

# CSP

## Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

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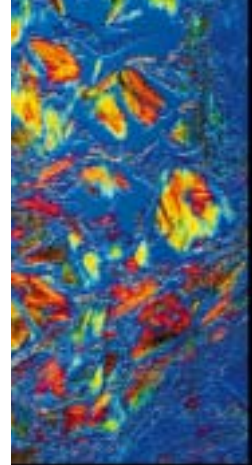
- When to test
- What tests to order
- How to interpret them

## Laboratory investigation of arthritis

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# Laboratory investigation of arthritis

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## Introduction

The laboratory evaluation of arthritis is evolving with the availability of new tests and new methods for old tests. The diagnosis of arthritis requires an understanding of the possible aetiological factors and pathophysiology, as well as knowledge of the laboratory tests available and their correct application.

For most causes of arthritis, there is no single diagnostic test. In general, diagnosis depends on the constellation of clinical, laboratory and radiological features, and investigations must be guided by history and clinical examination. However, septic arthritis and crystal arthritis are exceptions that can be diagnosed by joint aspiration. Septic arthritis is a medical emergency and diagnosis must be made promptly.

The focus of this article is on the role of the pathology laboratory in the investigation of arthritis. Radiological investigations of arthritis are also often required, but an in-depth discussion of these radiological studies is beyond the scope of this article.

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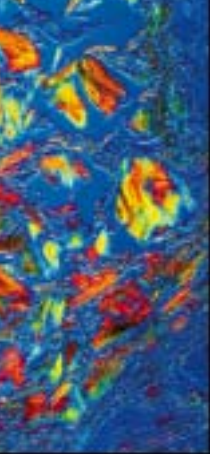
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Cover: Light micrograph of uric acid crystals from a gouty joint.

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### Case 1

A 19-year-old female complains of painful hands, wrists and knees, as well as fatigue and recurrent mouth ulcers. She has developed a scaly, erythematous rash over her face and arms after a beachside holiday. Examination demonstrates warmth, tenderness and swelling of her proximal interphalangeal joints, wrist joints and knee joints bilaterally. A urinalysis shows 1+ of protein. Investigations include a positive ANA (antinuclear antibody) with a titre of 1:2560 homogenous pattern and 1:640 speckled pattern.

#### 1. What is the differential diagnosis?

This presentation is suggestive of a connective tissue disorder, for example, SLE or mixed connective tissue disease.

#### 2. What is the significance of the elevated ANA?

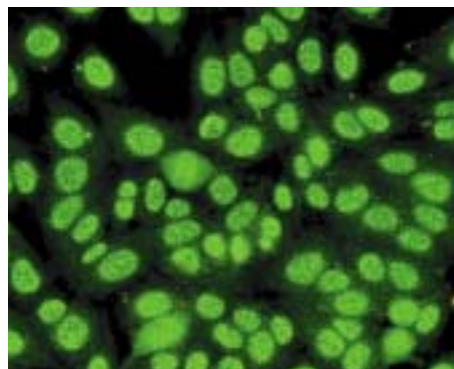
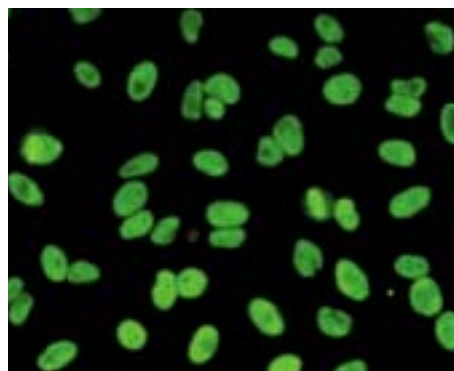
The ANA test is a sensitive, but not specific, test for connective tissue disease (figure 1). An elevated ANA is a common finding in healthy subjects without connective tissue disease, and is more commonly found in females and with increasing age. However, a negative ANA test makes the diagnosis of SLE unlikely. In general, the specificity for disease increases with increasing titre, which is usually reported in increments such as 1:40, 1:160, 1:640, 1:2560 and >1:2560. The pattern of the ANA (eg, homogenous or speckled) reflects the particular nuclear antigen or antigens recognised by the ANA. For example, antibodies to DNA cause a homogenous ANA pattern and antibodies to SSA or SSB cause a speckled ANA pattern. Therefore, the ANA pattern is an approximate indicator of the underlying antibody response.

Further clarification of the ANA result can be obtained by testing for anti-dsDNA (anti-double-stranded DNA antibodies) and anti-ENA (anti-extractable nuclear antigens). There is considerable overlap of measurable antibody responses among the various connective tissue disorders (see table 1, next page). For example, antibodies to SSA are found in Sjogren's syndrome and may also be found in SLE. Nonetheless, the ANA pattern and anti-ENA (if positive) provide a guide to diagnosis, but must be correlated with clinical features for interpretation.

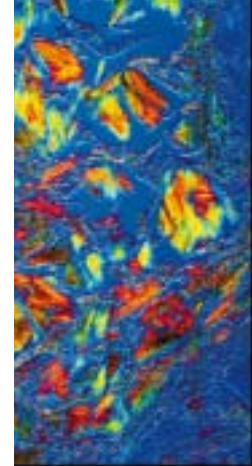
The anti-ENA test measures antibodies to a panel of nuclear antigens, typically SSA (Ro), SSB (La), Scl70, Sm, RNP and Jo-1. Various techniques may be used for anti-ENA testing that may affect the sensitivity and specificity of a positive result.<sup>1</sup> Note that different laboratories also measure anti-dsDNA by different techniques, with different specificities and sensitivities. Discussion with an immunopathologist is recommended if there is uncertainty regarding the significance of a result.

#### 3. What other tests may be indicated?

Other useful tests in the diagnosis and assessment of



Indirect immunofluorescence (IIF) microscopy for anti-nuclear antibody (ANA) testing.  
Top: Homogenous ANA pattern.  
Bottom: Speckled ANA pattern.

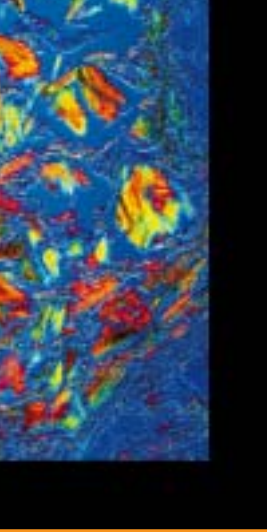


**Table 1: Clinical associations of the most common anti-extractable nuclear antigen (anti-ENA) antibodies (modified from Phan TG, et al 2002).**

Anti-ENA	Major clinical associations	Technique used for anti-ENA testing				Comments
		CIEP		ELISA		
		Se (%) <sup>*</sup>	Sp (%) <sup>*</sup>	Se (%)	Sp (%)	
SS-A/Ro	Sjogren's syndrome SLE Neonatal lupus syndromes C2/C4 deficiency	85-95 25-30	50-60 50-60	90-97 35-60	45-50 45-50	Anti-SSA/Ro antibodies are the most commonly detected anti-ENA antibody. There is some controversy as to whether anti-SSA antibodies may be detected in otherwise healthy people.
SS-B/La	Sjogren's syndrome SLE Neonatal lupus syndromes	70-80 10-15	60-70 50-55	75-85 20-30	50-60 45-50	Anti-SSB/La antibodies are generally only found along with anti-SSA/Ro antibodies. Thus an isolated anti-SSB/La result should be interpreted with caution.
Sm	SLE	30-35	98-100	35-50	55-99	Anti-Sm antibodies are considered to be highly specific for SLE, but only occur in about one-third of cases. They are more likely to be detected in subjects of Afro-Caribbean or Asian ethnic backgrounds, in whom they are associated with an increased risk of developing more severe disease (including renal lupus).
U1-RNP	MCTD, SLE	90-95 15-35	60-75 55-75	95-98 50-60	50-60 50-55	While anti-U1-RNP antibodies are classically associated with MCTD, they can also be found in individuals with SLE (often in association with anti-Sm antibodies).
Scl-70	Scleroderma	25-35	95-99	30-45	80-90	In patients with scleroderma, anti-Scl-70 antibodies are associated with an increased risk of developing internal organ involvement (including lung and renal disease) and extensive cutaneous disease. However, anti-Scl antibodies are occasionally detected in the absence of scleroderma.
Jo-1	Polymyositis/ dermatomyositis	25-40	95-99	35-45	90-95	Anti-Jo-1 antibodies are associated with an increased risk of developing interstitial lung disease. Because Jo-1 is only found in the cytoplasm of cells, the ANA may be reported as negative.

<sup>\*</sup> Se = sensitivity and Sp = specificity for the associated clinical conditions. CIEP = counter-current immunoelectrophoresis; ELISA = enzyme linked immunosorbent assay; MCTD = mixed connective tissue disease; SLE = systemic lupus erythematosus; SS = Sjogren's syndrome.

arthritis related to the connective tissue diseases include the complement components C3 and C4, ESR, CRP and rheumatoid factor. C3 and C4 are consumed in active SLE as a result of activation of the classical complement pathway by immune complexes. Thus, low C3 and C4 levels may be an indicator of activity in SLE, although normal or high complement levels may also be found because complement



proteins are also produced as part of the acute-phase response. Similarly, elevated ESR and CRP, which track the acute phase response, are often indices of disease activity. However, in contrast to most other systemic inflammatory disorders, the CRP may be paradoxically low or normal in some cases of active SLE.

### Case 2

A 19-year-old man presents three weeks after resolution of a diarrhoeal illness with conjunctivitis, a penile rash and swollen, painful knee joints bilaterally. Stool cultures from the time of the illness had grown *Campylobacter jejuni*.

#### 1. What is the likely diagnosis?

The history is suggestive of Reiter's syndrome (arthritis, conjunctivitis, and balanitis — penile kerato-derma blenorrhagicum) in the context of a reactive arthritis.

#### 2. What is the role of HLA-B27 testing?

The frequency of HLA-B27 is about 90% in ankylosing spondylitis and 50-90% in other reactive arthritides, but is also present in about 10% of the healthy Caucasian population and thus should not be considered diagnostic. The diagnostic utility of HLA-B27 testing depends on the pre-test probability of the patient having the disease. When the pre-test probability is high (ie, when Reiter's syndrome is likely on clinical grounds, such as in the case above), a positive or negative HLA-B27 result will not significantly



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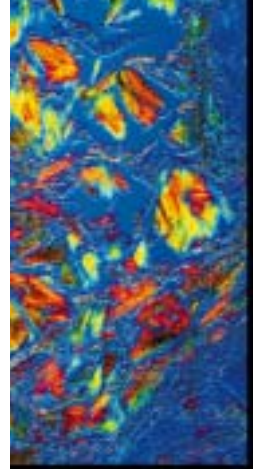


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change the likelihood of the diagnosis. Similarly, if the pre-test probability is low, a positive HLA-B27 result should not be relied on alone to diagnose the presence of Reiter's syndrome or ankylosing spondylitis. Therefore, the HLA-B27 test in the diagnosis of Reiter's syndrome is most useful when the pre-test probability is intermediate; that is, the diagnosis of Reiter's syndrome is uncertain, but possible on clinical grounds.

HLA-B27 assessment also has prognostic value. In the setting of a reactive arthritis, a positive HLA-B27 increases the likelihood of progression to a chronic spondyloarthritis, rather than resolution.

### Case 3

A 42-year-old female describes a 12-month history of joint pains in her hands and wrists. She notices stiffness of these joints, particularly in the mornings, and episodic swellings of the affected areas. Examination confirms tenderness of the wrist and proximal interphalangeal joints, but no other abnormal findings. Radiographs of the hands and wrists demonstrate periarticular osteopaenia, but no joint erosions.

#### 1. What is the differential diagnosis?

The patient has a chronic, symmetrical, small-joint polyarthritis. Conditions to consider include rheumatoid arthritis, psoriatic arthritis and viral arthritis, although the prolonged symptom duration makes the latter less likely.

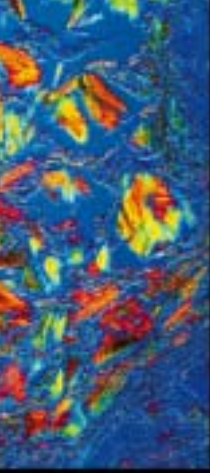
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## 2. Which tests may be helpful?

The diagnostic workup should include ANA and rheumatoid factor estimation. The ANA test is frequently positive in rheumatoid arthritis, but typically the anti-ENA and anti-dsDNA tests will be negative. The rheumatoid factor test is positive in 50-75% of patients with rheumatoid arthritis. A rheumatoid factor is an antibody directed against IgG. This antibody is predominantly of IgM isotype, but the measurement of IgA isotype rheumatoid factors has also been described.

The rheumatoid factor test also appears to have prognostic value in patients with rheumatoid arthritis. A strongly positive rheumatoid factor has been consistently reported to be associated with an increased risk of developing joint erosions and more aggressive disease. Some reports indicate that the presence of a positive IgA rheumatoid factor may predict an earlier onset of erosive joint disease, but these findings need to be investigated further.<sup>3</sup> Notably, rheumatoid factors can be detected in other conditions such as Sjogren's syndrome, cryoglobulinaemia and various other inflammatory and infectious diseases. While the level of the rheumatoid factors in these conditions is generally low, in Sjogren's syndrome they may be high. Therefore, a positive rheumatoid factor result should not be used in isolation to diagnose rheumatoid arthritis.

## 3. What is the role of anti-CCP (cyclic-citrullinated peptide) measurement?

Anti-CCP is a new ELISA-based test used in the diagnosis of rheumatoid arthritis. However, the literature is equivocal regarding its additional benefit to standard rheumatoid factor estimation. It may be useful in the diagnosis of a subset of patients who are rheumatoid factor negative.<sup>4, 5, 6</sup>

### Case 4

A 26-year-old, previously well woman complains of six days of generalised arthralgia associated with fatigue, myalgia and morning stiffness. The predominant joints involved include wrists, fingers, knees and ankles. She is now unable to work because of joint pain. She has not travelled anywhere outside her local area recently.

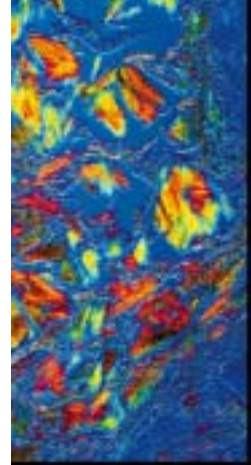
On examination, she has no evidence of lymphadenopathy, but has a temperature of 37.8°C and a fine macular rash over her torso and upper limbs. There is joint swelling over the metacarpals, but no joint effusions elsewhere. There is pain on moving the wrists, knees and ankles through their full range of motion.

## 1. What is the differential diagnosis?

Viral polyarthrititis is the most likely diagnosis. Many viral infections are associated with joint symptoms and low-grade fevers with a rash are features that suggest this diagnosis. Viruses that commonly cause significant joint symptoms are Ross River virus, Barmah Forest virus, parvovirus B19, and hepatitis B and C viruses. Many other viruses have been associated with arthritis, including cytomegalovirus, HIV, Epstein-Barr virus and rubella. The latter has been associated with post-vaccination disease. A history of exposure to people with similar illness can sometimes be obtained in cases of viral polyarthrititis.

Without a history of immunosuppression, multifocal septic arthritis from bacterial infection is unlikely to cause the above clinical presentation. However, in at-risk populations, brucella and *Coxiella burnetii* (Q fever) should be considered.

Primary polyarthrititis such as RA and SLE are also possible diagnoses, but these are far less likely in view of the brief, six-week duration. Testing for these conditions in the presence of a low likelihood of disease, such as in this case, would not be indicated.



## 2. What tests should be performed?

The short symptom duration indicates that a limited set of initial investigations is appropriate. Ross River<sup>8</sup> as well as Barmah Forest virus serology may be indicated, depending on where the patient lives.<sup>9</sup> If the symptoms develop in late winter and spring, or there is a history of exposure to children with viral infections, parvovirus B19 serology should be considered. An ESR and CRP will not reliably discern viral polyarthrititis from other causes of arthritis.

In most viral infections, specific IgM antibodies against the virus become detectable in the first week of the clinical illness. Serological diagnosis also requires demonstration of seroconversion from an IgG negative status to an IgG positive status. For some viral infections, specific IgM antibodies may persist for up to six months without being indicative of chronic viral infection. In contrast, the combination of negative-specific antiviral IgM, but positive-specific antiviral IgG is generally indicative of previous exposure to the virus and makes new infection unlikely. If risk factors for hepatitis B, hepatitis C, or HIV are present, tests for these viruses should be performed. However, these tests can be initially falsely negative if performed in the early stages of seroconversion illness, and follow-up repeat testing may be required.

### Case 5

A 65-year-old man attends because of a two-day history of a painful left knee. The knee became swollen, hot and red overnight and the pain has increased to the point that he is having trouble walking. His history is significant for type 2 diabetes, hypertension and obesity. He gives a history of being treated for gout in his right big toe a few years previously. On examination, his left knee is erythematous and tender to touch, and there is a large effusion. His temperature is 37.7° C. The initial differential diagnosis is between infectious and crystal arthritis. The time course favours crystal arthritis, but the fever could be explained by either condition.

### 1. What is the most important diagnostic procedure for this patient?

A joint aspiration.

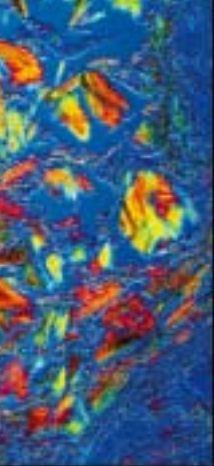
### 2. If a joint aspiration is performed, what tests should be ordered on the synovial fluid?

The specimen should be sent for crystal examination — laboratories will examine for urate crystals and calcium pyrophosphate crystals for gout and pseudogout respectively. Microscopy, culture and sensitivities for bacterial infection should also be requested.

### 3. How should the specimens be sent to the laboratory?

The synovial fluid sample is inoculated into a 5mL EDTA blood tube for the cell count and a yellow-top jar.

Unfortunately, laboratory detection and identification of crystals in synovial fluid may be unreliable<sup>10</sup>, particularly if a laboratory does not perform many synovial fluid examinations. Thus, the lack of crystals need not necessarily exclude crystal arthritis. Furthermore, the possibility of concurrent infectious and crystal arthritis in a joint should always be considered, especially if the patient fails to respond to treatment for crystal disease. Rarer causes of mono-arthritis, including unusual infections (fungae, brucella) or autoimmune disorders may need to be considered if there is a lack of response to initial therapy.



## Summary

The above cases cover the most common scenarios where the pathology laboratory can assist in the assessment, diagnosis and classification of arthritis.

As mentioned above, septic arthritis is the most important diagnosis to consider in patients presenting with mono-arthritis or oligoarthritis. Liaison with a pathologist is often useful to discuss test selection and interpretation, especially if there is clinical suspicion of an underlying connective tissue disease.

## Suggested further reading

*Manual of Use and Interpretation of Pathology Tests*, prepared by the Board of Education. McPherson J. (ed). Royal College of Pathologists of Australasia. 2001.

## References

1. Phan TG, Adelstein S. Autoantibodies to Extractable Nuclear Antigens: Making detection and interpretation more meaningful. *Clinical and Diagnostic Laboratory Immunology* 2002; 9 (1):1-7.
2. IgA Rheumatoid Factor as Predictor of Disease Activity. *Scandinavian Journal of Rheumatology Supplement* 1988; 75:233-37.
3. Vincent C, et al. Detection of Antibodies to Deiminated Recombinant Rat Filaggrin by Enzyme-Linked Immunosorbent Assay. *Arthritis and Rheumatism* 2002; 46 (8):2051-58.
4. Schellekens GA, et al. The Diagnostic Properties of Rheumatoid Arthritis Antibodies Recognising a Cyclic Citrullinated Peptide. *Arthritis and Rheumatism* 2000; 43 (1):155-63.
5. Kroot EJ, et al. The Prognostic Value of Anticyclic Citrullinated Peptide Antibody in Patients with Recent Onset Rheumatoid Arthritis. *Arthritis and Rheumatism* 2000; 43 (8):1831-35.
6. Vittecoq O, Krzanowska K. Rheumatoid Factor is the Strongest Predictor of Radiological Progression of Rheumatoid Arthritis in a Three-Year Prospective Study in Community-Recruited Patients. *Rheumatology* 2003; 42 (8):939-46.
7. Mylonas AD, et al. Natural History of Ross River Virus-Induced Epidemic Polyarthritis. *Medical Journal of Australia* 2002; 177 (7):356-60.
8. Blumer C, et al. Australia's Notifiable Disease Status. *Communicable Diseases Intelligence* 2003; 27 (1):1-78.
9. Gordon C, Dieppe P. Detection of Crystals in Synovial Fluids by Light Microscopy: Sensitivity and Reliability. *Annals of Rheumatoid Diseases* 1989; 48 (9):737-42.