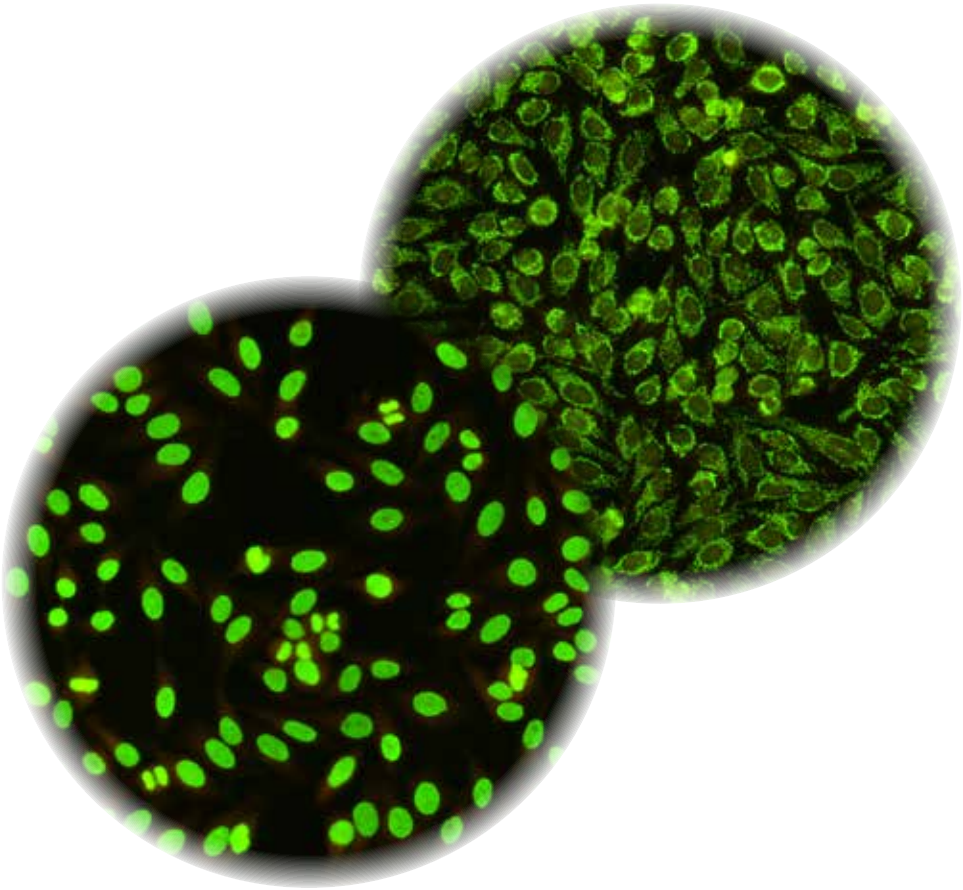
Comprehensive Atlas of HEp-2 cells



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The new language of life

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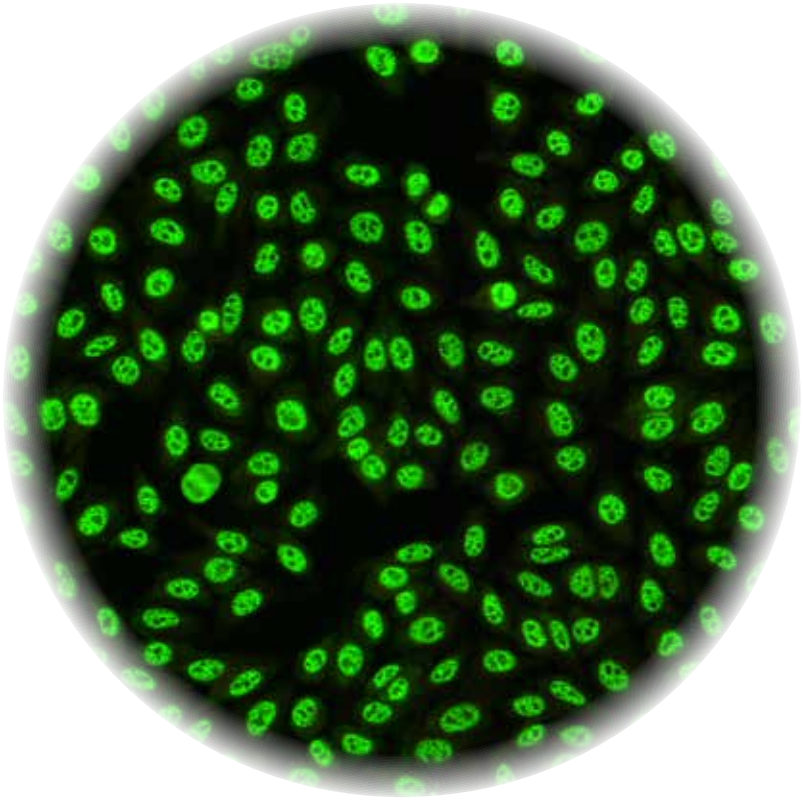


Antinuclear antibodies (**ANAs**) are valuable laboratory markers to screen for and support the diagnosis of various rheumatic diseases (known as ANA-associated rheumatic diseases). ANAs are usually detected by indirect immunofluorescence assay (**IFA**) using HEp-2 cells (**HEp-2 IFA**) and there are many variables influencing the results, such as subjective visual reading, serum screening dilution, substrate manufacturing, microscope components and conjugate.

The method of ANA detection was optimized by using the immortal HEp-2 cell line derived from a human larynx epidermal carcinoma. The use of the HEp-2 cells substrate in IFA increased the sensitivity of the autoantibodies detection and contributed to diagnosis of systemic autoimmune diseases and description of new ones, thanks to the possibility to detect over 100 antigens in their nucleus and cytoplasm.

The **importance of ANA testing** has been reinforced by the inclusion of ANA positivity as an entry criterion in the 2019 Systemic Lupus Erythematosus (SLE) classification criteria. In addition, specific ANAs (such as antibodies to Sm, double-stranded DNA (dsDNA), SSA/Ro60, U1RNP, topoisomerase I, centromere protein B (CENPB), RNA polymerase III and Jo1) are included in classification criteria for other rheumatic diseases.

Antigens Localization in HEp-2 cell

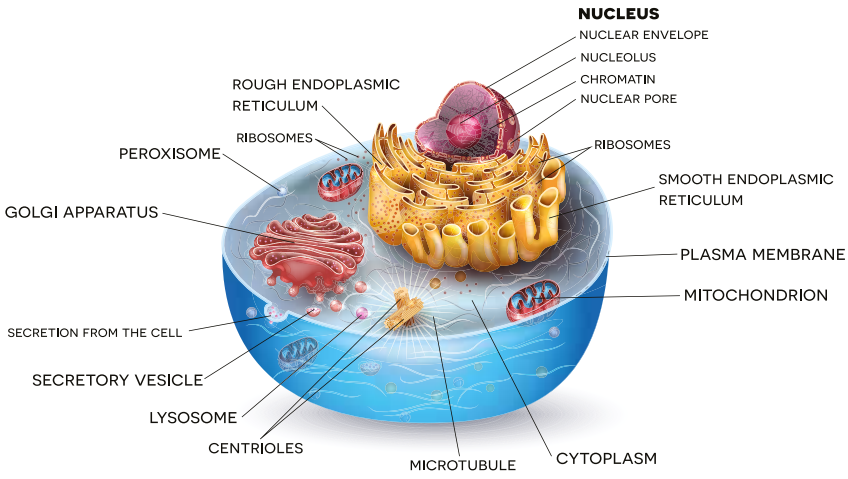


Figure 1. Structure of eukaryotic cell (e.g. HEp-2) and distribution of potential target antigens of autoantibodies

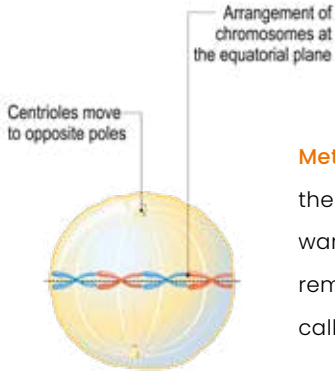
ANAs	Localisation	Antigens	SARDs
Anti-ss-DNA	Chromatin	ss-DNA	SLE
Anti-ds-DNA		ds-DNA	SLE
Anti-Scl-70		DNA topoisomerase I	SSc
Anti-Centromere		Centromere	CREST syndrome
Anti-Histones		Histones	Drug-Induced SLE
Anti-U1RNP	Nucleus	U1RNP	SSc, MCTD, overlap syndrome, UCTD
Anti-Sm		U1, U2, U4/U6, U5 RNP	SLE
Anti-U2 RNP		U2 RNP	Overlap syndrome
Anti-SS-A/Ro		hY1~hY5 RNP	SjS
Anti-SS-B/La		RNA polymerase III transcription factor terminator	SjS
Anti-PCNA		DNA polymerase δ	SLE
Anti-Ku		DNA-binding nuclear protein complex	SjS, SSc, DM, overlap syndrome
Anti-U3RNP	Nucleolus	U3RNP (Fibrillarin)	SSc
Anti-7-2-(Th)RNP		RNaseP, RNaseMRP	SSc
Anti-RNA Polimerasi I		RNA polymerase I	SSc
Anti-PM-Scl		Protein complex (20-110 kDa)	SSc, overlap syndrome
Anti-NOR-90		human upstream binding factor (hUBF)	SSc
Anti-Jo-1	Cytoplasmic	Istidil-tRNA Sintetase	DM/PM
Anti-ribosome		Ribosomal P proteins P0 – P2	LES
Anti-mitochondria		PDC-E2, OGDC-E2, E3BP, PDC-E1α	PBC
Anti-SS-A/Ro		hY1~hY5 RNP	SjS
Others (e.g. actin)		F-actin	AIH

Table 1. ANAs: type, localization and associated systemic autoimmune rheumatic disease (SARDs).

Cell Cycle: Mitosis

Prophase: is the first phase of mitosis, the process that separates the duplicated genetic material carried in the nucleus of a parent cell into two identical daughter cells. During prophase, the complex of DNA and proteins contained in the nucleus, known as chromatin, condenses. The chromatin coils and becomes increasingly compact, resulting in the formation of visible chromosomes. The replicated chromosomes have an X shape and are called sister chromatids. The sister chromatids are pairs of identical copies of DNA joined at a point called centromere. Then, a structure called mitotic spindle begins to form. The mitotic spindle is made of long proteins called microtubules that begin forming at opposite ends of the cell. The spindle will be responsible for separating the sister chromatids into two cells.

PROPHASE

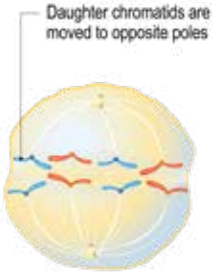


METAPHASE

Metaphase: the cell's chromosomes align themselves in the middle of the cell through a type of cellular «tug of war.» The chromosomes, which have been replicated and remain joined at a central point called the centromere, are called sister chromatids.

Cell Cycle: Mitosis

Anaphase: each pair of chromosomes is separated into two identical, independent chromosomes. The chromosomes are separated by a structure called the mitotic spindle. The sister chromatids are separated simultaneously at their centromeres. The separated chromosomes are then pulled by the spindle to opposite poles of the cell.



ANAPHASE

TELOPHASE

Telophase: it begins once the replicated, paired chromosomes have been separated and pulled to opposite sides, or poles, of the cell. During telophase, a nuclear membrane forms around each set of chromosomes to separate the nuclear DNA from the cytoplasm. The chromosomes begin to uncoil, which makes them diffuse and less compact.

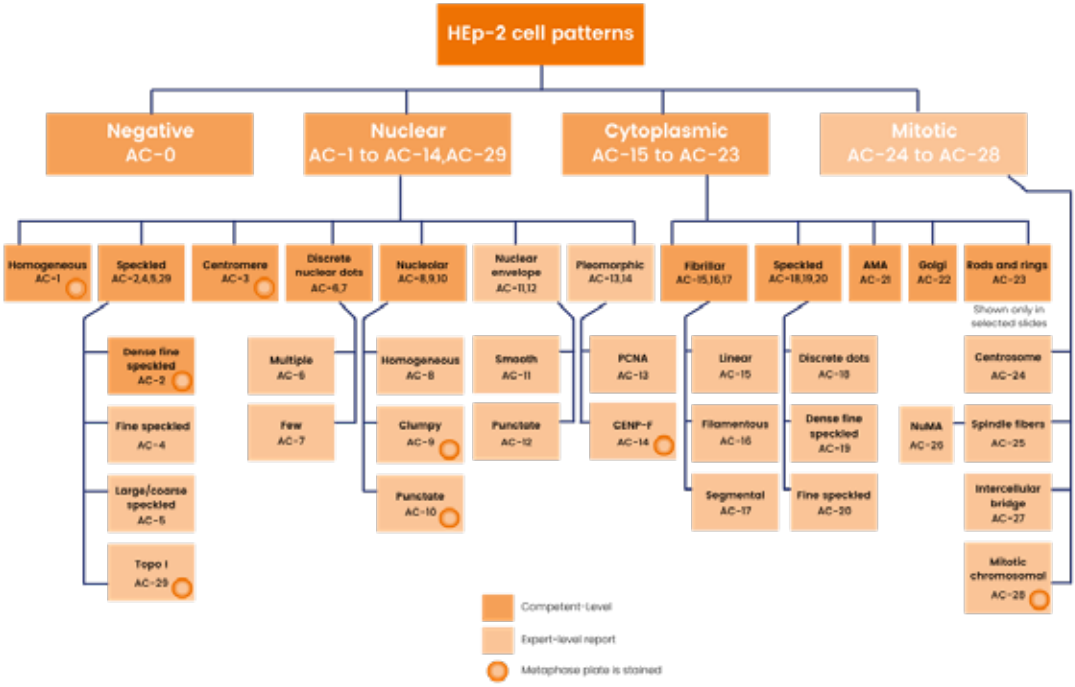
PAIR OF SEPARATE DAUGHTER CELLS

Cytokinesis: it is the physical process of cell division, which divides the cytoplasm of a parental cell into two daughter cells

ICAP Nomenclature

Based on the International Consensus on Antinuclear Antibody Patterns (ICAP), the several ANAs patterns are sorted into 3 main categories, based on the localization of the antigens in the HEp-2 cells:

1. Nuclear (AC-1/AC-14 and AC-29)
2. Cytoplasmic (AC-15/AC-23)
3. Mitotic (AC-24/AC-28)



Glossary

AIH: Autoimmune Hepatitis
AIM: Autoimmune Myositis
ANAs: Anti-Nuclear Antibodies
CREST syndrome: Limited cutaneous form of Systemic Sclerosis
DM: Dermatomyositis
HCV: Hepatitis C Virus
IFA: Indirect Immunofluorescence Assay
MCTD: Mixed Connective Tissue Disease
PM: Polymiositis
PBC: Primary Biliary Cholangitis
RA: Rheumatoid Arthritis
SARDs: Systemic Autoimmune Rheumatic Disorders
SjS: Sjögren's Syndrome
SLE: Systemic Lupus Erythematosus
SSc: Systemic Sclerosis
UCTD: Undifferentiated Connective Tissue Disease

References

International Consensus on Antinuclear Antibody Patterns (ICAP) website: https://www.anapatterns.org/index.php;

Agmon-Levin, N., et al., International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis, 2014. 73(1): p. 17-23;

Damoiseaux, J., et al., Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis, 2019. 78(7): p.879-889;

Bossuyt, X, De Langhe, E, Borghi, MO, Meroni, PL. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol 2020; 16:715–26;

Allan S. Wiik, et al., Antinuclear antibodies: a contemporary nomenclature using HEp-2 cells, Journal of Autoimmunity 2010, Volume 35, Issue 3;

Bloch DB, Nobre RA, Yang WH. GW/P-bodies and autoimmune disease. Adv Exp Med Biol. 2013;768:61-70. doi: 10.1007/978-1-4614-5107-5_5. PMID: 23224965.

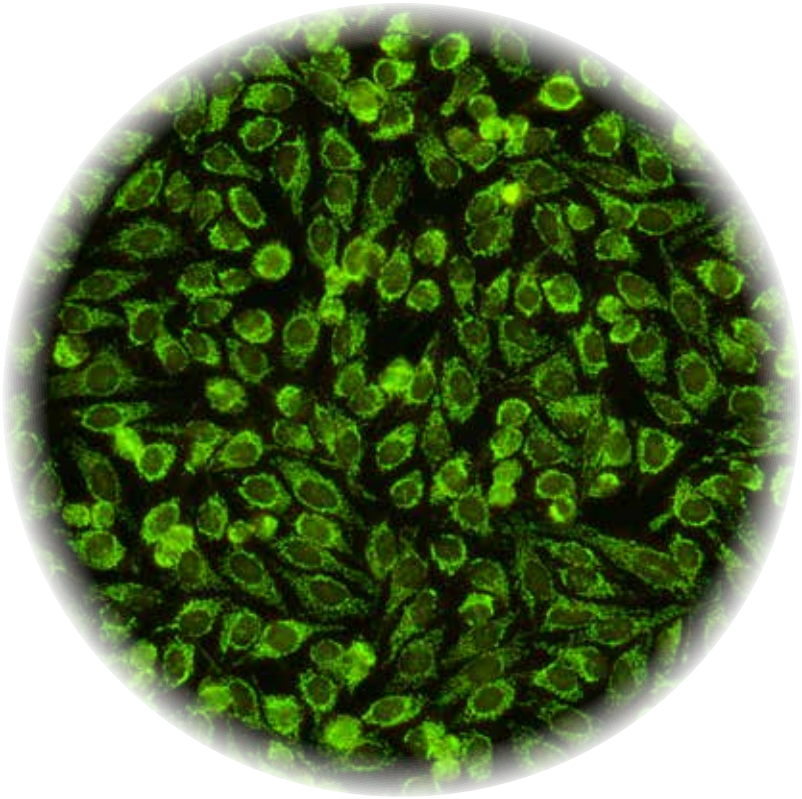
Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016;280:8-23;

European Association for the Study of the Liver. EASL clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis. J Hepatol 2017;67:145-72;

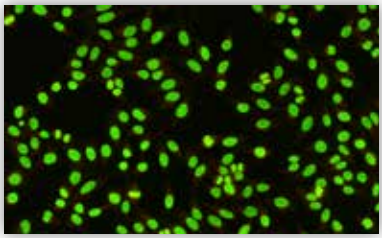
Szalat R, Ghillani-Dalbin P, Jallouli M, et al. Anti-NuMA1 and anti-NuMA2 (anti-HsEg5) antibodies: clinical and Immunological features: a propos of 40 new cases and review of the literature. Autoimmun Rev 2010;9:652-6;

Conrad K, Schössler W, Hiepe F. Autoantibodies in organ specific autoimmune diseases. a diagnostic reference. 8. 2th edn. Autoantigens autoantibodies autoimmunity, 2017.

HEp-2 cells ATLAS
a world of autoantibodies



AC-1 NUCLEAR HOMOGENEOUS



Pattern AC-1:
(dIFine® acquired image)

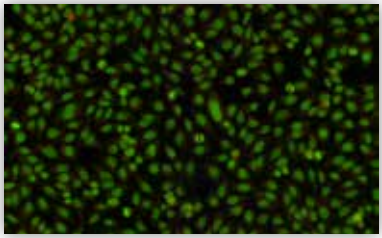
Previous Nomenclature: *diffuse*

Description: homogeneous and regular fluorescence across all nucleoplasm. Mitotic cells have the chromatin mass intensely stained in a homogeneous hyaline fashion.

Antigens: dsDNA, nucleosomes, histones

Clinical value: SLE, chronic AIH or juvenile idiopathic arthritis.

AC-2 NUCLEAR DENSE FINE SPECKLED



Pattern AC-2:
(dIFine® acquired image)

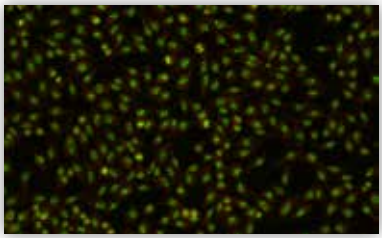
Previous Nomenclature: *none*

Description: speckled pattern distributed throughout the interphase nucleus with characteristic heterogeneity in the size, brightness, and distribution of the speckles. Throughout the interphase nucleus, there are some denser and looser areas of speckles (characteristic feature). The cells in metaphase and telophase show a strong speckled fluorescence of the chromosomes.

Antigens: DFS70/LEDGF

Clinical value: commonly found as high titer HEp-2 IFA-positive in apparently healthy individuals or in patients who do not have a SARDs.

AC-3 CENTROMERE



Pattern AC-3:
(dIFine® acquired image)

Previous Nomenclature: *kinetochore*

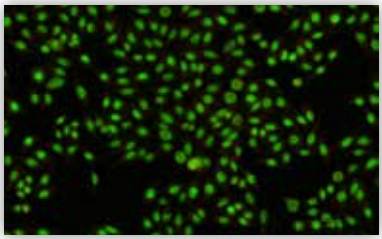
Description: discrete coarse speckles scattered in interphase cells and aligned at the chromatin mass on mitotic cells. e.g. anti-CENP-B.

Antigens: CENP-A/B (C)

Clinical value: patients with CREST syndrome.

NUCLEAR PATTERNS *(AC-1/AC-14 & AC-29)*

AC-4 NUCLEAR FINE SPECKLED



Pattern AC-4:
(dIFine® acquired image)

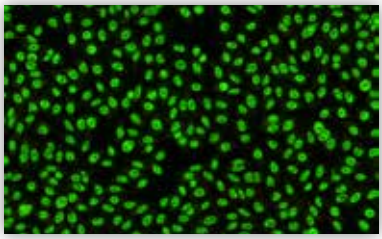
Previous Nomenclature: *fine granular*

Description: fine speckles across all nucleoplasm. The nucleoli may be stained or not. Mitotic cells have the chromatin mass not stained. e.g. anti-SS-A/Ro, anti-SS-B/La.

Antigens: SS-A/Ro, SS-B/La, Mi-2, TIF1γ, TIF1β, Ku

Clinical value: present to a varying degree in distinct SARDs, SjS, SLE, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, DM, SSc, and SSc-AIM overlap syndrome.

AC-5 NUCLEAR LARGE/COARSE SPECKLED



Pattern AC-5:
(dIFine® acquired image)

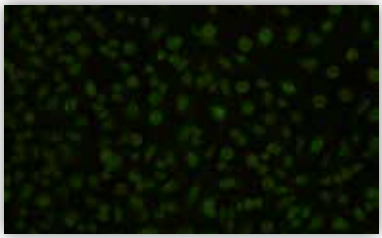
Previous Nomenclature: *spliceosome/ nuclear matrix*

Description: coarse speckles across all nucleoplasm. The nucleoli may be stained or not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained.

Antigens: hnRNP, U1RNP, Sm, RNA polymerase III

Clinical value: present to a varying degree in SARDs, particularly SLE, SSc, MCTD, overlap syndrome, and UCTD.

AC-6 MULTIPLE NUCLEAR DOTS



Pattern AC-6:
(dIFine® acquired image)

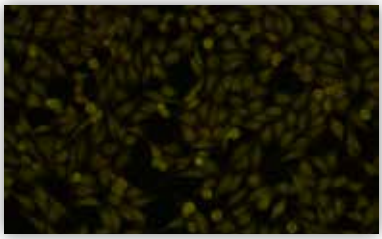
Previous Nomenclature: *6-20 nuclear dots, NSpI, PML bodies*

Description: discrete number of nuclear dots in interphase nuclei (6 to 20 nuclear dots/cell).

Antigens: Sp-100, PML proteins, MJ/NXP-2

Clinical value: found in a broad spectrum of disorders, including PBC, DM, as well as other inflammatory autoimmune diseases.

AC-7 FEW NUCLEAR DOTS



Pattern AC-7:
(dIFine® acquired image)

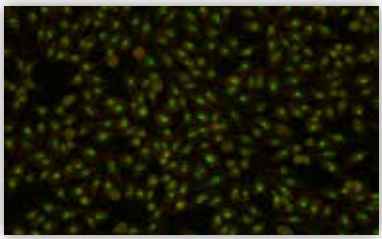
Previous Nomenclature: *1 to 6 nuclear dots, coiled body, Cajal bodies*

Description: few numbers of dots (1 to 6 nuclear dots/cell) in interphase nuclei. These are known as Cajal bodies or coiled bodies.

Antigens: p80-coilin, SMN complex

Clinical value: anti-p80-coilin antibodies may rarely occur in SLE, SSc, and SjS.

AC-8 HOMOGENOUS NUCLEOLAR



Pattern AC-8:
(dIFine® acquired image)

Previous Nomenclature: *none*

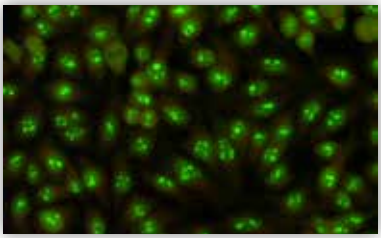
Description: diffuse fluorescence of the entire nucleolus, while the metaphase plate shows no staining. e.g. anti-PM-Scl, anti-Th/To.

Antigens: PM/Scl-75, PM/Scl-100, Th/To, B23/nucleophosmin, nucleolin, No55/SC65

Clinical value: found in patients with SSc, SSc-AIM overlap syndrome, and patients with clinical manifestations of other SARDs.

NUCLEAR PATTERNS *(AC-1/AC-14 & AC-29)*

AC-9 CLUMPY NUCLEOLAR



Pattern AC-9:
(dIFine® acquired image)

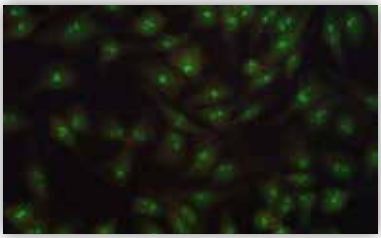
Previous Nomenclature: *none*

Description: irregular staining of the interphase nucleoli and Cajal bodies with a peri-chromosomal staining at the metaphase plates.

Antigens: U3-snoRNP/fibrillarin

Clinical value: found in patients with SSc.

AC-10 PUNCTATE NUCLEOLAR



Pattern AC-10:
(dIFine® acquired image)

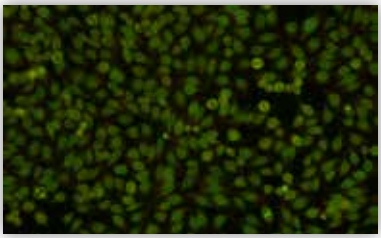
Previous Nomenclature: *nucleolar speckled*

Description: distinct grains seen in the nucleoli of interphase cells. In metaphase cells, up to 5 bright pairs of the nucleolar organizer regions (NOR) can be seen within the chromatin body. The cytoplasm of mitotic cells may be slightly positive.

Antigens: RNA polymerase I, hUBF/NOR-90

Clinical value: various conditions, including SSc, Raynaud's phenomenon, SjS, and cancer.

AC-11 SMOOTH NUCLEAR ENVELOPE



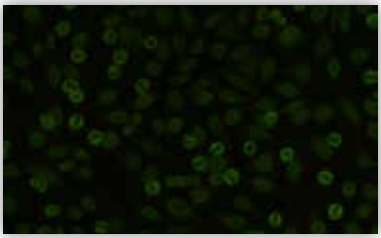
Pattern AC-11:
(dIFine® acquired image)

Previous Nomenclature: *nuclear rim, nuclear membrane, membranous*

Description: homogeneous staining of the nucleus with greater intensity at its outer rim and no staining at the metaphase and anaphase chromatin plates. There is a peculiar accentuation of the fluorescence at the points where adjacent cells touch each other.

Antigens: lamins A,B,C, or lamin-associated proteins
Clinical value: autoimmune liver diseases, linear scleroderma, autoimmune-cytopenia, and SARDs.

AC-12 PUNCTATE NUCLEAR ENVELOPE



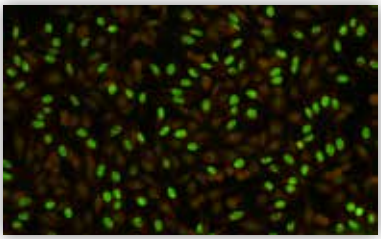
Pattern AC-12:
(dIFine® acquired image)

Previous Nomenclature: *nuclear membrane pores*

Description: nuclear envelope reveals a punctate staining in interphase cells, with accentuation of fluorescence at the points where adjacent cells touch each other. No staining of the metaphase and anaphase chromatin plates.

Antigens: nuclear pore complex proteins (e.g. gp210)
Clinical value: PBC, as well as patients with other autoimmune liver diseases and SARDs.

AC-13 PCNA-like



Pattern AC-13:
(dIFine® acquired image)

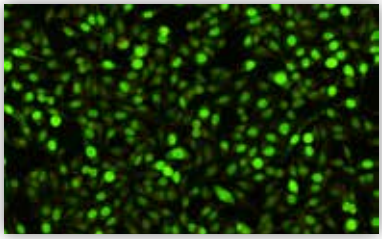
Previous Nomenclature: *none*

Description: speckled nucleoplasm staining, with variability in size and brightness of the speckles. In interphase, some cells are negative (G1 phase), some are intensely stained (S-phase) and some present rare and scattered speckles with occasional nucleolar staining (late S and early G2 phases). Mitotic cells are not stained.

Antigens: PCNA
Clinical value: specific for SLE

NUCLEAR PATTERNS *(AC-1/AC-14 & AC-29)*

AC-14 CENP-F-like



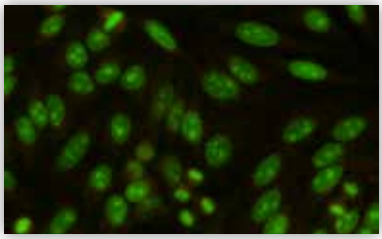
Pattern AC-14:
(dIFine® acquired image)

Previous Nomenclature: *MSA-3, NSp-II*

Description: nuclear speckled pattern with striking variability in intensity. The centromeres are positive only in prometaphase and metaphase, revealing multiple aligned small and faint dots. Prometaphase cells frequently show a weak staining of the nuclear envelope. During anaphase and telophase, some sera demonstrate intense staining in the ring located at the midzone (i.e. mid-body, stem body) where the division of the daughter cells is taking place.

Antigens: CENP-F
Clinical value: diversity of neoplastic conditions (breast, lung, colon, lymphoma, ovary, brain)

AC-29 DNA TOPOISOMERASE I (topo-I)-like



Pattern AC-29:
(dIFine® acquired image)

Previous Nomenclature: *Scl-70-like, Scl-86, DNA toposimerase I*

Description: five subcellular regions are showing a characteristic staining
1) Fine speckled AC-4 type nuclear staining in interphase cells;
2) Strong staining of nucleolar organizing region (NOR) associated on condensed chromosomes in mitotic cells. This NOR staining may be obscured by the bright chromosomal staining as NORs are not always on the same focal plane;
3) Strong fine speckled staining of condensed chromatin in mitotic cells. The mitotic chromatin staining may appear homogeneous, depending on the serum dilution;
4) Weak cytoplasmic staining in interphase (and mitotic) cells;
5) Variable nucleolar staining that can appear as a punctate nucleolar or peri-nucleolar staining in interphase cells. Nucleolar staining is not a universal feature of this pattern.

Antigens: DNA topoisomerase I
Clinical value: highly specific for SSc, with diffuse cutaneous SSc and more aggressive forms of SSc.

AC-16 CYTOPLASMIC FIBRILLAR FILAMENTOUS

Pattern AC-16:
(dIFine® acquired image)

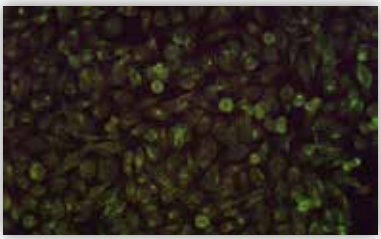
Previous Nomenclature: *none*

Description: staining of microtubules and intermediate filaments spreading from the nuclear rim.

Antigens: vimentin, cytokeratin, tropomyosin

Clinical value: several inflammatory and infectious conditions, rare in patient with SARDs.

AC-18 CYTOPLASMIC DISCRETE DOTS/GW BODY-like



Pattern AC-18:
(dIFine® acquired image)

Previous Nomenclature: *GW body, processing body, lysosome*

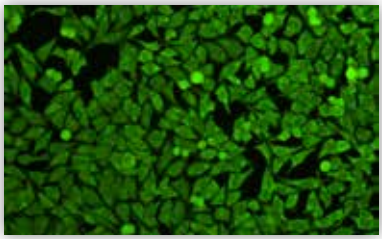
Description: staining of GW bodies in the cytoplasm of interphase cells with high numbers in late S/G2 cells.

Antigens: GW182, GW2, GW3

Clinical value: LES, SjS, neurological symptoms.

CYTOPLASMIC PATTERNS *(AC-16 & AC-18-AC-23)*

AC-19 CYTOPLASMIC DENSE FINE SPECKLED



Pattern AC-19:
(dIFine® acquired image)

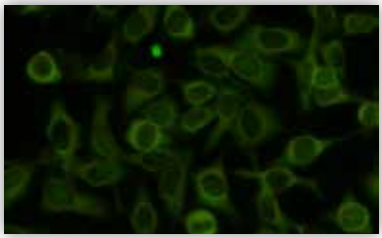
Previous Nomenclature: *cytoplasmic homogeneous*

Description: cloudy, almost homogeneous throughout the cytoplasm.

Antigens: PL-7, PL-12, ribosomal P proteins

Clinical value: SLE and the anti-synthetase syndrome, interstitial lung disease, polyarthritis, Raynaud's phenomenon.

AC-20 CYTOPLASMIC FINE SPECKLED



Pattern AC-20:
(dIFine® acquired image)

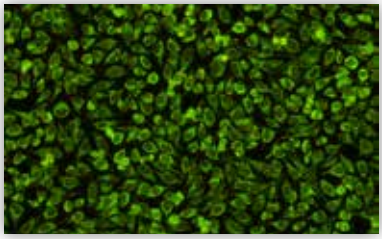
Previous Nomenclature: *cytoplasmic speckled*

Description: scattered small speckles in the cytoplasm mostly with homogeneous or dense fine speckled background.

Antigens: Jo-1/histidyl-tRNA synthetase

Clinical value: anti-synthetase syndrome, DM/PM, limited SSc.

AC-21 CYTOPLASMIC RETICULAR/AMA



Pattern AC-21:
(dIFine® acquired image)

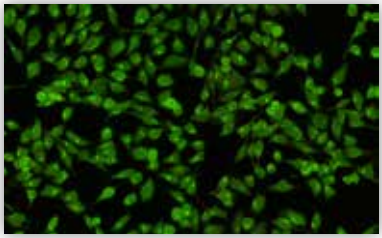
Previous Nomenclature: *mitochondria-like*

Description: coarse granular filamentous staining extending throughout the cytoplasm

Antigens: PDC-E2/M2, BCOADC-E2, OGDC-E2, E1α subunit of PDC, E3BP/protein X

Clinical value: PBC, SSc, including PBC-SSc and PBC-SjS overlap syndromes.

AC-22 POLAR/GOLGI-like



Pattern AC-22:
(dIFine® acquired image)

Previous Nomenclature: *none*

Description: speckled or granular perinuclear ribbon-like staining with polar distribution in the cytoplasm.

Antigens: giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245

Clinical value: rare and not specific in SARDs.

AC-23 RODS AND RINGS

Pattern AC-23:
(dIFine® acquired image)

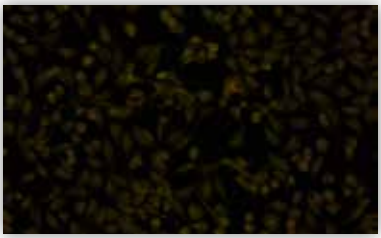
Previous Nomenclature: *none*

Description: distinct rod and ring structures in the cytoplasm of interphase cells. Some smaller rods and rings also reported in the nucleus.

Antigens: IMPDH2

Clinical value: found in HCV patients treated with interferon-α/ribavirin combination therapy, rare and not specific in SARDs.

AC-24 CENTROSOME



Pattern AC-24:
(dIFine® acquired image)

Previous Nomenclature: *centrioles*

Description: distinct centrioles (1-2/cell) in cytoplasm and at the poles of mitotic spindle.

Antigens: Cep250, Cep110, pericentrin

Clinical value: patients with Raynaud's phenomenon, localized scleroderma, SSc, SLE and RA, either alone or in combination with other SSc-associated antibodies.

AC-25 SPINDLE FIBERS

Pattern AC-25:
(dIFine® acquired image)

Previous Nomenclature: *none*

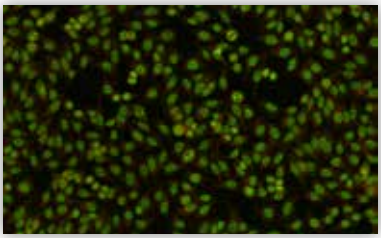
Description: the spindle fibers between the poles are stained in mitotic cells, associated with cone-shaped staining of the mitotic poles. Spindle fibers cover both NuMA-like and non-NuMA patterns. NuMA-like pattern has associated distinct nuclear speckles.

Antigens: HsEg5

Clinical value: low positive predictive value. Found rarely in SjS, LES and other connective tissue disorders.

MITOTIC PATTERNS *(AC-24/AC-27)*

AC-26 NUMA-like



Pattern AC-26:
(dIFine® acquired image)

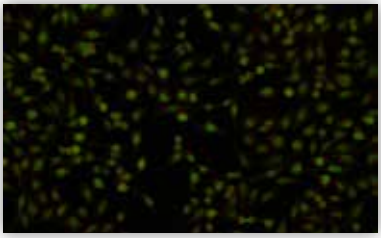
Previous Nomenclature: *MSA-1, centrophilin*

Description: nuclear speckled staining with spindle fibers.

Antigens: NuMA

Clinical value: LES, SjS and other SARDs.

AC-27 INTERCELLULAR BRIDGE



Pattern AC-27:
(dIFine® acquired image)

Previous Nomenclature: *stem body, midbody*

Description: staining of the intercellular bridge that connects daughter cells by the end of cell division, but before cell separation.

Antigens: none

Clinical value: low positive predictive value for any SARDs.

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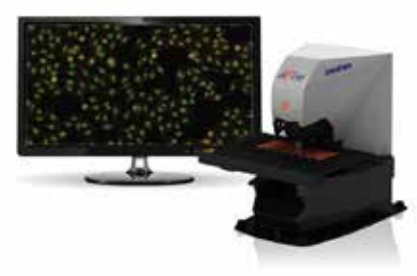

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