

# The use of laboratory tests in the diagnosis of SLE

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Systemic lupus erythematosus (SLE) is a protean autoimmune disease where autoantibodies are frequently targeted against intracellular antigens of the cell nucleus (double and single stranded DNA (dsDNA and ssDNA, respectively), histones, and extractable nuclear antigens (ENAs). Most of these autoantibodies are not specific for SLE and might be produced non-specifically as a result of polyclonal B cell activation. This article will focus on the evidence base for the most commonly used laboratory assays for the detection of these autoantibodies. Updated American Rheumatism Association (ARA) criteria for the diagnosis of SLE include several autoantibodies (table 1).<sup>1,2</sup> SLE is likely if four of 11 criteria are met over any time period. Importantly, the methods for detecting these antibodies are not specified by the ARA, and this article aims to highlight the fact that the particular assay used will crucially influence the interpretation of the test (table 2). Autoantibodies are usually polyclonal—of mixed isotype, affinity, and avidity—and are often directed against multiple targets. Different assays detect particular antibody properties, which are often quite different, and the clinical importance of this for pathogenesis or diagnosis is rarely fully understood. The use of laboratory tests in SLE is a perfect example of this dilemma. The prevalence of autoantibodies varies widely in cross sectional studies, perhaps partly as a result of such differences (table 3). Immunodiffusion (ID) detects high affinity antibodies, immunofluorescence (IIF) moderate and high affinity antibodies, and enzyme linked immunosorbent assay (ELISA) low and high affinity antibodies. Purified antigens might have contaminants, or might not contain the full complement of native proteins. Recombinant antigens might lack certain epitopes, have altered glycosylation or tertiary structure, or contain contaminating bacterial antigens. All assays require careful validation to determine whether they perform adequately for detecting human autoantibodies. An ideal test

would be specific (detects only those with disease), sensitive (detects all those with disease), have a high positive predictive value (PPV)—where most positives have disease, and a high negative predictive value (NPV)—where most negatives do not have disease. In addition, assay results may reflect disease activity, correlate with organ involvement, or predict relapse, thus allowing pre-emptive treatment. No test or test panel can currently perform all these tasks because increases in specificity usually lead to reciprocal decreases in sensitivity, and because some of the clinical features of SLE are not antibody mediated. Therefore, the information obtained from any test will reflect the types of antibody detected, the prevalence of the disease in the population being tested, and the question being asked of the test.

## Antinuclear antibodies (ANAs)

Any antibody to nuclear components is an ANA. Most patients with ANAs do not have SLE, but most people with SLE have ANAs. The most common screening test is IIF on rodent liver or human epithelial (HEp2) tissue,<sup>3</sup> although ELISA tests are available.<sup>4,5</sup> Lupus erythematosus cells simply represent nuclei opsonised by ANAs and are no longer used in diagnosis. Although ANAs are very sensitive for SLE, positive ANAs are common, especially in unwell elderly individuals.<sup>6,8</sup> Therefore, ANAs have low PPV for SLE in unselected populations or when present in low titres,<sup>6,9</sup> and are not diagnostic. One in three healthy people have detectable ANAs on HEp-2 cells at a screening dilution of 1/40 and one in 20 will be positive at 1/160. HEp-2 cells produce more positive ANAs than rat tissue, and some ANAs (for example, anticentromere antibodies) can only be reliably detected on HEp-2 substrate. Although “ANA negative” SLE is reported,<sup>10</sup> it is not clear whether this is the result of a technical artifact or whether a subgroup of SLE exists. Most ANA negative patients are positive in DNA or ENA assays or when screened by IIF on a different substrate. ARA criteria refer to “abnormal” titres of autoantibodies, but there is no cut off value that will absolutely distinguish normality from autoimmune disease. In general, higher titres are more meaningful, particularly in young patients. ANA measurement is at best semi-quantitative, and is poorly standardised between laboratories owing to the lack of suitable reference preparations. The precision and accuracy of the technique depends on the assay configuration, the quality control procedures, and the experience of the reader (table 4). Patterns might suggest antibody specificities but are not diagnostic (table 5). Most clinically relevant ANAs are IgG antibodies and the detection of IgM antibodies usually reduces the

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Table 1 The American Rheumatology Association (ARA) criteria

| ARA criteria 1982 (updated 1997)         | Detail  |
|--|---|
| Photosensitivity                         | Photosensitive skin rash  |
| Malar rash                               | Flat or raised fixed erythema   |
| Discoid rash                             | Raised with plugging/scarring/scaling                                   |
| Oral ulcers                              | Usually painless  |
| Arthritis                                | Non-erosive, 2+ peripheral joints                                       |
| Serositis                                | Pleural or cardiac  |
| Renal disorder                           | Proteinuria or cellular casts   |
| Neurological disorder                    | Convulsions or psychosis without other cause                            |
| Haematological disorder                  | Haemolysis, cytopenia   |
| “Immunological disorder” (modified 1997) | Anti-dsDNA, anti-Sm, antiphospholipid antibodies (ACAs, LA, or FP VDRL) |
| Antinuclear antibody (ANA)               | “Abnormal titre” ANA at any time point by IIF or equivalent assay       |

ACA, anticardiolipin antibody; ANA, antinuclear antigen; dsDNA, double stranded DNA; FP VDRL, false positive venereal disease reference laboratory test; IIF, immunofluorescence; LA, lupus anticoagulant.

Table 2 Common attributes of individual assay technologies used for the diagnosis and monitoring of SLE (all assays produce some false positive results)

| Assay                                       | Problems  | Advantages  | Result   |
|---|---|---|--|
| IIF rodent tissue                           | Subjective, Ro may be missed, semiquantitative, pattern not diagnostic, cannot detect cell cycle related patterns, not specific | Cheap, can be isotype specific  | Semiquantitative end point titration or qualitative result at screening titre + pattern                  |
| IIF HEp-2                                   | Subjective, Ro may be missed, semiquantitative (poor precision), pattern not diagnostic, not specific                           | Cheap, recombinant Ro60 expression to boost Ro sensitivity available, can be isotype specific | Semiquantitative end point titration or qualitative result at screening titre + pattern                  |
| Ouchterlony double diffusion (ID)           | Slow, crude antigens, subjective, qualitative, requires experience, not isotype specific, some false negatives                  | Specific, cheap   | Positive or negative + antigen specificity   |
| Counter current immunoelectrophoresis (CIE) | Slow, crude antigens, semiquantitative, requires experience, not isotype specific, some false negatives                         | As ID, but more sensitive   | Positive or negative + antigen specificity   |
| Haemagglutination                           | Detects IgG and IgM, semiquantitative, subjective, detects low affinity antibodies  | Cheap   | Positive or negative + semiquantitative titre  |
| Immunoblotting (IB)                         | Qualitative, may be insensitive for Ro, crude antigen, labour intensive   | Sensitive, very specific for individual antigens  | Positive or negative + antigen specificity   |
| Immunoprecipitation (Farr)                  | Radioactive, labour intensive, expensive, technically difficult, no isotype specificity, false positivity                       | Quantitative, high specificity, detects high affinity antibodies                              | Quantitative result, potentially in standardised IU/l if reference preparation available                 |
| ELISA                                       | Detects low affinity antibodies, needs high purity well defined antigens (native $\nu$ recombinant), false positivity           | Sensitive, variable, can be polyspecific or IgG specific                                      | Qualitative or quantitative results, potentially in standardised IU/l if reference preparation available |

ELISA, enzyme linked immunosorbent assay; IIF, immunofluorescence.

clinical usefulness of the test.<sup>11 12</sup> Antibody class switching to IgG usually occurs in established autoimmunity, and many low titre, low affinity IgM autoantibodies are found in healthy individuals. The absence of ANAs at titres of 1/160 or less makes SLE very unlikely. Approximately 10% of SLE like disease is drug induced and potentially reversible. However, drug induced ANAs are more common than disease, and careful interpretation of the possible clinical relevance of an ANA in this context is needed. Each laboratory should configure its protocol for an appropriate sensitivity/specificity compromise, should perform adequately in local and national external quality assessment (EQA) schemes, and should not interpret results without reference to the clinical details. More specific and precise tests should be performed in ANA positive individuals to determine the autoantibody specificity (table 5).

### Anti-DNA antibodies

dsDNA antibodies are associated with systemic lupus and nephritis, but not subacute cutaneous lupus or discoid lupus. The best method for detecting anti-dsDNA remains controversial<sup>13-18</sup> (table 4). The most common techniques in the UK are dsDNA ELISA, *Critidia luciliae* IIF (CLIF), or Farr immunoprecipitation assays (table 2). Specific assays should be used for diagnosis, whereas sensitive assays might be more useful for monitoring.<sup>4</sup> There are several difficulties in the detection of

anti-dsDNA, which apply to other autoantibodies, including:

- (1) Substrate differences: many sources of mammalian and non-mammalian DNA are used, but each might detect a different set of antibodies.
- (2) The isotype of antibody detected: assays might detect different antibody isotypes (IgG, IgA, IgM, or any combination). All isotypes are detected by Farr assays, ELISA, or CLIF, which use polyspecific antisera. A positive polyspecific assay might have a different clinical relevance to that of an IgG specific assay.<sup>19 20</sup> IgM anti-dsDNA detected by ELISA might not be specific for SLE.<sup>20 21</sup>
- (3) Antibody affinity: high affinity anti-dsDNA might be more relevant to SLE pathogenesis, particularly in nephritis. Low affinity antibodies are not detected by Farr assays, but are detected by ELISA.<sup>22-29</sup>
- (4) Assay specific parameters: each assay has known causes of false positivity. For example, C reactive protein (CRP) or ssDNA contamination in the Farr; lipoprotein-IgG complexes in CLIF; antibodies to linkers in ELISA. Contamination with ssDNA leads to overestimation of anti-dsDNA titres, because anti-dsDNA antibodies regularly bind ssDNA, but ssDNA antibodies are not specific for SLE.<sup>30</sup> This might not be important for monitoring disease activity because ssDNA might reflect the overall anti-nucleosome immune responses,<sup>31 32</sup> but it might reduce the diagnostic usefulness of an assay because ssDNA antibodies are not specific for SLE.
- (5) Problems with standardisation and calibration: an assay has to be precise (give the same result on the same serum every time) to enable successful monitoring of serial titres. An assay should be accurate and produce the same result as other assays on all serum samples to enable comparison between different centres. An international reference preparation (IRP) for anti-

Table 3 Frequency of serological positivity in SLE

| Autoantibody target  | % Positive at any stage of disease (any assay) | Possible clinical association (see text)           |
|----------------------|--|--|
| dsDNA                | 30-70  | Nephritis, disease activity                        |
| Sm                   | 20-40  | Rarely seen outside SLE                            |
| RNP                  | 40-60  | MCTD/overlap features                              |
| Ro                   | 10-15  | Sjogren's/skin involvement/congenital heart block  |
| Ribosomal P0, P1, P2 | 5-10   | Neuropsychiatric SLE, disease activity             |
| Histone              | 30   | Drug induced SLE, idiopathic SLE, disease activity |
| ACA                  | 40-50  | Risk of thrombotic complications/fetal loss/ITP    |

ACA, anticardiolipin antibody; dsDNA, double stranded DNA; ITP, idiopathic thrombocytopenic purpura; MCTD, mixed connective tissue disease; RNP, ribonuclear protein.

Table 4 The clinical usefulness of commonly used autoantibody assays in SLE

| Test (EQA scheme?)                     | Technique                 | Diagnostic specificity  | Clinical usefulness                                      | Use in monitoring activity                        | Evidence base |
|--|---------------------------|---|--|---|---------------|
| ANA (EQA+)                             | IIF rodent tissue         | Moderate for homogenous or speckled pattern at $\geq 1/160$ in selected patients, IgG only                              | Initial screen for further testing                       | Poor (semiquantitative)                           | Extensive     |
|  | IIF HEp-2                 | Moderate for homogenous or speckled pattern at $\geq 1/160$ in selected patients, IgG only                              | Initial screen for further testing                       | Poor (semiquantitative)                           | Extensive     |
| dsDNA (EQA+)                           | IIF ELISA                 | Unknown   | Unknown  | Possible  | Limited       |
|  | Farr                      | Good, known causes of false positives   | Good for diagnosis and monitoring                        | Useful in subset of patients                      | Extensive     |
| Complement C3, C4 (EQA+)               | Crithidia IIF             | Good, known causes of false positives   | Good for diagnosis                                       | Poor (semiquantitative)                           | Extensive     |
|  | ELISA                     | Variable, assay dependent, IgG specific good, known causes of false positives   | Assay dependent, good for IgG specific                   | Assay dependent, good for IgG specific            | Moderate      |
| CRP (EQA+)                             | Nitrocellulose            | Moderate  | Moderate   | No  | Limited       |
|  | Nephelometry/turbidometry | None  | Moderate, low C3 in renal disease                        | Useful in subset of SLE, serial values essential  | Extensive     |
| ENA Ro/La/Sm/RNP (EQA+)                | Nephelometry/turbidometry | None  | CRP usually low in active SLE except in severe serositis | Useful to distinguish infection from SLE activity | Moderate      |
|  | Immunodiffusion/CIE       | Good for Sm, moderate for Ro/La, may be poor for RNP  | Good for defining ANA specificity                        | No, except where disease has evolved new features | Extensive     |
| Antiphospholipid antibodies (ACA EQA+) | IB                        | Good, but may be insensitive for Ro As ID/CIE but more sensitive for Ro/RNP, less specific if low titre or IgM positive | Good   | No  | Moderate      |
|  | ELISA                     | Good, but may be insensitive for Ro As ID/CIE but more sensitive for Ro/RNP, less specific if low titre or IgM positive | Good for IgG antibody at appropriate cut off             | No, except where disease has evolved new features | Extensive     |
| Anti- $\beta 2$ GP1 ELISA              | Haemagglutination         | Poor, less specific, detects IgM  | Poor   | No, semiquantitative                              | Limited       |
|  | ACA ELISA                 | Poor, even for IgG antibodies   | Moderate, especially IgG specific                        | Possible  | Extensive     |
| Antihistone antibodies                 | Anti- $\beta 2$ GP1 ELISA | Moderate for IgG antibodies   | Moderate, especially IgG specific                        | Unknown   | Moderate      |
|  | Lupus anticoagulant ELISA | Poor  | Moderate   | Unknown   | Moderate      |
| Antihistone antibodies                 | ELISA                     | Poor, even for drug induced SLE   | Poor   | Possible  | Limited       |
|  | IB                        | Poor, even for drug induced SLE   | Poor   | No  | Limited       |

EQA +, UK external quality assessment scheme available.

ACA, anticardiolipin antibody; ANA, antinuclear antigen;  $\beta 2$ GP1,  $\beta 2$  glycoprotein 1; CIE, counter current immunoelectrophoresis; CRP, C reactive protein; dsDNA, double stranded DNA; ELISA, enzyme linked immunosorbent assay; ENA, extractable nuclear antigen; IB, immunoblotting; ID, immunodiffusion; IIF, immunofluorescence; RNP, ribonuclear protein.

dsDNA (Wo80) is available to improve standardisation between assays (table 6), but there remains poor correlation between dsDNA assays in EQA schemes, even within a single technology.<sup>20</sup> All laboratories should participate in EQA schemes and maintain adequate internal quality control procedures.

#### DIAGNOSIS

Farr assays are quite specific and well documented, but also detect high affinity IgM anti-dsDNA. IgG specific ELISA or CLIF methods are commonly used in UK laboratories, and may produce similar results to Farr assays. Polyspecific ELISAs also detect low affinity IgM antibodies of dubious clinical relevance and are less useful. ELISA results (particularly those detecting IgM) should be confirmed by IgG specific CLIF or Farr assays.<sup>33-36</sup> CLIF is less likely than ELISA to detect low affinity

anti-dsDNA of uncertain clinical relevance,<sup>37-42</sup> especially if IgG specific conjugates are used. Local validation of each assay is essential to ensure adequate diagnostic performance.

#### MONITORING OF DISEASE

Laboratory tests are no more effective than clinical review for detecting disease relapse,<sup>43-50</sup> but are helpful in confirming the activity of SLE. The rapidity of clinical relapse clouds the interpretation of the few prospective studies available, and regular sampling every six to eight weeks would be required to predict relapse reliably. dsDNA antibodies rise in active disease and in the evolution of lupus nephritis in most patients.<sup>51-55</sup> dsDNA antibody assays can be negative early in disease, after treatment, or when the patient is in clinical remission; therefore, not all patients with SLE are seropositive at any one time.<sup>56</sup> The absence of antibodies at any one time would not

Table 5 Common antinuclear antibody (ANA) HEp-2 patterns and their clinical use in SLE

| Pattern  | Autoantibody association                | Clinical association                       |
|--|---|--|
| Homogenous   | dsDNA, ssDNA, histones, nucleosomes, Ku | SLE, AICAH, and many non-pathological ANAs |
| Rim/peripheral   | dsDNA, laminin, nuclear pore            | SLE/AICAH                                  |
| Speckled (coarse)  | RNP/Sm                                  | SLE/MCTD                                   |
| Speckled (fine) may be missed on IIF owing to variable Ro expression on tissue | Ro/La (also cytoplasmic)                | SLE/SCLE/scleroderma                       |
| Speckled (centromere)  | Centromere A, B, C kinetochore proteins | Primary Raynauds, scleroderma, SLE         |

The main use of the ANA pattern on screening is to determine the need for further antibody specificity testing or to distinguish possible false positive ELISA.

Patterns are not diagnostic, most homogenous or speckled ANAs have no detectable dsDNA or ENA antibodies. AICAH, autoimmune chronic active hepatitis; dsDNA, double stranded DNA; ENA, extractable nuclear antigen; IIF, immunofluorescence; MCTD, mixed connective tissue disease; RNP, ribonuclear protein; SCLE, subacute cutaneous lupus erythematosus; ssDNA, single stranded DNA.

Table 6 International reference preparations for autoantibodies

| Reference preparation                     | Specificity  |
|---|--|
| WHO W680                                  | dsDNA 100 IU/ampoule                               |
| WHO1064 or MRC research standard A 66/233 | Homogeneous ANA 100 IU/ampoule                     |
| WHO1063                                   | nRNP   |
| WHO1061                                   | IgM ANA 100 IU/ampoule                             |
| CDC reference preparations                | dsDNA, La, U1-RNP, Sm, Ro, centromere, Scl70, Jo-1 |

ANAs, antinuclear antibodies; CDC, Centers for Disease Control; dsDNA, double stranded DNA; nRNP, nuclear ribonuclear protein.

exclude a previous diagnosis of SLE, and ARA criteria include events that occur sequentially over time. Prophylactic treatment on the basis of rising titres of anti-dsDNA cannot be widely justified at present, but rises in titre merit closer clinical monitoring for relapse. IgM and low affinity anti-dsDNA antibodies might be less specific for SLE.<sup>19 57 58</sup> High avidity IgA antibodies are seen in active SLE,<sup>59</sup> but are no more helpful than IgG assays. Some IgG specific ELISAs have similar usefulness to the Farr assay,<sup>23 60</sup> but this requires validation on an assay by assay basis. It remains to be proved whether low affinity anti-dsDNA antibodies are specific for subclinical or mild SLE.<sup>29 61</sup>

Anti-ssDNA activity might be useful in monitoring activity,<sup>62 63</sup> but it is not clear that this offers any advantage over anti-dsDNA measurements and no EQA/IRP for ssDNA is available.<sup>17 60</sup>

#### Antihistone antibodies

Around 50–80% of patients with SLE have IgG and IgM antihistone antibodies detectable by immunoblotting (IB) or ELISA. ELISAs are described that detect antibodies to total histones or to subfractions (H1, H2a, H2b, H3, and H4),<sup>64 65</sup> but the clinical specificity is not well established for any subfraction. Titres of antihistone antibody might reflect disease activity, but are not specific for SLE and cannot distinguish drug induced SLE from idiopathic SLE.<sup>52 66 67</sup> Drug induced antibodies are often IgM and occur without any clinical manifestations.

#### Antibodies to ENAs

##### ANTI-Ro/La ANTIBODIES

##### Diagnosis

Antibodies to Ro(SS-A) and La(SS-B) are found in SLE and Sjogren's syndrome. Neither is specific for SLE, but both are very useful when anti-dsDNA is absent.<sup>68</sup> ID/CIE (counter-current immunoelectrophoresis) assays were frequently used in the past, but are now superseded by more sensitive ELISA or IB assays (table 2). Some IB assays might be insensitive for anti-Ro. ELISAs are more sensitive for anti-Ro, anti-La, and anti-RNP, but are positive in other diseases also (table 4).<sup>3 69 70</sup> Disease specificity is improved by excluding weak positives. Unlike anti-dsDNA, anti-ENA antibody affinity does not appear to be important,<sup>71</sup> but IgG antibodies are of greater clinical relevance.<sup>12</sup> Little is known about the importance of IgA or IgM anti-Ro. Ro exists in two forms: Ro52 and Ro60. In SLE, anti-Ro60 antibodies predominate, whereas both are

present in Sjogren's syndrome.<sup>72</sup> Ro60 contains conformational epitopes that are absent in some assay substrates. Newer enzyme immunoassays and western blotting are capable of discriminating antibodies to Ro52 and Ro60 individually, but it is not clear how clinically useful this will be. Bovine spleen is less sensitive than human substrate in ID/CIE,<sup>5 34</sup> and can cause problems in detection.

##### Monitoring

There is little evidence that anti-ENA specificity or titres reflect SLE activity,<sup>68 73</sup> but anti-Ro is associated with cutaneous involvement in subacute cutaneous lupus erythematosus,<sup>74</sup> and with congenital heart block (CHB).<sup>75</sup> Anti-Ro52 in isolation is associated with CHB but is not detected by assays containing only Ro-60,<sup>76</sup> such as HEp-2000 cells.

Anti-La is rarely detected without anti-Ro<sup>77 78</sup> because both proteins associate with a common type of human RNA called hYRNA. There is little evidence that anti-La is associated with reduced renal disease. Titres of anti-Ro and anti-La increase more slowly than anti-dsDNA in relapse and quantitative reporting is unlikely to be useful.<sup>79</sup> Standardisation of results between laboratories is still problematic in EQA schemes. Centers for Disease Control anti-ENA reference preparations of defined specificity, but no IRP with defined units, are available.

##### Neonatal SLE and CHB

Anti-Ro52, anti-Ro60, anti-La, or anti-Sm IgG is transferred across the placenta in the last trimester and on rare occasions can lead to pathology in the child.<sup>75 80–82</sup> Serology can be performed on cord blood or the mother's blood antenatally, and intrauterine monitoring can be instituted in high risk seropositive pregnancies.

##### ANTIBODIES TO SM/RNP

##### Diagnosis

High titre anti-Sm constitutes an ARA criterion for SLE and is highly SLE-specific, although low titre anti-Sm in ELISA/immunoprecipitation assays has been reported in other diseases.<sup>5 83–85</sup> Anti-Sm antibodies are rarely found without anti-RNP (ribonucleoprotein)<sup>86</sup> because both proteins associate with common snRNA species in the spliceosome. Anti-RNP is more common and less specific for SLE.<sup>83 87</sup> Anti-RNP ELISAs are more sensitive than ID, but ELISA and ID might be equivalent for anti-Sm<sup>70 88 89</sup> (table 2). Bovine thymus substrate has similar sensitivity to human thymus extract for Sm/RNP. The importance of antibody isotype or affinity is unknown.<sup>90</sup>

##### Monitoring

Anti-RNP or anti-Sm antibodies are not strongly associated with specific clinical features of SLE, outside mixed connective tissue disease (MCTD),<sup>73 91–95</sup> but anti-Sm might appear with disease evolution.<sup>96</sup> Titres can fluctuate with disease activity and

treatment,<sup>97–99</sup> but serial monitoring does not effectively predict relapse.<sup>100–101</sup>

There are insufficient data on the more rare ENA specificities to justify routine clinical use.

#### **Ribosomal P antibodies**

Anti-ribosomal antibodies detected by ELISA or IB are associated with neuropsychiatric SLE,<sup>102–106</sup> but their predictive value is uncertain and controversial.<sup>107–108</sup> Titres rise in active SLE.<sup>109</sup> Data associating lymphocytotoxic antibodies with cognitive dysfunction are very limited.<sup>110</sup>

#### **Antiphospholipid antibodies**

Anticardiolipin antibodies (ACAs) of all isotypes are seen in 16–60% of patients with SLE. IgG ACAs<sup>111–115</sup> are a risk factor for thrombosis and the antiphospholipid syndrome, but are controversial risk factors for renovascular events.<sup>116–117</sup> Not all ACA positive patients with SLE have an antiphospholipid syndrome and ACA negative patients can have thrombotic complications. ACAs might be an additional risk factor for pregnancy outcome in SLE.<sup>118–119</sup> Titres vary with disease activity, perhaps explaining an association with severe renal disease.<sup>120–121</sup> EQA schemes reveal poor standardisation, despite the availability of reference materials for calibration.

IgG anti-β2 glycoprotein 1 antibodies are more closely associated with thrombosis in the primary antiphospholipid syndrome and SLE,<sup>122</sup> and approximately 25% of SLE patients may be positive. Other antiphospholipid antibodies are of uncertain importance including antiphosphatidyl serine, anti-annexin V, and antithromboplastin.

Additional lupus anticoagulant testing (for example, dilute Russell's viper venom test or activated partial thromboplastin time) is essential because lupus anticoagulant might predispose to thrombosis, and might occur without ACAs.<sup>112</sup> The reproducibility of the lupus anticoagulant test is variable and it cannot be used to monitor disease activity.<sup>123</sup>

#### **Acute phase proteins/cytokines**

The erythrocyte sedimentation rate is a sensitive but non-specific indicator of activity in SLE, and is slow to reflect changes in disease activity. CRP has a short half life and rapidly reflects acute inflammation. A high CRP can distinguish bacterial infection from active SLE, where the CRP is usually low,<sup>124–126</sup> but CRP might be raised in severe lupus serositis.<sup>127</sup> Soluble interleukin 2 receptor or tumour necrosis factor receptor values might reflect disease activity, but are not specific for SLE, and are of uncertain clinical relevance.<sup>128–129</sup>

#### **Complement**

Although immune complexes are seen in SLE, immune complex assays are poorly reproducible, non-specific, and rarely useful,<sup>130</sup> except for cryoglobulins. Complement assays are occasionally useful.<sup>18–28–131–136</sup> A single C4 is not informative and serial monitoring is necessary because C4 null alleles are common in SLE, so that the baseline C4 may be chronically low.<sup>137</sup>

SLE can also be active without causing changes in C3 and C4.<sup>48</sup> Persistently low C3 is associated with chronic renal disease.<sup>138–139</sup> Classical pathway assays (CH100/CH50) cannot distinguish deficiency from severe consumption, but can exclude early pathway complement deficiencies (C1, C2, or C4), which are associated with SLE. In contrast, C3 and C4 values are precise and economical, even if useful in some patients only.

Complement activation products (C3d, C3a, C4a, C5a, iC3, C4d, Bb, C5b–9, and erythrocyte CR1) are raised in active disease,<sup>140–143</sup> but assays to measure these molecules are not widely available and require special sample handling to a degree that makes routine clinical use impracticable.

#### **Anti-C1q antibodies**

Anti-C1q antibodies are detected by ELISA in 90% of patients with SLE,<sup>144</sup> but are also found in membranoproliferative glomerulonephritis and rheumatoid vasculitis. High titres are associated with proliferative glomerulonephritis,<sup>145–146</sup> but these antibodies are of limited clinical use.

#### **Anti-endothelial cell antibodies**

Anti-endothelial cell antibodies may reflect disease activity,<sup>147</sup> but are poorly characterised.

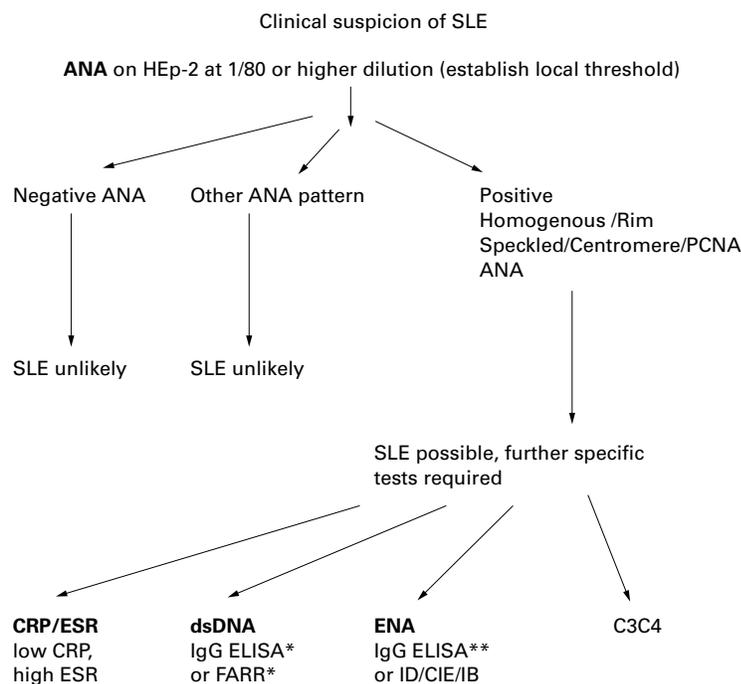
#### **Antineutrophil cytoplasmic antibodies**

Antineutrophil cytoplasmic antibodies of all types are found in SLE and are not clinically relevant.<sup>148</sup>

#### **Summary**

ANA IIF is an effective screening assay in patients with clinical features of SLE and will detect most anti-ssDNA, anti-dsDNA, ENAs, and other autoantibodies. False positives are common. The clinical importance cannot be extrapolated from the ANA titre or pattern, although higher titres (> 1/160) are more likely to be important. HEP-2 cells are the most sensitive substrate for ANA detection, but this must be balanced against an increased incidence of insignificant positivity.

ANA positive samples should be subjected to more specific assays for the diagnosis of SLE. A combination of ENA (Ro/La/Sm/RNP) and dsDNA assays will detect most patients with SLE<sup>5–18–149</sup> as long as the characteristics of the assays used are well understood. ESR and CRP measurements provide useful additional information. Sjogren's syndrome and MCTD will produce overlapping serology with SLE, and anti-dsDNA titres are sometimes seen in autoimmune hepatitis and rheumatoid arthritis. All results should be reported in the light of the clinical details, by an experienced immunologist. A suggested diagnostic protocol is outlined in fig 1. The type of assay used crucially influences the predictive value of the tests. ELISA technology dominates routine laboratory practice, but tends to produce more false positive and true weak positive results, which may reduce the PPV of the test. This can be minimised by using IgG specific conjugates and careful assay validation. The NPV for SLE



**Figure 1** Suggested diagnostic protocol for investigation of suspected SLE. \*Confirm weak positives or possible false positives by IgG CLIF; \*\*confirm weak positives or possible false positives by ID/CIE/IB. Antiphospholipid/lupus anticoagulant assays might be necessary if relevant clinical features of an antiphospholipid syndrome are present. ANA, antinuclear antibody; CIE, counter-current immunoelectrophoresis; CLIF, *Crithidia luciliae* immunofluorescence; CRP, C reactive protein; dsDNA, double stranded DNA; ELISA, enzyme linked immunosorbent assay; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; ID, immunodiffusion; IB, immunoblotting; PCNA, proliferating cell nuclear antigen.

is high for most assays but the PPV varies. Where necessary, laboratories should use crithidia or Farr dsDNA assays to confirm dubious ELISA dsDNA results, and ID/IB to confirm dubious ENA results.

For monitoring, a precise, quantitative assay is required. It is unclear whether the detection of IgM or low affinity antibodies has a role here. A combination of anti-dsDNA, C3, C4, CRP, and ESR assays provides the most useful clinical information. Anti-ssDNA assays are likely to be useful, and are potentially more robust than anti-dsDNA assays, but require more validation.

Local validation of individual assays and EQA participation is essential. Not all assays that apparently measure the same antibody specificities have equal clinical relevance, even within a single technology. Insufficient international or national reference preparations are currently available for many antibody specificities to enable effective standardisation. Quality assurance schemes reveal large differences in units reported by different assays for some analytes, even when calibrated against an IRP or equivalent reference preparation. Serial results can therefore only be compared from the same laboratory at present. Most autoantibodies increase during active disease, but few prospective data are currently available to justify treatment on the basis of rising titres. Further randomised prospective studies are required to examine the importance of antibody isotype and affinity in the monitoring of SLE by individual assay methods. The most

important aspect of the appropriate use of laboratory assays is to become familiar with the limitations of the technology currently in use in your local laboratory, and to consult with your clinical immunologist in cases of doubt, preferably before commencing serological screening.

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