Investigation play an important role in diagnosis of clinical conditions. However indiscriminate use of tests due to lack of knowledge of false positive and false negative test results, predictive value of a test etc can cause more problem than benefit. It is imperative for the clinician to know, when to order a test, how to interpret it and what can be the methodological problems with the test. In this report we have tried to provide a ready reference for office use, for the commonly employed immunological tests, the international units in which they should be reported for inter-lab comparisons and what are the methods employed to detect these tests etc. For more details reader should refer to articles in suggested reading.

**Rheumatoid factor (RF)**

**Methods:** Latex agglutination Turbidimetry/Nephelometry ELISA

**How to report:** It should be reported as IU per ml rather than as end titres

**Pitfalls:** Can be present in normals (5%), prevalence increases with age various chronic infections like TB, other autoimmune disease like Sjogren syndrome, systemic lupus

**Utility:** Presence of RF in a patient with polyarthritis supports a diagnosis of RA

Presence of RF in a patient with rheumatoid arthritis (RA): suggests poorer prognosis

Absence of RF does not exclude a diagnosis of RA

NO NEED TO REPEAT RF IF ONCE POSITIVE IN A PATIENT WITH RA IF NEGATIVE REPEAT AFTER 3-6 MONTHS

**Antinuclear antibodies**

**Methods:** Indirect Immunofluorescence assay, ELISA

**How to report:** It should be reported as positive or negative If done by double diffusion report titre. If done by ELISA report units

**Utility:** Presence of ANA in a patient with polyarthritis or multisystem involvement supports/increases likelihood of diagnosis of connective tissue disease.

Mere presence of ANA does not make a diagnosis of SLE

Since titres do not correlate with disease activity, serial measurements are not indicated

**ANA test is very useful in excluding a diagnosis of SLE if it is negative in a patient suspected to have SLE.**

**Anti dsDNA antibodies**

**Methods:** ELISA, Farr assay, IIF using crithidiae lucillae

**How to report:** It should be reported in IU/ml Give the method used and normal range.

**Pitfalls:** Can be present in chronic hepatitis if done by ELISA

**Utility:** Present in 60% of patients with SLE

Correlates well with activity of lupus nephritis thus serial measurements helpful

NO ROLE IN ANY OTHER RHEUMATIC DISEASE

**Anti ENA antibodies**

**Methods:** Counter immunoelectrophoresis and double diffusion, ELISA, Immunoblotting

**How to report:** Usually interpreted as positive or negative If done by double diffusion report titre. If done by ELISA report units

**Utility:** % prevalence in different autoimmune diseases

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Pattern on IIF</th>
<th>SLE</th>
<th>IIM</th>
<th>SS</th>
<th>SSc</th>
<th>MCTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm</td>
<td>Speckled</td>
<td>30-50</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>U1RNP</td>
<td>Speckled</td>
<td>40</td>
<td>&lt;5</td>
<td>5</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>Speckled</td>
<td>30</td>
<td>&lt;5</td>
<td>50</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>Speckled</td>
<td>10</td>
<td>&lt;5</td>
<td>50</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Scl-70</td>
<td>Fine</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>40-60</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

IIM - Inflammatorymyositis, SS - Sjogren's Syndrome, SSC Systemic sclerosis, MCTD - Mixed connective tissue diseases

Should be done only if there is ANA positivity, anti-Ro is a notable exception

Indications for anti-Ro testing: Mother of baby with congenital heart block, ANA negative lupus, Subacute cutaneous lupus, Sjogren’s syndrome
Anti-neutrophil cytoplasmic antibodies
Methods: IIF using human neutrophils ELISA
How to report: It should be reported as positive with end titre dilution and the pattern seen on IIF. The three patterns are Cytoplasmic Perinuclear Atypical
All samples positive on IIF should be tested on ELISA for PR3 (cytoplasmic pattern) or MPO (if perinuclear/atypical pattern).
In addition if there is a strong clinical suspicion then ELISA can be done even in absence of IIF positivity.
Pitfalls: can be seen with infections especially bacterial infections
Utility: C-ANCA is highly suggestive of a diagnosis of Wegners disease in proper clinical setting. p-ANCA is seen in various autoimmune diseases and microscopic polyangiitis. ANCA is usually absent in classical PAN

Anti-cardiolipin antibodies
Methods: ELISA
How to report: It should be reported in GPL units for IgG ACl MPL units for IgM Acl Mild elevation 10-20 GPL Moderate elevation 20-40GPL Marked elevation >40 GPL
Pitfalls: can be seen transiently with infections, drugs Can be falsely low in catastrophic APL syndrome
Utility: It should be repeated after 6 weeks to confirm persistent high levels for a diagnosis of anti-phospholipid syndrome in a patient with recurrent venous, arterial thrombosis or recurrent fetal loss. Levels do not correlate with activity so serial testing not useful

Complement levels
Methods: Radial immunodiffusion Turbidimetry Nephelometry
How to report: It should be reported as g/L or mg/dL Normal range should be given in brackets
Utility: Low complement levels suggest complement activation and rarely complement deficiencies
-Elevated complement levels are seen in different rheumatic diseases as part of acute phase response
-In SLE elevated levels suggest infection or severe arthritis DECREASED COMPLEMENT LEVELS HAVE MORE CLINICAL UTILITY THAN ELEVATED LEVELS. THUS A LOW C3 IN SLE SUGGESTS ACTIVE DISEASE

C reactive protein
Methods: Latex agglutination, Turbidimetry/Nephelometry
How to report: It should be reported as G/L or mg/dL.
Pitfalls: increased in any inflammation due to trauma, infection, malignancies etc
Utility: In a patient with joint disease elevated CRP suggests inflammatory arthritis. In SLE elevated CRP suggests infection. In rheumatoid arthritis CRP is a good indicator of disease activity thus serial measurements are useful. It is markedly elevated in Still’s disease

Immunoglobulins
Methods: Single radial immunodiffusion, Turbidimetry/ Nephelometry
How to report: It should be reported as G/L or mg/dL
Pitfalls: increased in any inflammation as part of acute phase response
Utility: Clinical utility of measuring immunoglobulins is limited. In children with arthritis and history of recurrent infection should be done to exclude hypo gammaglobulinemia. In elderly presenting with diffuse aches and pains and have raised globulins to exclude myeloma (serum electrophoresis is better than immunoglobulin levels)

Cryoglobulins
Method: Qualitative assay
How to report: It should be reported as present/absent % cryocrit nature of cryoglobulin
Pitfalls: present in infections, autoimmune diseases, malignancy
Utility: Indicated in a patient with multisystem disease to exclude cryoglobulenemia

Synovial fluid examinations
Methods: Cell count Crystal examination under polarizing microscope Gram stain and culture
How to report: colour, turbidity, cell count with differential, mucin clot test, gram stain report and presence/absence & type of crystals
Pitfalls: Debris, glove powder etc can look like crystals
Utility: Useful in differentiating inflammatory and non-inflammatory arthritis. Useful in excluding infection. Confirmation of diagnosis of crystal disease (negative birefringent crystals —monosodium urate)

Immune complexes
Methods: Physical, PEG precipitation, Immunological, RAJI cell assay, C1q based assay, C3d based assay.
How to report: Reported as mg/dL of proteins. Need to be done by atleast 2 methods.
Pitfalls: can be seen with infections especially with bacterial infections

Utility: Very little clinical utility due to variability in results obtained from different assays as well as rise in diverse clinical conditions

Suggested reading: