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INTEGRATION OF SCREENING AND CONFIRMATORY PHASES IN THE LABORATORY DIAGNOSIS OF SYSTEMIC AUTOIMMUNE DISEASES

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Abstract. The laboratory diagnosis of autoimmune diseases is a complex and multi-stage process. The non-specific nature of clinical manifestations makes early diagnosis difficult. Therefore, the detection of autoantibodies constitutes one of the main pillars of diagnostics. The modern approach is based on the sequential and integrative application of screening and confirmation stages. Indirect immunofluorescence is widely used as the gold standard method for ANA screening due to its high sensitivity. This method provides not only the detection of autoantibodies but also additional information about their potential target antigens through fluorescence patterns. However, indirect immunofluorescence results are not sufficient for the precise identification of specific autoantibodies and require additional confirmatory tests. At this stage, line blot and other immunoblot-based methods play a significant role. These technologies enable the determination of specific autoantibody profiles and facilitate the differential diagnosis of various systemic autoimmune diseases. Proper integration of screening and confirmation stages reduces false-positive results, increases diagnostic accuracy and optimizes clinical decision-making. At the same time, correct interpretation of ANA patterns and evaluation of autoantibodies in a clinical context are of particular importance. Low-titer ANA positivity and certain non-specific patterns may lead to misinterpretation. Therefore, integration of laboratory findings with clinical data is essential. In conclusion, the integrative application of screening and confirmation stages in autoimmune diagnostics allows for more reliable and clinically valuable results. This approach significantly improves early diagnosis, accurate differential assessment and effective patient management.

Keywords: *Antinuclear antibodies (ANA), autoantibodies, autoimmune diseases, Indirect Immunofluorescence (IIF), ICAP classification (International Consensus on ANA Patterns).*

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1. Introduction

The immune system is a complex network of cells, tissues and organs that protects the body from bacteria, viruses, parasites and other harmful external agents. It includes leukocytes, lymph nodes, the spleen, bone marrow and various biological molecules. The main function of the immune system is to accurately distinguish between "self" and "non-self" structures and to effectively eliminate foreign antigens (Wang *et al*., 2015; 2024). This balanced mechanism is known as immune tolerance and prevents immune reactions against the body's own tissues (Wang *et al*., 2015). However, in some cases, disruption of immune tolerance mechanisms leads the immune system to recognize its own cells and tissues as foreign and to generate a pathological immune response against them, which marks the onset of autoimmune processes (Pisetsky, 2023). This condition is referred to as autoimmunity and often presents clinically with non-specific symptoms (Song *et al*., 2025).

2. Immunological basis of autoimmunity

The development of autoimmunity is usually associated not with a single factor but with the complex and interactive influence of multiple factors (Miller, 2023). Genetic predisposition plays a crucial role in this process. In some individuals, alterations in genes involved in the regulation of the immune system increase susceptibility to autoimmune reactions (Tizaoui *et al*., 2021). In addition, environmental factors such as infections (viruses and bacteria), smoking, harmful chemicals, dietary habits, stress and hormonal changes may disrupt the functional balance of the immune system and direct it toward a pathological state (Miller, 2023). Furthermore, the mechanism of molecular mimicry also plays an important role in the development of autoimmunity. In this case, since the antigenic structures of certain microorganisms resemble the body's own components, the immune system may attack its own tissues while responding to the pathogen (Wang *et al*., 2015; Pisetsky, 2023). As a result, T and B lymphocytes become activated, autoantibodies are produced and a chronic inflammatory process develops (Song *et al*., 2025; Pisetsky, 2023).

3. Laboratory and clinical significance of autoantibodies

Autoantibodies are immunoglobulins produced by the immune system against the body's own antigens and they play a role both in tissue damage and as important laboratory biomarkers (Xiao *et al*., 2021; Mulhearn *et al*., 2020). These antibodies may be directed against nuclear components (ANA), cytoplasmic structures, cell surface receptors and phospholipids. For example, anti-dsDNA antibodies are associated with systemic lupus erythematosus (SLE), Anti-Cyclic Citrullinated Peptide (anti-CCP) with rheumatoid arthritis and Anti-thyroid Peroxidase (anti-TPO) with thyroid diseases (Xiao *et al*., 2021). ANA (anti-nuclear antibodies) target components of the cell nucleus and are widely observed in the early stages of systemic autoimmune diseases (Dinse *et al*., 2020). In addition, antiphospholipid antibodies increase the risk of thrombosis and are of significant clinical importance (Xiao *et al*., 2021). Autoantibodies are not only diagnostic markers but also actively participate in pathogenesis by contributing to immune complex formation, enhancing inflammation and causing damage to various organs (Song *et al*., 2025; Pisetsky, 2023). In some cases, autoantibodies may block or alter cellular functions.

Their detection plays a crucial role in the early diagnosis of autoimmune diseases. One important point is that autoantibodies can be detected in some individuals without any clinical symptoms; this condition is referred to as preclinical autoimmunity (Olsen *et al*., 2022).

4. Etiopathogenesis of autoimmune diseases

Autoimmune diseases are pathological conditions that develop as a result of an abnormal immune response directed against the body's own components (Wang *et al*., 2015). Clinically, this process manifests with inflammation, tissue damage and various systemic or organ-specific symptoms (Song *et al*., 2025). Both T and B lymphocytes are involved in the pathogenesis of these diseases (Pisetsky, 2023). From a clinical perspective, the autoimmune response may present in two main forms: systemic form (involving multiple organs such as the skin, joints, kidneys and hematological system) and organ-specific form (damage to a single organ such as the thyroid gland, pancreas or gastric mucosa). In addition, the diseases may have either an acute or chronic course. Although autoimmune diseases are widespread and affect all age groups, they are observed more frequently in women (Miller, 2023). In general, autoimmune diseases develop as a result of the interaction between genetic predisposition and environmental factors (Tizaoui *et al*., 2021). The genetic component is complex and may involve functional alterations in several genes responsible for immune regulation. Approximately 5% of the global population is affected by these diseases (Wang *et al*., 2015). Autoimmune diseases are usually long-term and progressive in nature and similar to other complex diseases (such as cancer), develop gradually (Bieber *et al*., 2023). The main stages of pathogenesis include the formation of autoantigen-specific lymphocytes and autoantibodies, followed by the development of clinical disease (Bieber *et al*., 2023; Pisetsky, 2023).

5. Principles of laboratory diagnosis of autoimmune diseases

The diagnosis of these diseases is not based on a single test and requires a comprehensive evaluation of clinical findings, laboratory analyses and instrumental investigations. The main goal is to confirm the activity of the immune system against self-antigens and to determine organ damage. Various laboratory methods are used for the detection of autoantibodies, including Enzyme-Linked Immunosorbent Assay (ELISA), indirect immunofluorescence (IIF), Western blot and chemiluminescence immunoassay (CLIA) (Mulhearn *et al*., 2020). Among these serological methods, IIF is considered the gold standard in autoimmune laboratory diagnostics, allowing both visual detection of autoantibodies and pattern analysis. ELISA is used for quantitative and semi-quantitative determination of specific autoantibodies, while immunoblot (line blot) is used for precise identification of autoantibody profiles and differential diagnosis. Laboratory diagnostics play a decisive role in the early detection and effective management of autoimmune diseases. Since these diseases often begin with non-specific clinical symptoms (fatigue, pain, weakness, etc.), it is difficult to establish an accurate diagnosis based solely on clinical findings. Therefore, laboratory tests constitute the cornerstone of the diagnostic process.

6. Dynamics of the screening stage in the laboratory diagnosis of autoimmune diseases

The most important advantage of laboratory diagnostics is early detection and in many cases, autoantibodies can be identified in the blood years before clinical symptoms appear (Olsen *et al*., 2022; Bieber *et al*., 2023). This allows the disease to be detected at an early stage and enables timely intervention to prevent complications. Another important aspect is accurate differential diagnosis, as different autoimmune diseases may present with similar clinical symptoms. The determination of autoantibodies (e.g., ANA, anti-dsDNA, anti-CCP, etc.) plays a key role in distinguishing these diseases from one another. In this context, IIF performed on human epithelial carcinoma cells (HEp-2 and less frequently HEp-2000) is considered the gold standard and the most widely used screening method, particularly for the detection of ANA (Dinse *et al*., 2020; Mulhearn *et al*., 2020). The use of HEp-2 cells as a substrate allows not only the identification of nuclear patterns but also cytoplasmic and mitotic cell patterns. HEp-2000 is a modified substrate designed to increase sensitivity to certain specific autoantibodies such as anti-Ro/SS-A. However, in terms of the overall detection profile of other ANA specificities, no significant difference is observed between HEp-2 and HEp-2000 (Kądziela *et al*., 2025).

In the indirect IIF test, autoantibodies present in the patient's serum bind to target antigens localized within HEp-2 cells. The resulting antigen-antibody complex is then recognized and visualized using secondary antibodies labeled with a fluorochrome specific to the Fc fragment of human immunoglobulin G (IgG). The lowest dilution showing fluorescence in any given pattern is recorded as the ANA titer. Furthermore, the indirect IIF test enables determination of the intracellular localization of the antigen based on the fluorescence pattern. Therefore, the clinical value of the ANA test is not limited to a simple positive or negative result. Immunofluorescence patterns observed on HEp-2 cells provide more specific information about the target antigen of the autoantibody and the associated autoimmune disease. For this reason, correct interpretation of fluorescence patterns is considered an integral part of laboratory diagnostics (Chan, 2022; KLIMUD, 2020).

7. Interpretation of ANA fluorescence patterns and ICAP classification

In order to standardize the interpretation of ANA-IIF patterns, the International Consensus on ANA Patterns (ICAP) system was developed. The ICAP initiative has established a unified consensus for the nomenclature and standard definitions of HEp-2 indirect immunofluorescence patterns. Currently, 29 different ANA patterns have been described by ICAP and the clinical significance of each is evaluated in the context of suspected diseases. This classification aims to eliminate terminological differences between laboratories, ensure uniform expression of results using a standardized nomenclature and improve the accuracy of clinical interpretation. Within the ICAP framework, each fluorescence pattern is classified with a specific Anti-Cell (AC) code (for example, AC-1 homogeneous, AC-2 dense fine speckled, AC-3 centromere pattern). This coding system facilitates the harmonization of laboratory reports, guides the selection of reflex tests and contributes to the optimization of clinical decision-making (Chan *et al*., 2022).

8. Diagnostic significance of ANA patterns

According to ICAP, ANA patterns are mainly divided into nuclear, cytoplasmic and mitotic groups. Although each ANA pattern has certain clinical associations, their interpretation should not rely solely on laboratory findings but must be evaluated together with the patient's clinical symptoms and other serological markers. Nuclear patterns, in particular, are among the most frequently observed and clinically significant fluorescence patterns in the initial laboratory assessment of systemic autoimmune rheumatic diseases (Ranjan *et al*., 2025).

In the nuclear homogeneous pattern, the nuclei of HEp-2 cells are uniformly stained during the interphase stage (Figure 1). Strong homogeneous fluorescence of chromatin structures in the metaphase stage confirms this pattern. It is mainly associated with anti-dsDNA, anti-histone and anti-nucleosome antibodies and is particularly observed in SLE and drug-induced lupus (Kądziela *et al*., 2025).

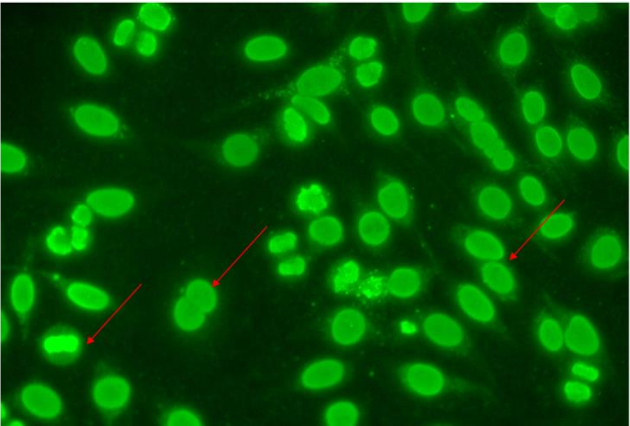

Figure 1. AC-1 nuclear homogeneous pattern (IIF)

In the dense fine speckled (DFS-70) pattern, dense and fine granular staining is observed throughout the nucleoplasm of interphase nuclei (Figure 2). The detection of DFS70 pattern is more often associated with non-autoimmune conditions (such as infections and malignancies) rather than systemic autoimmune rheumatic diseases (Kądziela *et al*., 2025).

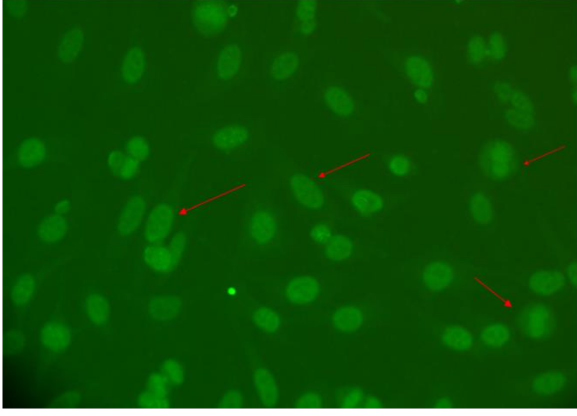

Figure 2. AC-2 nuclear dense fine speckled pattern (IIF)

In the centromere pattern, discrete punctate staining corresponding to the number of chromosomes is observed in interphase nuclei, while distinct dotted fluorescence appears on chromatin in metaphase (Figure 3) (https://www.anapatterns.org/index.php). This pattern is mainly associated with autoantibodies against CENP-A, CENP-B and CENP-C antigens. It is most commonly linked to CREST syndrome, systemic sclerosis (SSc) and Raynaud's phenomenon. Anti-centromere antibodies have high diagnostic specificity and are important markers, particularly in limited forms of systemic sclerosis (Kądziela *et al*., 2025; KLIMUD, 2020).

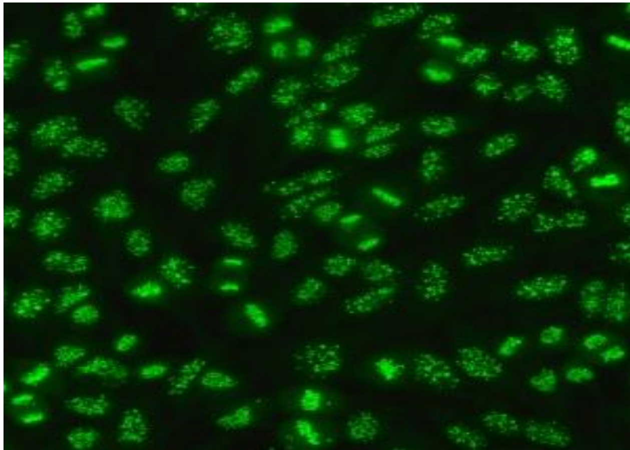

Figure 3. AC-3 centromere pattern (IIF)

The nuclear fine speckled pattern (AC-4) shows small, uniformly distributed fine fluorescent granules throughout the nucleoplasm. It is mainly associated with anti-SS-A/Ro and anti-SS-B/La autoantibodies (Figure 4) (https://www.anapatterns.org/index.php). These antibodies are strongly associated with Sjögren's syndrome and SLE. In Sjögren's syndrome, AC-4 is one of the most characteristic ANA patterns and additional testing for anti-Ro/SSA and anti-La/SSB is recommended (Chan *et al*., 2022; Vílchez-Oya *et al*., 2022).

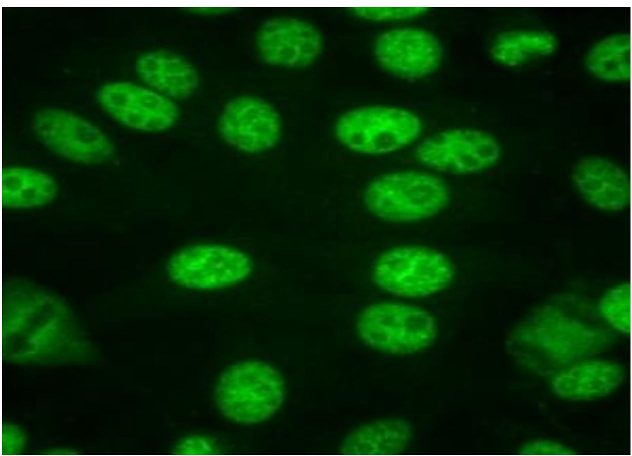

Figure 4. AC-4 nuclear fine speckled pattern (IIF)

In the **nuclear coarse speckled pattern (AC-5),** larger and more prominent coarse granular fluorescence is observed throughout the nucleus (Figure 5). This pattern is

mainly associated with anti-Sm and anti-U1-RNP autoantibodies. Anti-Sm antibodies have high specificity for systemic lupus erythematosus (SLE), whereas anti-U1-RNP is particularly characteristic of mixed connective tissue disease (MCTD). In addition, the AC-5 pattern may also be observed in some cases of systemic sclerosis and overlap syndromes. According to ICAP data, the AC-5 pattern is one of the patterns most strongly associated with anti-Sm and anti-U1-RNP and it has high diagnostic value in clinical interpretation (Chan *et al*., 2022; Andrade *et al*., 2024).

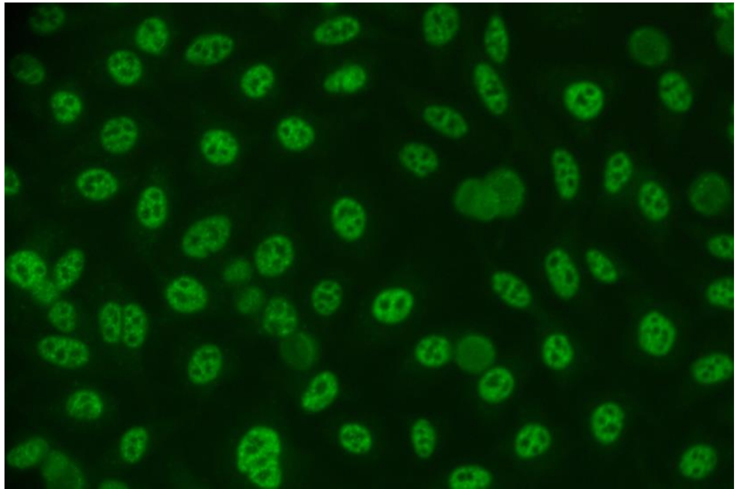

Figure 5. AC-5 nuclear coarse speckled pattern (IIF)

In the nuclear dot pattern, typically 6-10 discrete dots are observed in interphase nuclei, with no staining in metaphase chromatin (Figure 6) (https://www.anapatterns.org/index.php). This pattern is associated with autoantibodies against nuclear antigens such as Sp100, PML and NXP-2. It is most commonly associated with primary biliary cholangitis (PBC), but may also be observed in Sjögren's syndrome (SS), SLE and other chronic inflammatory connective tissue diseases (Kądziela *et al*., 2025; KLIMUD, 2020).

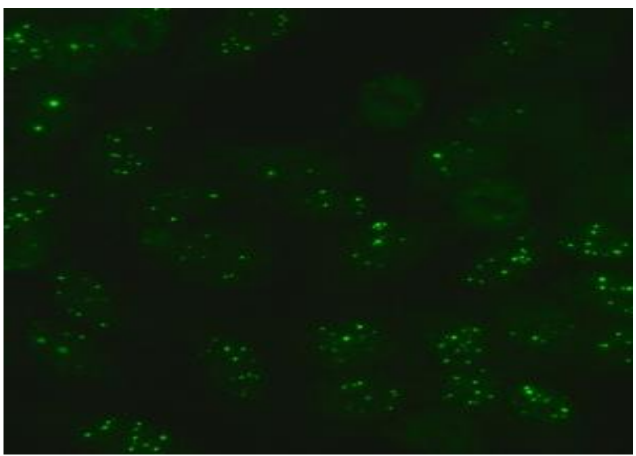

Figure 6. AC-6 multiple nuclear dot pattern (IIF)

In the **homogeneous nucleolar pattern (AC-8),** the nucleoli within the nuclei of interphase cells are distinguished by uniform, smooth and homogeneous fluorescence

staining (Figure 7). This staining typically appears as several large nucleoli, while the metaphase chromatin remains negative. The AC-8 pattern is mainly associated with autoantibodies directed against the PM/Scl complex, Th/To and fibrillarin. In particular, PM/Scl antibodies are linked to polymyositis-scleroderma overlap syndrome, whereas fibrillarin (U3-RNP) antibodies are associated with diffuse systemic sclerosis and pulmonary hypertension. This pattern is considered one of the early immunological markers of systemic sclerosis and has prognostic significance in clinical practice (Chan *et al*., 2022; Mahler *et al*., 2021).

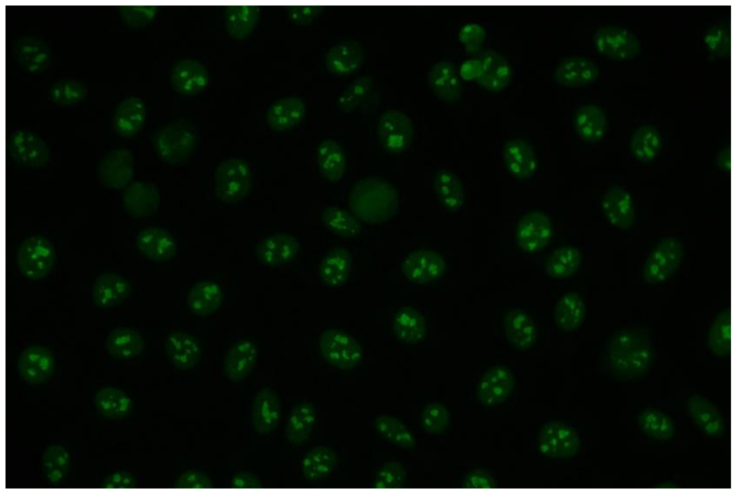

Figure 7. AC-8 homogeneous nucleolar pattern (IIF)

In the **clumpy nucleolar pattern (AC-9),** large, irregular and coarse fluorescent aggregates are observed within the nucleoli. The staining is non-homogeneous and appears as rough, clustered accumulations inside the nucleolus. This pattern is primarily associated with autoantibodies against fibrillarin (U3-RNP) and is especially seen in diffuse forms of systemic sclerosis. Fibrillarin antibodies are more commonly linked to internal organ involvement, particularly affecting the lungs and cardiovascular system.

Detection of the AC-9 pattern may indicate a more aggressive disease course and therefore requires special attention in clinical interpretation ((https://www.anapatterns.org/index.php; Sobanski *et al*., 2021).

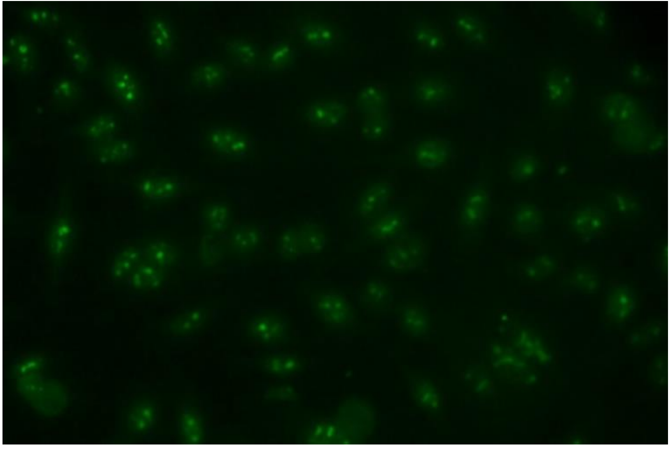

Figure 8. AC-9 clumpy nucleolar pattern (IIF)

In the **punctate nucleolar pattern (AC-10),** multiple fine punctate fluorescent staining is observed within the nucleoli (Figure 9). This pattern has a more delicate and speckled appearance, distinguishing it from AC-8 and AC-9 patterns. It is mainly associated with autoantibodies directed against RNA polymerase I and NOR-90 antigens. NOR-90 antibodies may be seen in systemic sclerosis, Sjögren's syndrome and certain overlap syndromes. RNA polymerase-related autoantibodies are particularly associated with systemic sclerosis and are linked to an increased risk of renal crisis and rapidly progressive skin fibrosis. Therefore, the AC-10 pattern is considered not only a laboratory marker but also a clinically relevant indicator of disease risk (Chan *et al*., 2022).

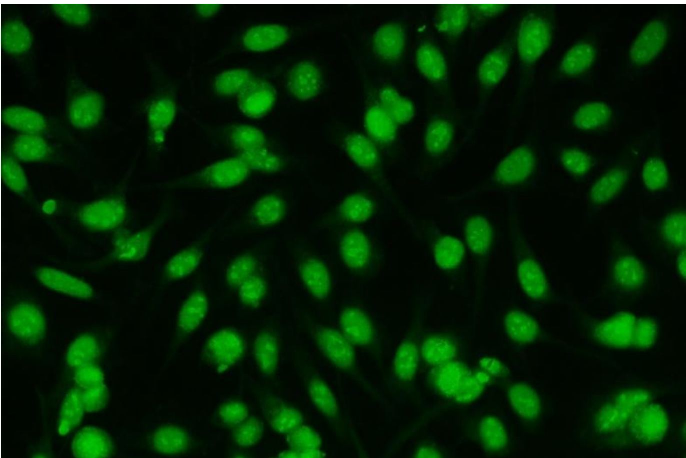

Figure 9. AC-10 punctate nucleolar pattern (IIF)

In the **nuclear membrane pattern**, ring-shaped or punctate staining is observed at the periphery of the interphase nuclei of HEp-2 cells. In the metaphase stage, no chromatin staining is detected (Figure 10) (https://www.anapatterns.org/index.php). This pattern is mainly associated with autoantibodies directed against antigens of the nuclear membrane and nuclear pore complex, including proteins such as lamins, gp210, p62 and the lamin B receptor. It is associated with primary biliary cholangitis (PBC), systemic lupus erythematosus (SLE), chronic active hepatitis, vasculitides and thrombocytopenia (KLIMUD, 2020).

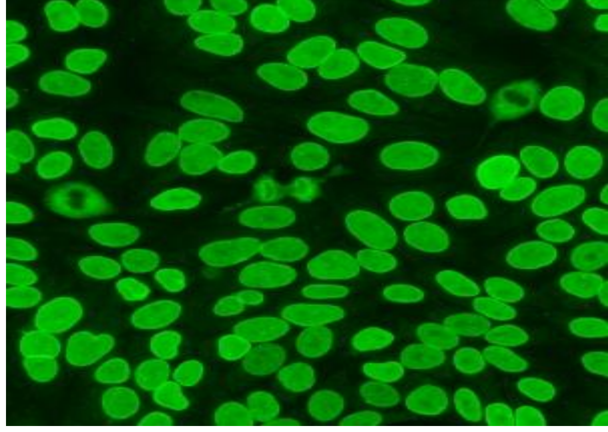

Figure 10. AC-11 smooth nuclear membrane pattern (IIF)

The most commonly observed patterns among cytoplasmic fluorescence characteristics are filamentous, granular, reticular patterns associated with anti-mitochondrial antibodies, the Golgi pattern, as well as “rods and rings” structures.

In the filamentous cytoplasmic pattern, a network-like staining composed of fine fibrils is observed within the cell cytoplasm (Figure 11) (https://www.anapatterns.org/index.php). This pattern is mainly associated with autoantibodies directed against antigens such as vimentin, cytokeratin and tropomyosin. In the presence of anti-vimentin antibodies, the fibrils appear denser particularly around the nucleus, while in metaphase cells, bright spots may be observed outside the chromosomal region. In anti-tropomyosin antibodies, stress fibers extending beneath the plasma membrane are stained. Although not specific to a particular autoimmune disease, this pattern can be detected in various inflammatory and autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Crohn’s disease, ulcerative colitis, Behçet’s disease and systemic sclerosis.

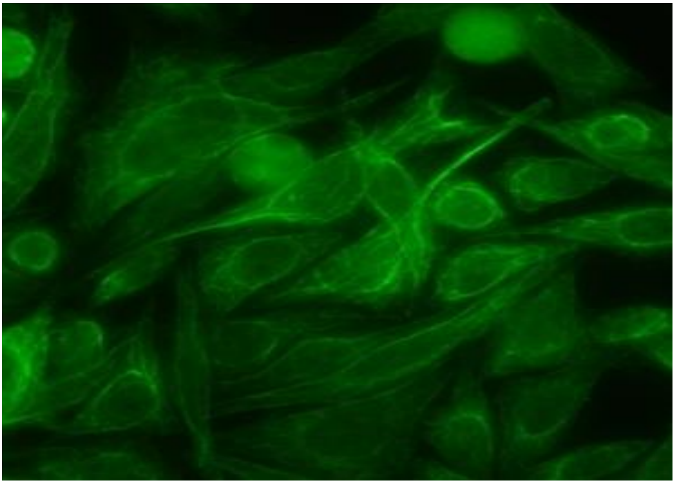

Figure 11. AC-16 filamentous cytoplasmic pattern (IIF)

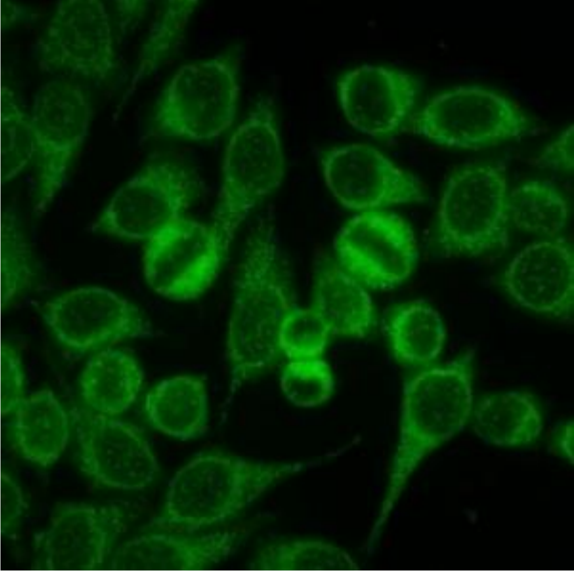

Figure 12. AC-20 cytoplasmic fine speckled pattern (IIF)

In the cytoplasmic granular pattern, fine or coarse granular fluorescence staining is observed throughout the cell cytoplasm, sometimes appearing more intense in the perinuclear region (Figure 12) (https://www.anapatterns.org/index.php). This pattern is associated with autoantibodies directed against cytoplasmic antigens such as ribosomal P and anti-Jo-1. Ribosomal P antibodies are most commonly associated with SLE, particularly with neuropsychiatric manifestations and renal involvement. Anti-Jo-1 antibodies are mainly associated with polymyositis, anti-synthetase syndrome and interstitial lung disease (KLIMUD, 2020).

In the reticular pattern, coarse granular fluorescence is observed in the cytoplasm of HEp-2 cells, particularly more prominent around the nucleus. In liver tissue, intense staining is observed in the cytoplasm of hepatocytes (Figure 13). This pattern is mainly associated with anti-mitochondrial antibodies, especially AMA-M2. It is most characteristic of primary biliary cholangitis (PBC), but may also be observed in scleroderma and certain autoimmune liver diseases (Hsiao *et al*., 2022).

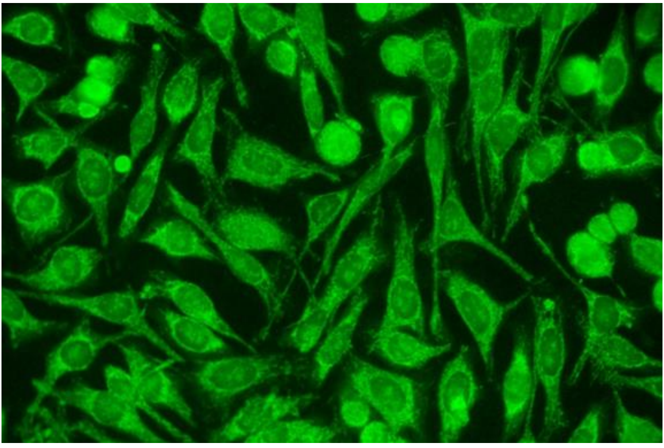

Figure 13. AC-21 cytoplasmic reticular pattern (IIF)

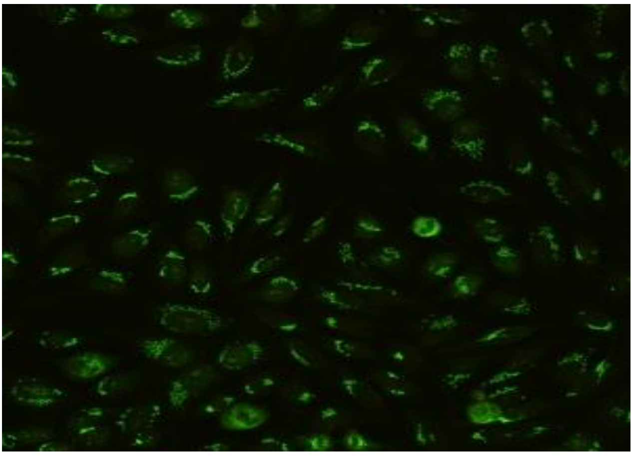

Figure 14. AC-22 cytoplasmic polar speckled pattern (IIF)

In the cytoplasmic polar speckled pattern, granular staining is observed in the cytoplasm with a polar localization, positioned close to or sometimes in contact with one side of the nucleus (Figure 14). This fluorescence consists of large and irregular granules. This pattern is mainly associated with autoantibodies directed against antigens located in

the peripheral membrane of the Golgi complex. Although it can be observed in SLE, Sjögren's syndrome (SS) and some chronic rheumatic diseases, its clinical specificity is low (Salman & Dinç, 2024).

In the cytoplasmic rods and rings pattern, characteristic rod- and ring-shaped fluorescent structures are observed in the cytoplasm of HEp-2 cells. In metaphase cells, chromatin staining is not observed (Figure 15) (https://www.anapatterns.org/index.php). This pattern is mainly associated with autoantibodies directed against the inosine monophosphate dehydrogenase 2 (IMPDH2) antigen. It is most commonly observed in patients with hepatitis C following interferon and ribavirin therapy and more rarely in SLE, Hashimoto's thyroiditis and even in healthy individuals (Calise & Chan, 2020).

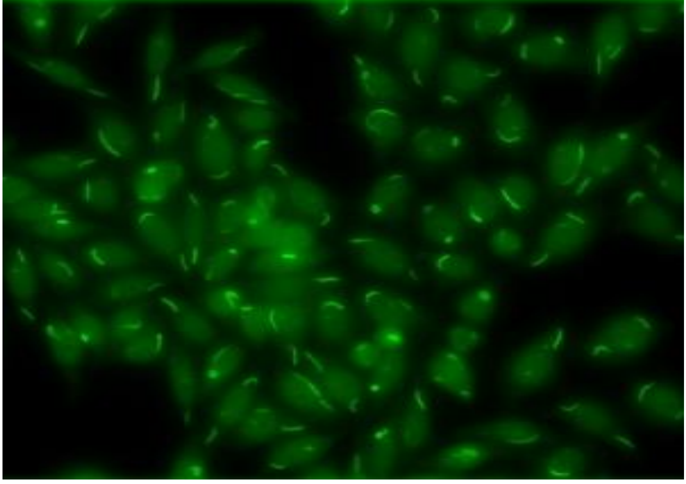

Figure 15. AC-23 cytoplasmic rods and rings pattern (IIF)

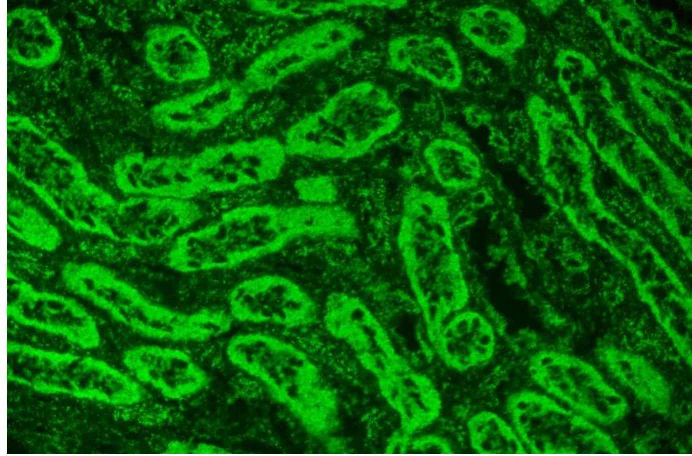

Figure 16. Characteristic fluorescence staining of AMA (Antimitochondrial antibodies) (IIF)

Mitotic patterns are observed in dividing HEp-2 cells and are associated with autoantibodies directed against structures of the mitotic apparatus. These mainly include centrosome, spindle fiber and intercellular bridge patterns. In the centrosome pattern, punctate staining is observed near the nucleus, while in the spindle fiber pattern, fluorescence is seen along the mitotic spindle fibers (Figure 16). The intercellular bridge pattern is characterized by staining of intercellular bridges during the telophase stage. These patterns are most commonly associated with Raynaud's phenomenon, systemic

sclerosis, CREST syndrome, Sjögren's syndrome and mixed connective tissue disease (Hsiao *et al*., 2022; Kądziela *et al*., 2025). Anti-mitochondrial antibodies (AMA) are directed against proteins of the inner and outer mitochondrial membranes and are classified into several subtypes, designated AMA-M1 through AMA-M9. Among these, AMA-M2 has the greatest clinical significance and is primarily directed against the E2 subunit of the 2-oxo acid dehydrogenase complex (PDC-E2). During indirect immunofluorescence (IIF), granular cytoplasmic staining is observed in the proximal and distal tubules of the kidney, in the hepatocyte cytoplasm of the liver and in gastric tissue. This pattern is particularly characteristic of primary biliary cholangitis (PBC) (KLIMUD, 2020; Li *et al*., 2025).

In addition, high AMA-M2 titers may also be observed in SLE, systemic sclerosis (SSc), Sjögren's syndrome (SS), inflammatory myopathies, type 1 autoimmune hepatitis and chronic viral hepatitis. However, since they do not correlate with disease activity or treatment response in primary biliary cholangitis (PBC), they are not considered a prognostic marker (Colapietro *et al*., 2022).

The *Crithidia luciliae* (*C. luciliae*) IIF test is one of the confirmatory methods with high specificity for the detection of anti-dsDNA antibodies. In this method, the protozoan *C. luciliae*, which contains only double-stranded DNA (dsDNA) in its kinetoplast, is used as the substrate (Figure 17). When anti-dsDNA antibodies are present in the patient's serum, they bind to the kinetoplast DNA and after the addition of an anti-IgG conjugate, characteristic fluorescence staining is observed under a fluorescence microscope (KLIMUD, 2020).

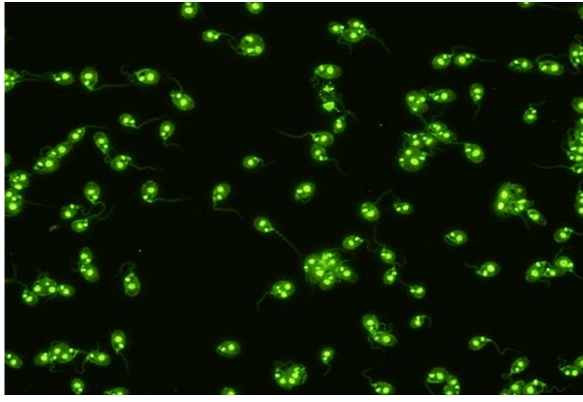

Figure 17. Fluorescence staining on *Crithidia luciliae* substrate (IIF)

Anti-DNA antibodies exist in two forms: those directed against single-stranded DNA (ssDNA) and those against double-stranded DNA (dsDNA). Clinically, anti-dsDNA antibodies are more significant and represent one of the most important and highly specific serological biomarkers for systemic lupus erythematosus (SLE) (Orme *et al*., 2021; Rekvig, 2020). These antibodies are particularly associated with lupus nephritis, disease activity and flares and high titers are linked to increased risk of renal involvement and nephritis (Al-Mughales, 2022; Bruschi *et al*., 2024).

The *C. luciliae* test is mainly used to confirm anti-dsDNA results in SLE. Despite its high specificity, it has limited value in monitoring disease activity because it provides semi-quantitative results. Since anti-dsDNA antibodies may also be observed in certain infections and other autoimmune diseases, not every positive result should be directly interpreted as SLE (Rekvig, 2025).

Antineutrophil cytoplasmic antibodies (ANCA) are specific autoantibodies directed against antigens in the cytoplasmic granules of polymorphonuclear neutrophils (Figure 18). These autoantibodies are considered important serological markers, particularly in the diagnosis of small-vessel vasculitides. ANCA autoantibodies activate neutrophils, leading to endothelial damage and the development of vasculitis. A positive result, when combined with clinical suspicion, has diagnostic significance, while a rising titer may indicate relapse. Since ANA positivity can mask p-ANCA, confirmation with MPO-ANCA and PR3-ANCA by ELISA is recommended in such cases (Wang *et al*., n.d.).

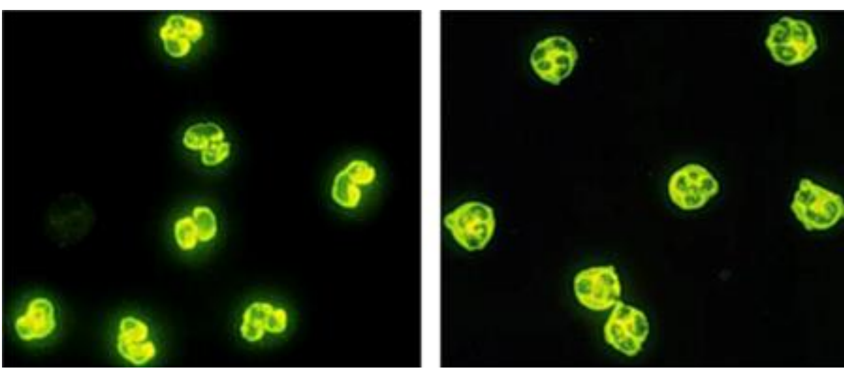

Figure 18. Characteristic fluorescence staining of p-ANCA and c-ANCA (IIF)

The initial evaluation of ANCA is performed by IIF on ethanol-fixed peripheral blood neutrophils. Two main patterns are typically observed: cytoplasmic c-ANCA and perinuclear p-ANCA patterns (Figure 19). The c-ANCA pattern is characterized by diffuse fluorescence staining throughout the cytoplasm. This pattern is primarily directed against the PR3 antigen and is most commonly associated with granulomatosis with polyangiitis (GPA). In contrast, the p-ANCA pattern shows staining around the nucleus, is mainly directed against the MPO antigen and is more frequently observed in cases of microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) (Khalid & Aeddula, 2024).

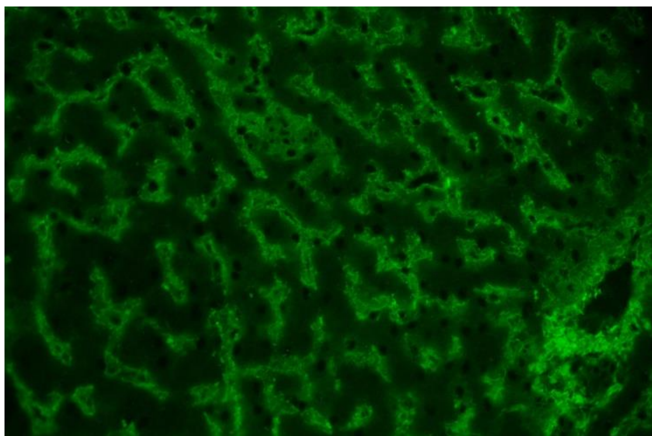

Figure 19. Characteristic fluorescence staining of Anti-EMA (Endomysial antibodies) (IIF)

Anti-endomysial antibodies (anti-EMA) are mainly of the IgA class and are directed against the endomysium surrounding intestinal smooth muscle fibers, with the primary target antigen being tissue transglutaminase (tTG) (Figure 20). EMA is considered an

important serological marker with high specificity, particularly in the diagnosis of celiac disease and is used as a confirmatory test (Zanchi *et al*., 2024).

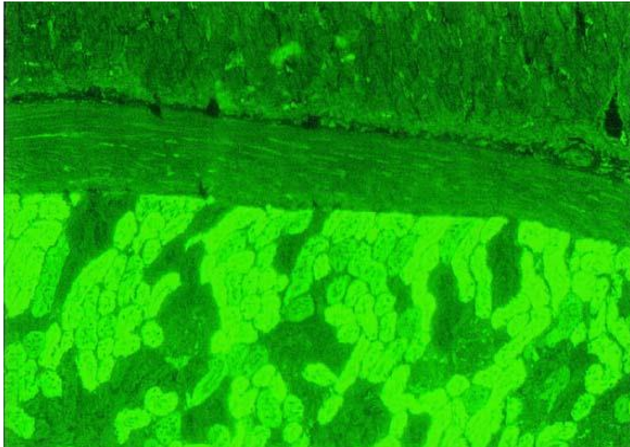

Figure 20. Characteristic fluorescence staining of Anti-LKM (liver kidney microsomal antibodies) (IIF)

The detection of EMA is primarily performed by IIF. Substrates such as primate esophagus, monkey liver or human umbilical cord tissue may be used. In EMA positivity, characteristic fluorescence is observed along the endomysial fibers. Although anti-tTG is more commonly used for screening, EMA strongly supports the diagnosis due to its high specificity. However, since false-negative results may occur in cases of selective IgA deficiency, evaluation of total IgA levels is essential (Zanchi *et al*., 2024).

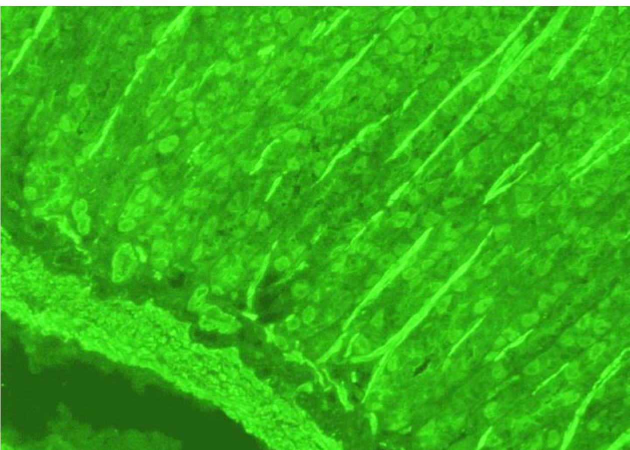

Figure 21. Characteristic fluorescence staining of ASMA (Anti-smooth muscle antibodies) (IIF)

Anti-liver kidney microsomal (anti-LKM) antibodies are autoantibodies directed against microsomal antigens of the liver and kidney and are considered important serological markers, particularly in the diagnosis of autoimmune hepatitis. Clinically, the most significant subtype is anti-LKM-1, with the primary target antigen being cytochrome P450 2D6 (CYP2D6). This autoantibody is closely associated with autoimmune hepatitis type 2 (AIH-2) and has diagnostic value (Bock *et al*., 2025).

During indirect IIF, anti-LKM antibodies are mainly evaluated on kidney sections. Characteristically, in mouse kidney, proximal tubules are stained, whereas distal tubules

remain unstained. In addition, strong homogeneous staining is also observed in the cytoplasm of hepatocytes in liver tissue.

Anti-smooth muscle antibodies (ASMA) are autoantibodies directed against structural components of smooth muscle cells, particularly filamentous actin (F-actin) and are considered important serological markers mainly for autoimmune hepatitis type 1 (AIH-1).

During IIF, ASMA is typically evaluated on mouse or rat stomach, kidney and liver tissue sections. In stomach sections, staining of smooth muscle cells produces a characteristic “dry soil” or “tree bark” appearance. In kidney tissue, simultaneous staining of vessel walls, glomeruli and tubules is known as the VGT pattern (KLIMUD, 2020).

Anti-parietal cell antibodies (APCA) are autoantibodies directed against the parietal cells of the stomach and are mainly associated with pernicious anemia and autoimmune gastritis. These antibodies can be detected in 80-90% of cases in the early stages of the disease, while their positivity decreases in more advanced stages.

The presence of APCA is evaluated by indirect immunofluorescence (IIF). For this purpose, mouse or monkey stomach tissue is commonly used as the substrate. In positive cases, characteristic cytoplasmic staining is observed in gastric parietal cells. These antibodies are considered important serological markers, particularly in the early diagnosis of pernicious anemia.

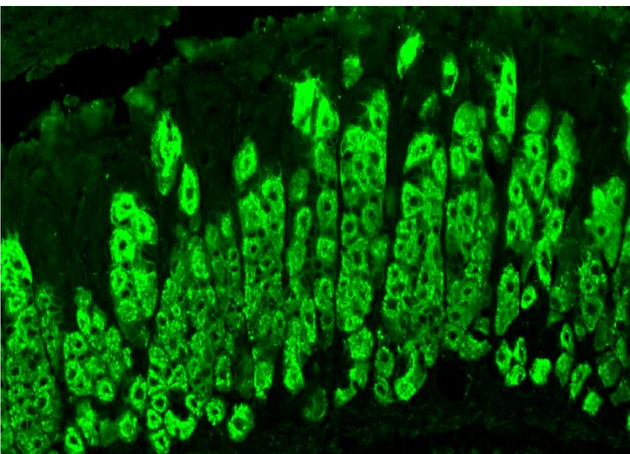

Figure 22. Characteristic fluorescence staining of APCA (Anti-parietal cell antibodies) (IIF)

Although indirect IIF is highly sensitive in ANA screening, it is not always sufficient to precisely determine the specific target antigen of the autoantibody based solely on fluorescence patterns. Therefore, additional confirmatory tests are required to establish a definitive diagnosis. One of the most commonly used confirmatory methods in clinical practice is the Extractable Nuclear Antigen (ENA) panel. The ENA panel enables the detection of specific autoantibodies directed against extractable nuclear antigens and plays a crucial role in the differential diagnosis of systemic autoimmune rheumatic diseases. The identification of specific markers such as anti-Sm, anti-RNP, anti-SSA/Ro, anti-SSB/La, anti-Scl-70 and anti-Jo-1 not only confirms ANA positivity but also allows for a more accurate assessment of the clinical course and disease direction.

9. Line blot technology and determination of autoantibody profiles

Anti-DFS70 antibodies are widespread in the body and target the DFS70 protein (70 kDa), commonly known as the lens epithelium-derived growth factor (LEDGF) (Panafidina *et al*., 2024). The presence of anti-DFS70 antibodies in high titers and in isolation is considered a reliable marker for differentiating ANA-positive healthy individuals from those with connective tissue diseases (CTD) (Cheng *et al*., 2021). Furthermore, the detection of anti-DFS70 antibodies alongside other ANA subtypes is regarded as a prognostic indicator of a milder clinical course of CTD. Anti-DFS70 antibodies can be detected in both healthy individuals and patients with various inflammatory pathologies, such as atopic dermatitis, asthma and rheumatic diseases (Santler *et al*., 2023) (Figure 23).

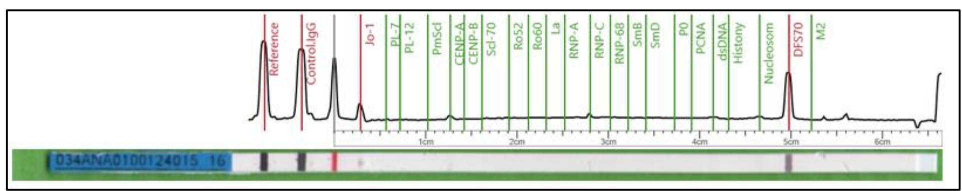

Figure 23. Determination of Jo-1 and DFS70 autoantibodies in the ENA line immunoblot panel

Anti-nRNP antibodies react with U1 RNA-associated proteins (70 kDa, A and C) to form the U1snRNP complex and serve as the primary diagnostic marker for Mixed Connective Tissue Disease (MCTD). These antibodies are detected in approximately 95% of patients (Elhani *et al*., 2023).

In contrast, anti-nRNP antibodies are also detected in approximately 30% of SLE patients. It has been reported that anti-nRNP positive SLE patients exhibit a lower incidence of severe central nervous system (CNS) involvement or renal damage compared to patients with anti-dsDNA antibodies (Kądziela *et al*., 2025).

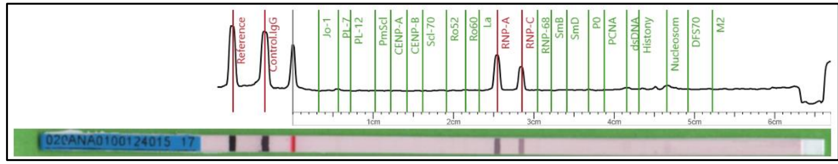

Figure 24. Determination of U1-snRNP complex autoantibodies in the ENA line immunoblot panel

Anti-nRNP antibodies are also associated with arterial hypertension, vascular damage, thrombotic complications and Raynaud's phenomenon, which increases their significance in assessing the clinical course and prognosis (Al-Mayouf *et al*., 2024) (Figure 24).

Anti-Sm antibodies are one of the critical serological biomarkers with high specificity for SLE and target the spliceosomal small nuclear ribonucleoproteins (snRNP). Although the specificity of these antibodies is very high (96-98%), positivity is observed in only a subset of patients (5-40%), making them particularly valuable for confirming the diagnosis (Guimarães *et al*., 2022; Kraev *et al*., 2024).

Furthermore, high titers of anti-Sm antibodies are associated with clinical disease activity, particularly renal, pulmonary and central nervous system involvement, as well as thrombotic complications. Their detection alongside anti-dsDNA and anti-RNP/Sm

antibodies may indicate a more severe clinical course. Additionally, some studies suggest that Epstein-Barr virus infection may stimulate the production of anti-Sm antibodies through molecular mimicry (Kądziela *et al*., 2025; Zamora-Medina *et al*., 2019). Therefore, anti-Sm antibodies are considered important markers not only for diagnostic but also for prognostic purposes.

Anti-Ro/SS-A and Anti-La/SS-B antibodies are important autoantibodies directed against extractable nuclear antigens and are most commonly associated with Sjögren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE). These antibodies can appear years before the onset of clinical symptoms and hold high diagnostic significance. Particularly in Sjögren's syndrome, they are associated with early onset, lymphocytic infiltration of the glands, parotid enlargement and systemic complications (Dragoutsos *et al*., 2024; Vílchez-Oya *et al*., 2022) (Figure 25).

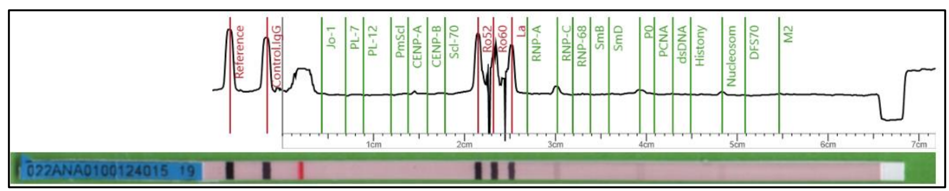

Figure 25. Determination of Anti-SS-A/Ro-60, Anti-SS-A/Ro-52 and Anti-SS-B/La autoantibodies in the ENA line immunoblot panel

Furthermore, they require special attention in pregnant women as they are associated with a risk of neonatal lupus and congenital heart block (Wu *et al*., 2022). The anti-Ro-52 subtype is more closely linked to autoimmune myositis, systemic sclerosis and primary biliary cholangitis. Since ANA can be negative in some cases due to weak expression in HEp-2 cells, it is essential to perform additional serological tests when there is clinical suspicion (Huang *et al*., 2020).

Anti-Scl-70 has been one of the crucial ANA subtypes used in the diagnosis of SSc for approximately thirty years. These antibodies target topoisomerase I, a nuclear enzyme essential for DNA replication and transcription. In indirect immunofluorescence (IIF), they are typically observed with a fine speckled pattern (Cavazzana *et al*., 2023). These antibodies possess high specificity for SSc and are detected in approximately 60% of patients. They are particularly associated with the diffuse cutaneous form of systemic sclerosis, which involves extensive skin involvement and a more severe clinical course (Muruganandam *et al*., 2023; Watad *et al*., 2019).

Anti-Scl-70 positivity shows a stronger association with ulcers, flexion contractures, interstitial lung disease, pulmonary fibrosis, synovitis, muscle involvement and heart blocks. Furthermore, it is linked to increased disease activity and a higher risk of pulmonary hypertension and nephritis and it is considered a vital prognostic indicator for extensive skin involvement and pulmonary fibrosis (Boonstra *et al*., 2020).

Anti-PM/Scl antibodies target the PM/Scl-75 and PM/Scl-100 proteins, which are the primary components of the PM/Scl complex and are associated with RNA processing. In indirect immunofluorescence (IIF), they typically produce a homogeneous nucleolar fluorescence (AC-8) pattern (Cavazzana *et al*., 2023) (Figure 26).

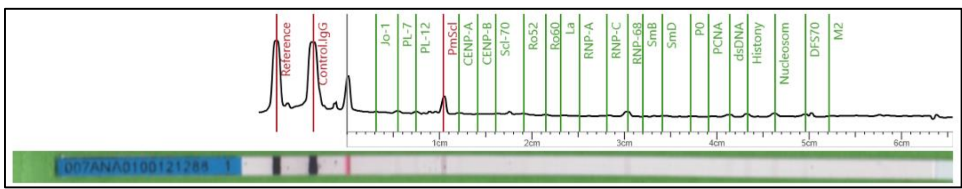

Figure 26. Determination of Anti-PM/Scl autoantibodies in the ENA line immunoblot panel

These antibodies are most frequently observed in SSc, polymyositis, dermatomyositis and especially in SSc/PM overlap syndrome. According to meta-analyses, they are detected in 31% of overla syndrome cases, 11% in dermatomyositis, 8% in polymyositis and 2% in SSc (Wielosz *et al.*, 2021). Anti-PM/Scl positivity is associated with interstitial lung disease (ILD), muscle weakness, myalgia, arthritis, Raynaud's phenomenon, dysphagia and sclerodactyly and is considered a particularly important indicator for ILD. Anti-PM/Scl-75 shows a stronger association with joint contractures, while anti-PM/Scl-100 is associated with elevated creatine kinase levels.

Anti-Jo-1 is the most common anti-synthetase antibody, targeting the cytoplasmic protein histidyl-tRNA synthetase. This enzyme is involved in the attachment of histidine to tRNA during protein synthesis. Anti-Jo-1 antibodies are typically detected in approximately 15-30% of patients with idiopathic inflammatory myositis (IIM) and are considered particularly characteristic of polymyositis (PM) and dermatomyositis (DM) (Kądziela *et al.*, 2025) (Figure 23).

Due to the high expression of the target antigen in lung and muscle tissues, these antibodies are associated with interstitial lung disease (ILD) and a more severe clinical course. Anti-Jo-1 positivity is primarily associated with the classic triad of anti-synthetase syndrome: idiopathic ILD, inflammatory muscle disease and non-erosive arthritis. Furthermore, it may be accompanied by Raynaud's phenomenon, 'mechanic's hands,' fever and relapses (Yang *et al.*, 2024).

In some cases, anti-synthetase syndrome (ASyS) can also be observed as an overlap syndrome with SSc, RA or SS. Although it has been reported that there may be a positive correlation between anti-Jo-1 antibody titers and disease activity, further studies are required to fully confirm this (Huang & Aggarwal, 2020).

Anti-ribosomal P protein antibodies (anti-Rib-P) are considered markers for SLE with low sensitivity but high specificity, being detected in approximately 10-40% of patients. They are rarely found in healthy individuals or in other autoimmune diseases. These antibodies target the P0, P1 and P2 phosphoproteins located in the 60S subunit of the ribosome and are involved in the translation process. In some cases, they may also be observed in autoimmune hepatitis (Ge *et al.*, 2022).

Anti-Rib-P positivity in SLE may be associated with neuropsychiatric, renal, articular, hepatic, cutaneous and hematological manifestations, as well as juvenile SLE. Its detection in some patients who are negative for anti-dsDNA and anti-sm further enhances its diagnostic significance. According to some reports, patients with concurrent anti-dsDNA and anti-Rib-P positivity may experience milder renal involvement, suggesting that these antibodies could potentially play a nephroprotective role (Shi *et al.*, 2020).

Anti-centromere antibodies (ACA) are significant serological markers found in various autoimmune diseases and are most commonly associated with the limited cutaneous form of systemic sclerosis (SSc). ACA positivity is observed in approximately 80% of these patients, whereas it is found in only 10% of those with diffuse systemic

sclerosis (Kajio *et al*., 2021). ACA can also be detected in Sjögren's syndrome (SS), primary biliary cholangitis (PBC), systemic lupus erythematosus (SLE) and other overlap syndromes.

In indirect immunofluorescence (IIF), ACA produces a typical centromere pattern. The primary target antigens are CENP-A, CENP-B and CENP-C. Particularly, anti-CENP-B antibodies are closely linked to CREST syndrome, which is characterized by calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia (Kądziela *et al*., 2025) (Figure 27).

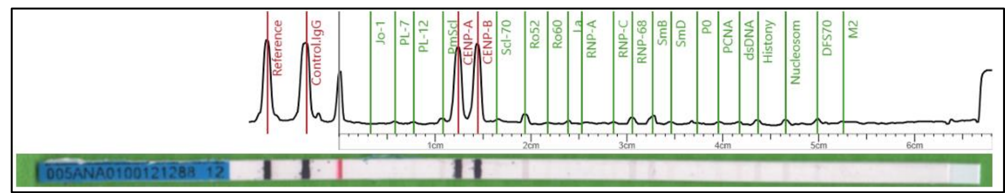

Figure 27. Determination of Anti-CENP-A and Anti-CENP-B autoantibodies in the ENA immunoblot panel

Anti-nucleosome antibodies (ANuA) are antinuclear antibodies directed against the nucleosome, the basic unit of chromatin consisting of DNA and histones. While these antibodies are found in various connective tissue diseases, they exhibit the highest sensitivity and specificity for SLE. The increased immunogenicity of nucleosomes during apoptosis explains their critical role in the pathogenesis of SLE (Obaid *et al*., 2020) (Figure 28).

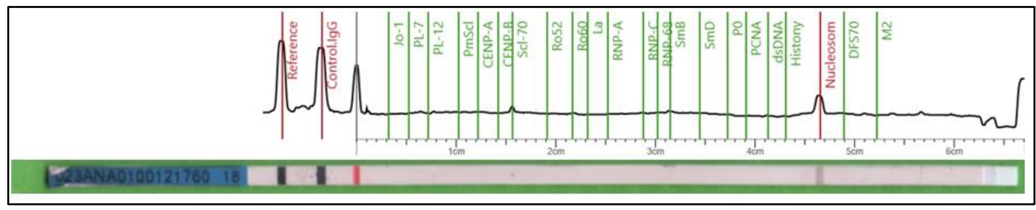

Figure 28. Determination of Anti-nucleosome autoantibodies in the ENA line immunoblot panel

Anti-histone antibodies (AHA) are antinuclear antibodies directed against histone proteins (H1, H2A, H2B, H3 and H4) that participate in immune responses by binding to DNA-protein complexes. AHAs are most commonly detected in the IgG class and typically produce a homogeneous pattern in indirect immunofluorescence (Lee, 2022). These antibodies are primarily associated with SLE and drug-induced lupus erythematosus (DILE). AHAs are observed with a higher frequency in DILE specifically, demonstrating approximately 67% sensitivity and 95% specificity (Kądziela *et al*., 2025).

AHA positivity is observed in approximately 50% of SLE patients and antibodies against H1, H3 and H4 histones are considered particularly characteristic from a diagnostic perspective (Lee, 2022). However, their sensitivity is lower than that of anti-nucleosome antibodies and their specificity is lower than that of anti-dsDNA. Nevertheless, they can support the diagnosis of SLE in complex diagnostic cases.

Proliferating Cell Nuclear Antigen (PCNA) is a nuclear protein involved in DNA synthesis and cell cycle regulation. Although antibodies against PCNA are detected in various systemic autoimmune rheumatic diseases, they are most frequently observed in

SLE. For PCNA, the sensitivity toward SLE is considered low, while the specificity is high (Kądziela *et al*., 2025).

In indirect immunofluorescence (IIF), PCNA antibodies produce a characteristic nuclear granular pattern. High PCNA titers in SLE may indicate a more severe clinical course associated with renal, central nervous system and hematological involvement. Since PCNA is also an indicator of cell proliferation, it is considered an important prognostic marker in oncology as well. Elevated PCNA levels are observed in malignant epithelial lesions of the breast, lung, duodenum and other sites, in some cases correlating with the tumor stage (Kądziela *et al*., 2025).

For this reason, the HEp-2 IIF method serves not only as a screening test but also plays a crucial role in characterizing autoantibodies directed against various cellular structures. This approach is widely applied in the identification of antibodies against specific cellular components, such as centromere antigens, proliferating cell nuclear antigen (PCNA), U3-ribonucleoprotein (fibrillarin), CENP-F, Cajal bodies, mitotic apparatus structures, as well as 'rods and rings' (Andrade *et al*., 2024).

10. Challenges and false positives in autoantibody interpretation

One of the primary challenges in interpreting autoantibodies is that laboratory positivity does not always equate to clinical disease. Specifically, certain patterns detected in the ANA-IIF test can be incorrectly evaluated as indicators of systemic autoimmune rheumatic diseases (SARD). The dense fine speckled (DFS70, AC-2) pattern is one of the most typical examples in this regard. The DFS70 pattern is frequently observed in healthy individuals, allergic diseases, infections and various non-autoimmune conditions and it is not considered specific for systemic autoimmune rheumatic diseases. However, this approach is only valid when the anti-DFS70 antibody is confirmed to be monospecific meaning it is present without other ENA or disease-related autoantibodies. If anti-DFS70 is detected alongside other pathological autoantibodies, clinical interpretation must be conducted with greater caution. Therefore, establishing a diagnosis based solely on the IIF pattern can lead to significant errors.

Another significant challenge is the misinterpretation of low-titer ANA positivity. Specifically, ANA positivity at a 1:80 serum dilution can be observed in approximately 25-30% of the healthy population and this prevalence increases with age, particularly among elderly women. Consequently, low-titer positivity often holds no clinical significance. The most common error is focusing excessively on laboratory results while neglecting clinical symptoms. However, the ANA test is a screening tool, not a diagnosis in itself. In the absence of clinical manifestations such as arthritis, photosensitivity, Raynaud's phenomenon, xerostomia (dry mouth), muscle weakness or interstitial lung disease, ANA positivity alone is insufficient for the diagnosis of an autoimmune disease.

Other factors contributing to false-positive results include age, gender, infections, certain medications and technical laboratory variations. Viral infections, chronic inflammatory conditions and certain antibiotics, antihypertensives and biological agents can cause transient ANA positivity. Furthermore, differences in substrates between laboratories, the quality of HEp-2 cells, expertise in microscopic interpretation and observer-dependent variability complicate the standardization of results. Specifically, the misidentification of AC-4 and AC-2 patterns - often confusing them with one another - is a frequent problem encountered in clinical practice.

Recent studies indicate that interpreting autoantibody results without a close correlation between clinical features and serological markers can lead to diagnostic errors. In healthy individuals, especially in the elderly population, nuclear fine speckled patterns, DFS70 (AC-2) and homogeneous fluorescence types are most commonly observed. Therefore, the diagnostic value of low-titer ANA positivity is limited and must be confirmed with antigen-specific tests (ENA panel, anti-dsDNA, anti-DFS70, etc.). In the modern approach, the objective is not merely to record a positive result, but to accurately determine the real clinical significance of that result for the patient. The greatest error in autoimmune diagnostics is the 'positive test = disease' mindset; instead, the correct approach follows the principle: 'positive test + matching clinical presentation + specific autoantibody profile = clinically significant result' (Ranjan *et al*., 2025).

11. Integration of laboratory results with clinical context

The interpretation of results in the laboratory diagnosis of autoimmune diseases is not limited to the evaluation of analytical parameters; it is essential to correlate these findings with the patient's clinical condition. Since the detection of serological markers does not always reflect an active disease process, an isolated interpretation of laboratory data can lead to erroneous diagnostic decisions. In particular, the presence of ANA and other autoantibodies can, in some cases, be identified without any clinical symptoms. This condition is most frequently encountered in the healthy population - especially among elderly individuals and women - as low-titer positivity and is considered a serological finding with limited clinical significance. Consequently, establishing a diagnosis based solely on laboratory results increases the risk of overdiagnosis and unjustified treatment.

The clinical value of autoantibodies is closely linked to their type, specificity and titer, as well as the observed immunofluorescence pattern. For instance, high-titer and persistent anti-dsDNA positivity is considered highly specific for systemic lupus erythematosus (SLE) and can be associated with disease activity. Conversely, low-titer and non-specific ANA patterns may be observed in various non-autoimmune conditions. Furthermore, the isolated detection of only the anti-DFS70 antibody is recognized as a crucial differential indicator that helps reduce the probability of systemic autoimmune rheumatic disease (Ranjan *et al*., 2025).

The consideration of clinical data is a fundamental prerequisite for the correct interpretation of laboratory results. The presence of clinical signs such as arthritis, photosensitivity, oral ulcers, Raynaud's phenomenon, interstitial lung involvement or muscle weakness enhances the diagnostic significance of specific autoantibodies. Conversely, in the absence of these clinical findings, the positivity of serological markers alone is insufficient for a diagnosis. Furthermore, monitoring the temporal variability of serological indicators is clinically significant. Certain autoantibodies, particularly anti-dsDNA, may fluctuate in parallel with disease activity and flares. Therefore, dynamic monitoring is considered more informative for clinical decision-making than a single test result. The integration of laboratory and clinical data also plays a vital role in the selection of reflex tests. Choosing additional specific tests based on screening results and clinical suspicion increases diagnostic accuracy and prevents unnecessary testing. This approach also enables more efficient use of laboratory resources. Consequently, evaluating laboratory results within the clinical context is a core principle in autoimmune diagnostics. Instead of an approach based solely on laboratory indicators, an integrative

analysis involving clinical signs, supplementary laboratory data and instrumental findings facilitates more accurate diagnostic outcomes.

12. Diagnostic integration of screening and confirmation phases

In connective tissue diseases, most ANA titers do not show a direct correlation with disease activity and routine monitoring is generally not recommended. The primary exception is anti-dsDNA antibodies, which correlate with SLE activity, particularly in cases of renal involvement, serositis and hematological changes. Diagnostically, anti-dsDNA and anti-Sm are more characteristic of SLE, anti-Mi2 and anti-Jo1 of dermatomyositis and anti-Scl-70 of systemic sclerosis. Anti-SSA (Ro) and anti-SSB (La) are frequently observed in SLE, Sjögren's syndrome and neonatal lupus, while anti-RNP is associated with mixed connective tissue disease and PM-Scl is more common in overlap syndromes. Therefore, the interpretation of autoantibodies must strictly be performed within the clinical context (Kądziela *et al*., 2025). In accordance with this principle, the Indirect Immunofluorescence laboratory at the central branch of Inci Laboratories is equipped with modern automated systems. At this facility, the high-sensitivity and high-precision determination of autoantibodies is carried out using the iPRO V2 (BioSystems, Spain) and RAYTO Blotray 866 (BioSystems, Spain) devices. These instruments operate in integration with the Indirect Immunofluorescence and ImmunoBlot (LineBlot) methods - considered the gold standards in autoimmune diagnostics - enabling the acquisition of results with high accuracy and reliability.

Figure 29. Inci Laboratories, Central Branch; Autoimmune Diagnostic Laboratory

The iPRO V2 is a high-precision analytical system that enables fully automated testing of autoimmune markers via the Indirect Immunofluorescence (IIF) method. The device automatically prepares serum dilutions using phosphate-buffered saline (PBS), dispenses the required volumes of reagents, performs incubation and washing stages and

ensures the reproducibility of results. The system provides high throughput with the capacity to process up to 96 slides simultaneously, compatibility with various sample tube sizes and barcode-based identification capabilities. It significantly reduces laboratory workload through automated reagent management and an optimized workflow. The device's automated IIF technology ensures high-sensitivity detection of markers such as ANA (anti-nuclear antibody), anti-dsDNA (double-stranded DNA), AMA (anti-mitochondrial antibody), ASMA (anti-smooth muscle antibody), ANCA-profile (anti-neutrophil cytoplasmic antibody), anti-EMA (anti-endomysial antibody), LKM (liver-kidney microsome) and APCA (anti-parietal cell antibody). Through these markers, the laboratory diagnosis of both systemic and organ-specific autoimmune diseases is conducted with exceptional accuracy and reliability.

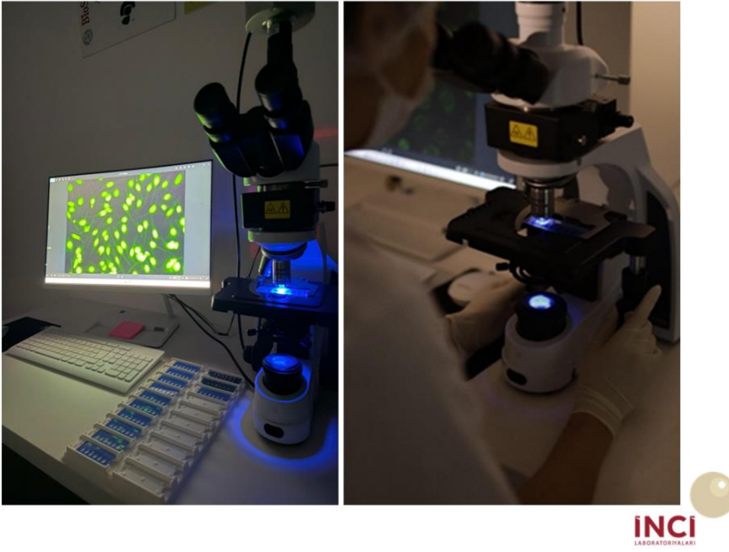

Figure 30. Visual identification of samples using the Fluorescence Manual Microscope

The Fluorescence Manual Microscope (BioSystems, Spain) is a laboratory instrument used for the visual (manual) evaluation of IIF assays. This microscope enables the detection of antigen-antibody interactions in samples placed on slides and allows for the determination of antibody titers based on fluorescence intensity. The manual fluorescence microscope provides laboratory specialists with the capability to evaluate fluorescence signals, identify staining patterns, verify sample quality and perform precise confirmation of results alongside automated systems. The observation process is conducted in a darkroom environment. This ensures that fluorescence signals are more clearly visible, minimizes the effect of background light and allows for the accurate detection of even weak signals.

The RAYTO Blotray 866 is a high-precision and reliable laboratory system designed for the automation of immunoblot assays. The device enables the loading and parallel processing of up to 40 strips within a single workflow, allowing immunoblot tests to be conducted in a rapid, standardized and reproducible manner.

The system executes the incubation and washing stages in a fully automated manner, which helps minimize errors resulting from the human factor. After scanning the test panels, the device's software automatically correlates the detected positive signals with the corresponding diseases or biomarkers and presents them on the user interface. This feature enables physicians and laboratory specialists to interpret results in a more

efficient, accurate and standardized fashion. The Blotray 866 is compatible with various autoimmune, infectious and allergen panels. Immunoblot tests, including extended ENA profiles, vasculitis panels, myositis panels and autoimmune liver panels, are actively performed using this system.

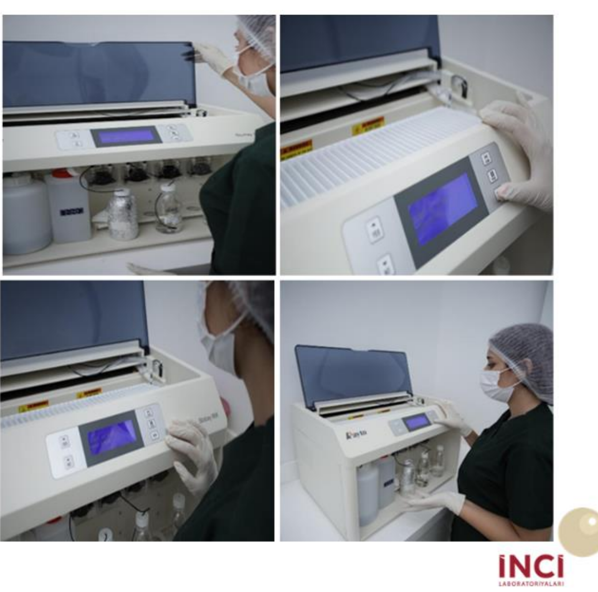

Figure 31. RAYTO Blotray 866; Operating principle of the automated immunoblot analysis system

The Immunoblot and iPRO V2 systems are utilized as a complementary two-stage approach in autoimmune diagnostics. The iPRO V2 performs the screening phase, identifying general autoantibody positivity in samples in a rapid and automated manner. In contrast, the Immunoblot system serves as the confirmatory stage. Samples that yield positive or borderline results during screening are analyzed via immunoblotting to clarify the specific autoantibody profile. The relationship between these two systems is built upon the screening-confirmation principle: the iPRO V2 ensures the initial selection and detection of at-risk samples, while the immunoblot performs result validation and identifies the precise immunological profile. This approach enhances both the speed and accuracy of laboratory diagnostics, enabling a more reliable assessment of systemic autoimmune diseases (International Symposium on Laboratory Diagnosis of Autoimmune Diseases, 2025).

13. Conclusion

The diagnosis of autoimmune diseases is one of the most complex areas of modern laboratory medicine, where the early and accurate interpretation of autoantibodies plays a decisive role. Research indicates that ANA and other autoantibodies serve as crucial biomarkers, not only for diagnostic confirmation but also for predicting the clinical course of the disease. However, establishing a diagnosis based on a single serological marker is scientifically and clinically insufficient. For an accurate assessment of autoimmune diseases, laboratory results must be analyzed in an integrated manner with clinical symptoms, organ involvement and supplementary immunological tests.

The application of modern automated systems significantly enhances accuracy and standardization in laboratory diagnostics, thereby reducing the risk of misinterpretation. However, technological advancement does not diminish the importance of the human factor - namely, clinical experience and immunological reasoning - in the clinical decision-making process.

In conclusion, the field of autoimmune diagnostics has transformed from a screening-based approach into an integrative, multi-marker and clinically oriented decision-making model. This approach establishes the fundamental scientific basis for early diagnosis, personalized treatment and more effective management of autoimmune diseases.

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