henoch-Schönlein purpura.

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Skin Diseases		
Psoriasis (see also Monitoring Therapy)		
6399	CBC (includes Differential and Platelets)	Baseline screening prior to methotrexate therapy
7943(X)	Creatinine Clearance	Baseline renal function testing prior to methotrexate therapy
10256	Hepatic Function Panel Includes alkaline phosphatase (234); ALT (823); AST (822); albumin (223); globulin (calculated); albumin to globulin ratio (calculated); total protein (754); and total (287), direct (285), and indirect (calculated) bilirubin.	Baseline and on-going liver function testing for guiding methotrexate therapy
4848	Hepatitis B Core Antibody (IgM)	Baseline screening for HBV prior to methotrexate therapy
498	Hepatitis B Surface Antigen with Reflex to Confirmation	Baseline screening for HBV prior to methotrexate therapy
8472	Hepatitis C Antibody with Reflex to HCV RNA, Quantitative Real-Time PCR	Baseline screening for HCV prior to methotrexate therapy
91431	HIV-1/2 Antigen and Antibodies, Fourth Generation, with Reflexes	Baseline screening for HIV prior to methotrexate therapy
Chronic Urticaria (see also Monitoring Therapy)		
16440	Chronic Urticaria Panel Includes histamine release, thyroid peroxidase and thyroglobulin antibodies, and TSH.	Diagnose chronic urticaria associated with thyroid disease
90123[a]	Chronic Urticaria Panel 2 (Comprehensive) Includes histamine release, thyroid peroxidase antibody, thyroglobulin antibody, TSH, and IgE antibody (anti-IgE IgG).	Diagnose chronic urticaria associated with thyroid disease and anti-IgE IgG
90139[a]	Chronic Urticaria Panel 3 Includes IgE antibody (anti-IgE IgG) and histamine release.	Diagnose chronic urticaria associated with anti-IgE IgG
6399	CBC (Includes Differential and Platelets)	Support a diagnosis of chronic urticaria
4420	C-Reactive Protein (CRP)	Support a diagnosis of chronic urticaria
809	Sed Rate by Modified Westergren	Support a diagnosis of chronic urticaria
10256	Hepatic Function Panel Includes alkaline phosphatase (234); ALT (823); AST (822); albumin (223); globulin (calculated); albumin to globulin ratio (calculated); total protein (754); and total (287), direct (285), and indirect (calculated) bilirubin.	Baseline and on-going liver function testing for guiding methotrexate therapy
16838[a]	Histamine Release (Chronic Urticaria)	Diagnose chronic urticaria
18877[a]	IgE Antibody (Anti-IgE IgG)	Diagnose chronic urticaria associated with anti-IgE IgG

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Skin Diseases		
Vitiligo		
899	TSH	Assist in diagnosis of vitiligo related to autoimmune thyroid disease.
15102	Thyroid Cascading Reflex Includes TSH and reflexes. If TSH is abnormal, reflexes to free T4. If TSH is elevated and free T4 is normal or low, reflexes to TPO antibody. If TSH is low and free T4 is normal or low, reflexes to free T3.	Assist in diagnosis of vitiligo related to autoimmune thyroid disease.
5081	Thyroid Peroxidase Antibodies (TPO)	Assist in diagnosis of vitiligo related to autoimmune thyroid disease.
Autoimmune Blistering Disease (see also Monitoring Therapy)		
16034	Bullous Pemphigoid Antigen (BP 180) Antibody	Assist in diagnosis of BP. Monitor disease activity.
16136	Bullous Pemphigoid BP230 IgG	Assist in diagnosis of pemphigoid and its variants Assist in monitoring disease activity
6399	CBC (includes Differential and Platelets)	Baseline screening prior to corticosteroid and immunosuppressive therapy
19955	Celiac Disease Comprehensive Panel Includes tissue transglutaminase antibody (IgA) (8821) with reflex(es) to endomysial antibody screen (IgA) and endomysial antibody titer (15064); also includes serum IgA (539) with reflex to tissue transglutaminase antibody (IgG) (11070).	Diagnose dermatitis herpetiformis
10231	Comprehensive Metabolic Panel Includes albumin (223), albumin to globulin ratio (calculated), alkaline phosphatase (234[X]), ALT (823), AST (822), BUN/creatinine ratio (296), calcium (303), carbon dioxide (310), chloride (330), creatinine (375[X]), eGFR (calculated), globulin (calculated), glucose (483[X]), potassium (733), sodium (836), total bilirubin (287), and total protein (754).	Baseline screening for liver and kidney function prior to corticosteroid and immunosuppressive therapy
375(X)	Creatinine	Baseline screening prior to corticosteroid and immunosuppressive therapy
18899(X)	Cutaneous Direct Immunofluorescence	Assist in diagnosis of autoimmune blistering disease
16033(X)	Desmoglein Antibodies (1 and 3)	Assist in diagnosis of pemphigus and its variants. Monitor disease activity
37097	Epidermal Antibodies with Reflex To Titers Includes intercellular substance antibody, basement membrane zone antibody, and reflex(es) to titer.	Assist in diagnosis. Titers correlate with disease activity in some diseases.
482(X)	Gamma Glutamyl Transferase (GGT)	Baseline screening for liver disease prior to corticosteroid and immunosuppressive therapy

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Skin Diseases		
Autoimmune Blistering Disease (see also Monitoring Therapy)		
500	Glucose-6-Phosphate Dehydrogenase, Quantitative	Baseline screening prior to dapsone therapy: unsafe patient has glucose-6-phosphate dehydrogenase deficiency
8396	hCG, Total, Quantitative	Pregnancy test prior to treatment
4848	Hepatitis B Core Antibody (IgM)	Baseline screening for HBV prior to methotrexate therapy
498	Hepatitis B Surface Antigen with Reflex to Confirmation	Baseline screening for HBV prior to methotrexate therapy
8472	Hepatitis C Antibody with Reflex to HCV RNA, Quantitative Real-Time PCR	Baseline screening for HCV prior to methotrexate therapy
91431	HIV-1/2 Antigen and Antibodies, Fourth Generation, with Reflexes	Baseline screening for HIV prior to methotrexate therapy
539	IgA	Baseline screening for IgA deficiency prior to intravenous immunoglobulin treatment
16690	IgG, IgA, Indirect Immunofluorescence	Assist in diagnosis of autoimmune blistering disease.
Autoimmune Rheumatic Diseases Involving the Skin		
Systemic lupus erythematosus (SLE)		
8561	Absolute Lymphocyte Count	Detect lymphopenia
90072	ANA Screen, IFA, with Reflex to Titer and Pattern (Lupus Panel 1) Includes ANA screen (IFA) with reflex to titer and pattern; also includes chromatin (nucleosomal), dsDNA, and Sm antibodies.	Diagnose SLE
29839	ANA Screen, IFA, with Reflex to Titer and Pattern (Lupus Panel 2) Includes ANA screen (IFA) with reflex to titer and pattern; also includes dsDNA, scleroderma (Scl-70), Sm, Sm/RNP, SS-A, and SS-B antibodies.	Diagnose SLE
19881(X)	ANA Screen, IFA, with Reflex to Titer and Pattern (Lupus Panel 3) Includes ANA screen (IFA) with reflex to titer and pattern; chromatin (nucleosomal), dsDNA, RNP, Sm, SS-A, and SS-B antibodies; complement components C3 and C4; and total complement (CH50).	Diagnose SLE
10716	ANA Screen, IFA, with Reflex to Titer and Pattern (Lupus Panel 4) Includes ANA screen (IFA) with reflex to titer and pattern; dsDNA, rheumatoid factor, ribosomal P, Scl-70, Sm, Sm/RNP, SS-A, SS-B, and thyroid peroxidase antibodies; and complement components C3 and C4.	Diagnose SLE

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Rheumatic Diseases Involving the Skin		
Systemic lupus erythematosus (SLE)		
37491(X)	ANA Screen, IFA, with Reflex to Titer and Pattern (Lupus Panel 5) Includes ANA screen (IFA) with reflex to titer and pattern; actin (IgG), gastric parietal cell, rheumatoid factor, ribosomal P, Scl-70, Sm, Sm/RNP, SS-A, SS-B, and thyroid peroxidase antibodies; dsDNA (Crithidia), mitochondrial, myocardial, reticulin, and striated muscle antibody screens with reflex to titers; and C3 and C4 complement components.	Diagnose SLE
30340(X)	Beta-2-Glycoprotein I Antibodies (IgG, IgA, IgM)	Diagnose SLE
7352	Cardiolipin Antibodies (IgA, IgG, IgM)	Diagnose SLE
34088	Chromatin (Nucleosomal) Antibody	Diagnose SLE
37859(X)	Complement Component C3, C4, CH50	Diagnose SLE
18899(X)	Cutaneous Direct Immunofluorescence	Assist in diagnosis of various forms of lupus, as well as other photosensitive rheumatic diseases
361	Direct Antiglobulin Test (DAT)	Determine presence of autoimmune hemolytic anemia
255	DNA (ds) Antibody	Diagnose SLE
427	Erythropoietin	Determine presence of hemolytic anemia
19654	Lupus Anticoagulant and Antiphospholipid Confirmation (non-Coumadin) with Consultation Includes beta2-glycoprotein I antibodies (IgG, IgM), cardiolipin antibodies (IgG, IgM), prolonged aPTT thrombotic evaluation, and coagulation consultation.	Diagnose SLE
17725(X)	Lupus Activity Panel 2 Includes complement component C3 and C4 and high avidity dsDNA antibody.	Diagnose SLE
91740[a]	Platelet Antibody, Direct (IgG)	Detect autoimmune thrombocytopenia
34283	Ribosomal P Antibody	Diagnose neuropsychiatric SLE
19887	RNP Antibody	Diagnose SLE or MCTD
38567	Sm/RNP Antibody	Diagnose SLE or MCTD
37923	Sm Antibody	Diagnose SLE
937	White Blood Cell Count (WBC)	Determine presence of leukopenia
Systemic sclerosis		
90073	ANA Screen, IFA with Reflex to Titer and Pattern (Systemic Sclerosis Panel 1) Includes ANA screen (IFA) with reflex to titer and pattern; also includes centromere B, RNA polymerase III, and Scl-70 antibodies.	Diagnose systemic sclerosis
94646[b]	Anti-PM/Scl-100 Antibody, EIA	Determine prognosis

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Rheumatic Diseases Involving the Skin		
Systemic sclerosis (continued)		
16088	Centromere B Antibody	Diagnose limited cutaneous systemic sclerosis (CREST)
91292[b]	Fibrillarin (U3 RNP)	Diagnose SSc; determine prognosis
18855	Ku Autoantibodies	Predict muscle and joint involvement and digital vasculopathy-related complications
19899(X)	RNA Polymerase III Antibody	Diagnose systemic sclerosis
4942	Scleroderma Antibody (Scl-70)	Diagnose systemic sclerosis
94685[a]	Systemic Sclerosis 12 Antibodies Panel 2 Includes centromere protein (CENP)-A, CENP-B, PM-Scl100, PM-Scl75, RP11 (RNA polymerase III), RP155 (RNA polymerase III), Scl-70, Th/To, U1 snRNP RNP70k, U1 snRNP RNP A, U1 snRNP RNP C, and U3-snRNP (fibrillarin) antibodies.	Diagnose systemic sclerosis
Dermatomyositis (DM)		
227	Aldolase	Diagnose DM
94646	Anti-PM/Scl-100 Antibody, EIA	Assist in diagnosis of myositis, polymyositis, and dermatomyositis or overlap syndromes
374	Creatine Kinase (CK), Total	Diagnose DM
18899(X)	Cutaneous Direct Immunofluorescence	Differentiate DM from some lupus phenotypes
90998	EJ Autoantibodies	Diagnose DM
5810(X)	Jo-1 Antibody	Diagnose DM
593	Lactate Dehydrogenase (LD)	Diagnose DM
17172	Mi-2 Autoantibodies	Diagnose DM
90995[a]	Myositis AssessR™ Includes autoantibodies for PL-7, PL-12, Mi-2, Ku, EJ, OJ, and SRP	Diagnose DM
10185(X)[a]	Myositis AssessR™ plus Jo-1 Antibodies Includes autoantibodies for PL-7, PL-12, Mi-2, Ku, EJ, OJ, SRP, and Jo-1	Diagnose DM
94777[a]	Myositis Specific 11 Antibodies Panel Includes autoantibodies for EJ, Jo-1, MDA-5, Mi-2α, Mi-2β, NXP-2, OJ, PL-7, PL-12, SRP, and TIF1-γ	Diagnose DM
90999	OJ Autoantibodies	Diagnose DM
90996	PL-7 Autoantibodies	Diagnose DM
90997	PL-12 Autoantibodies	Diagnose DM
16318	SRP Autoantibodies	Diagnose DM, determine prognosis

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Rheumatic Diseases Involving the Skin		
Mixed Connective Tissue Disease		
19875	ANA Screen, IFA, with Reflex to Titer and Pattern (Mixed Connective Panel 1) Includes ANA screen (IFA) with a reflex to titer and pattern and RNP antibody.	Diagnose MCTD
90074	ANA Screen, IFA, with Reflex to Titer and Pattern (Mixed Connective Panel 2) Includes ANA screen (IFA) with reflexes to titer and pattern; dsDNA, RNP, and Scl-70 antibodies.	Diagnose MCTD
18899(X)	Cutaneous Direct Immunofluorescence	Differentiate MCTD from some lupus phenotypes
19887	RNP Antibody	Diagnose SLE or MCTD
38567	Sm/RNP Antibody	Diagnose SLE or MCTD
Spondyloarthropathies (SpA; Reactive and Psoriatic Arthritis)/Juvenile Idiopathic Arthritis (see also Monitoring Therapy)		
4420	C-Reactive Protein (CRP)	Diagnose axial spondyloarthritis and assess disease activity
528	HLA-B27 Antigen	Diagnose SpA
15584	HLA-B27 DNA Typing	Diagnose SpA
Systemic Vasculitides		
70159	ANCA Screen with MPO and PR3, with Reflex to ANCA Titer	Differentiate types of systemic vasculitis
36733	ANCA Vasculitides Includes proteinase-3 and myeloperoxidase antibodies.	Diagnose ANCA-associated vasculitides
981[a]	Complement Component C1q	Assist in diagnosis of HUV
37859(X)	Complement Component C3, C4, CH50	Assist in diagnosis of HUV
353	Complement Component C4c	Assist in diagnosis of cryoglobulinemic vasculitis
4420	C-Reactive Protein (CRP)	Identify inflammatory conditions
375(X)	Creatinine	Assess renal function
36562(X)[a]	Cryoglobulin (% Cryocrit), Serum	Assist in diagnosis of cryoglobulinemia/ cryoglobulinemic vasculitis
37358[a]	Cryoglobulin Screen with Reflex to Cryoglobulin Profile, Serum Cryoglobulin profile includes % cryoglobulin (cryocrit), cryoglobulin immunofixation and immunodiffusion, and rheumatoid factor.	Assist in diagnosis of cryoglobulinemia/ cryoglobulinemic vasculitis
18899(X)	Cutaneous Direct Immunofluorescence	Assist in diagnosis of various vasculitides
425	Eosinophil Count, Blood	Assess eosinophilia
427	Erythropoietin	Determine presence of hemolytic anemia

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APPENDIX: TESTS FOR DIAGNOSIS AND MONITORING THERAPY

Test Code	Test Name	Clinical Use
Autoimmune Rheumatic Diseases Involving the Skin		
Systemic Vasculitides (continued)		
36735	Immune Complex Detection by C1q Binding	Assist in diagnosis of HUV
16690	IgG, IgA, Indirect Immunofluorescence	Assist in diagnosis of vasculitides
6517	Microalbumin, Random Urine with Creatinine	Assess renal impairment in IgA vasculitis
8796	Myeloperoxidase Antibody (MPO)	Diagnose ANCA-associated vasculitides
10671[c]	Pan-ANCA Plus with Reflex Includes an ANA screen (IFA) with reflex to titer and pattern; also includes ANCA total antibody and pattern, bactericidal/permeability increasing protein (BPI) IgG, myeloperoxidase antibody, and proteinase-3 antibody.	Differentiate types of systemic vasculitis
723	Platelet Count, EDTA	Assess thrombocytopenia
34151	Proteinase-3 Antibody	Diagnose ANCA-associated vasculitides
11320	Protein, Total, 24-Hour Urine without Creatinine	Assess renal impairment in IgA vasculitis
809	Sed Rate by Modified Westergren	Determine disease severity
294	Urea Nitrogen (BUN)	Assess renal function
8563	Urinalysis, Microscopic	Determine presence of microhematuria
937	White Blood Cell Count (WBC)	Assess leukopenia
Monitoring Therapy		
36295[a]	Adalimumab Anti-drug Antibody for Rheumatic Diseases	Monitor response to adalimumab therapy
36297[a]	Adalimumab Level and Anti-drug Antibody for Rheumatic Diseases	Monitor response to adalimumab therapy
36299[a]	Adalimumab Level for Rheumatic Diseases	Monitor response to adalimumab therapy
36302[a]	Infliximab Anti-drug Antibody for Rheumatic Diseases	Monitor response to infliximab therapy
36312[a]	Infliximab Level and Anti-drug Antibody for Rheumatic Diseases	Monitor response to infliximab therapy
36310[a]	Infliximab Level for Rheumatic Diseases	Monitor response to infliximab therapy
648	Methotrexate	Monitor response to folinic acid rescue in case of methotrexate overdose
18831[a]	TPMT Activity	Baseline screening prior to azathioprine therapy: activity determines dosing

[a] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the U.S. Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.

[b] This test was developed and its performance characteristics validated by Rheumatology Diagnostic Laboratory. There is no FDA approved assay for this test. As a lab developed test (LDT), approval or clearance by the FDA is not required. This test may be used for clinical purpose and should not be regarded as investigational or for research.

[c] This test was performed using a kit that has not been cleared or approved by the FDA. The analytical performance characteristics of this test have been determined by Quest Diagnostics. This test should not be used for diagnosis without confirmation by other medically established means.

Panel components may be ordered separately.

Reflex tests are performed at an additional charge and are associated with an additional CPT code.

Multiple test codes are available. Refer to the Quest Diagnostics Directory of Services or the online Test Center (QuestDiagnostics.com) for test information.

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