Laboratory Tests for Autoimmune Diseases Position Paper

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Overview of tests for autoimmune diseases

A diagnosis of autoimmune diseases is obtained by using a combination of:

- Detailed clinical history,
- Physical examination, and
- Other investigations, including laboratory testing, such as serological (antibody) tests, tests for acute phase reactants and in some cases, biopsies for histological confirmation of disease.

Laboratory test results do not usually have adequate predictive value to be diagnostic unless they are used in conjunction with the patient's clinical history and physical examination.

Therefore, they should not be used as screening tests in unselected populations.

Consensus diagnostic and/or classification criteria for autoimmune diseases have been developed for target populations and incorporate a range of findings, including clinical history, physical examination and serological test results.

Autoantibodies are markers of autoimmune diseases

Autoimmune diseases are due to a breakdown in the body's self-tolerance, either central or peripheral. This results in the immune system targeting self-peptides, leading to tissue damage or destruction.

Autoimmune diseases can be antibody mediated or immune cell mediated and are classified as systemic or organ specific.

Autoantibodies are referred to as markers of autoimmune disease, but they do not cause autoimmune diseases. Autoantibodies can be directly involved in the pathology of antibody mediated autoimmune diseases or they can be associated with autoimmune diseases.

Laboratory tests for autoimmune diseases

The laboratory can assist with diagnosis confirmation of autoimmune diseases by performing:

- Primary tests to identify autoantibodies or disease markers in patients with clinical features that are suggestive of autoimmune disease.
- Secondary tests for further autoantibody characterisation, diagnosis, prognosis or monitoring autoimmune disease activity.

Laboratory tests are also used in management of autoimmune diseases (particularly when they are antibody mediated) to predict flares, monitor efficacy of therapy and monitor disease progression.

Serial monitoring of autoimmune diseases is limited to a smaller number of tests which have been shown to fluctuate with disease status.

Types of laboratory tests

Multiple methods are used in the laboratory to detect the presence of autoantibodies, with descriptive and semi-quantitative or quantitative results.

Descriptive and semi-quantitative assays include:

- Indirect immunofluorescence assays (IFA) to measure tissue autoantibodies, antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA).
- Immunoblot (line) assays, such as antibodies to extractable nuclear antigens (ENA).
- Cell based assays for autoantigens expressed on cells engineered to express antigens otherwise difficult to detect (such as aquaporin-4, CASPR-2, DPPX, NMDA receptor, AMPA receptor, desmoglein-1, desmoglein-3 autoantibodies).

Depending on the diagnostic query and the pattern of immunofluorescence observed, positive ANA tests should be followed up by assays to determine the type of ANA present.

Autoantibodies recognising one or more of these nuclear antigens can also be individually assessed (antibodies to ENA or double stranded [ds] DNA). Antibodies to these individual nuclear components can have specific disease associations or prognostic value.

Quantitative assays include:

- Enzyme linked immunosorbent assays (ELISA) and assays which are based on a similar principle, including fluorescence enzymes (FEIA), and chemiluminescence (CLIA).
- Addressable laser bead immunoassays (ALBIA) which is a multiplex technology that can assess several antibody specificities simultaneously on a small serum sample volume.
- Radioimmunoassays (RIA).

Quantitative assays can detect specific immunoglobulin isotypes or all bound immunoglobulin. However, in most cases the assays are not standardised and numerical results cannot be directly compared between methods and different laboratories.

Clinicians should be mindful of variation between different test methods and laboratories, and what is defined as a clinically relevant change in the result for the autoantibody. Therefore, **it is important that the same quantitative test method and laboratory is used for monitoring of a patient's autoimmune disease**.

In a quantitative assay, the autoantigen is presented on either a tissue, as a molecule in solution or bound to a solid phase such as a membrane or polystyrene well. Patient serum is incubated with the antigen allowing the antigen specific antibody to bind to the substrate.

Following a washing step bound patient serum is detected using an anti-human immunoglobulin antibody labelled with a fluorescent tag or enzyme which allows quantitation and/or visualisation of the bound autoantibody. Alternatively, the antigen antibody complex may be immunoprecipitated and quantified.

Biopsy testing in autoimmunity

The deposition of immunoglobulin or complement components seen in skin and renal biopsies can also be used to inform the clinicians about the pathogenic mechanisms involved and which disease is present.

This involves a direct immunofluorescence staining method when using fresh frozen tissue and is reported qualitatively.

In some cases the degree of staining may be scored as a percentage of tissue affected in the biopsy.

Prevalence of some autoantibodies associated with autoimmune diseases

Advances in laboratory technology, reagents, alternative assay methods and expanding understanding autoimmune diseases have shown that many patients with no autoimmune diseases have a detectable autoantibody. An example of this is anti-nuclear antibodies (ANA) which occur with variable frequency in almost all autoimmune diseases, and in the context of neoplasia. They are also found in a small proportion of healthy subjects, usually in low titer, and frequency of detection increases with age.

Certain antibodies can be detected many years before clinical presentation of autoimmune diseases, such as centromere antibodies in limited scleroderma. Several international studies have reported prevalence of serum autoantibodies in their corresponding general population. Ethnicity, gender, age and geographical location may influence the prevalence of autoimmunity.

Turnaround times for autoantibody testing

Turnaround times for autoantibody testing can range from several hours to weeks. However, there are situations when identification and/or quantitation of the autoantibody warrants obtaining an urgent result.

For example, this may require phoning the laboratory if a patient presents with a rapidly evolving disease involving organ failure which may mimic other conditions or emergencies, including vasculitis, Goodpasture Disease and autoimmune encephalitis.

Types of autoimmune diseases

Localised (organ specific) autoimmune diseases mainly affect a single organ or tissue, although the effects frequently extend to other body systems and organs. These diseases are often managed by organ-specific medical specialists, such as endocrinologists, gastroenterologists, neurologists or rheumatologists.

Systemic autoimmune diseases can affect many body organs and tissues at the same time. They can be broadly classified into rheumatological disease and vasculitis disorders. These diseases are often managed by clinical immunology/allergy specialists and/or rheumatologists.

Examples of Localised Autoimmune Diseases	Examples of Systemic Autoimmune Diseases
Addison's disease (adrenal)	Antiphospholipid antibody syndromes (blood cells)
Autoimmune hepatitis (liver)	Dermatomyositis (skin, muscles)
Coeliac disease (gastrointestinal tract)	Mixed connective tissue disease
Crohn's disease (gastrointestinal tract)	Polymyalgia rheumatica (large muscle groups)
Diabetes Mellitis Type 1a (pancreas)	Polymyositis (skin, muscles)
Grave's disease (thyroid)	Rheumatoid arthritis (joints, less commonly lungs, skin, eyes)
Guillain-Barre syndrome (nervous system)	Scleroderma (skin, intestine, less commonly lungs, kidneys)
Hashimoto's thyroiditis (thyroid)	Sjögren's syndrome (salivary glands, tear glands, joints)
Multiple sclerosis (nervous system)	Systemic Lupus Erythematosus (skin, joints, kidneys, heart, brain, red blood cells, other)
Myasthenia gravis (nerves, muscles)	
Pernicious anaemia (stomach)	
Primary biliary cholangitis/ cirrhosis (liver)	
Sclerosing cholangitis (liver)	
Ulcerative colitis (gastrointestinal tract)	

Further information

RCPA - Pathology Tests this list is searchable and provides basic information on a wide range of pathology tests across all disciplines, including autoimmunity.

Information about Vasculitis www.anzvasculitis.org/medical-professionals/

ANA Consensus www.anapatterns.org

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For more information go to www.allergy.org.au/patients/autoimmunity

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