



GLOSSARY

Teória a prax
v Čechách
a na Slovensku

Vývoj testov autoprotilátok

- 1948 – LE test (Hargraves)
- 1957 – nepriamy imunofluorescenčný test NIF (Friou)
- 1960 – jadrové (bunkové extrakty) *ENA*
- 1969 – *Farr* – anti-dsDNA
- 1975 – *Crithidia luciliae* – anti-dsDNA
- >1979 - bunkové línie ako substrát pre NIF
- >1980 - ELISA

IMMUNOFLUORESCENT PATTERNS AND SPECIFICITY OF HUMAN ANTINUCLEAR ANTIBODIES

Y. B. BICKEL, E. V. BARNETT AND C. M. PEARSON

*Department of Medicine, University of California,
Los Angeles, California*

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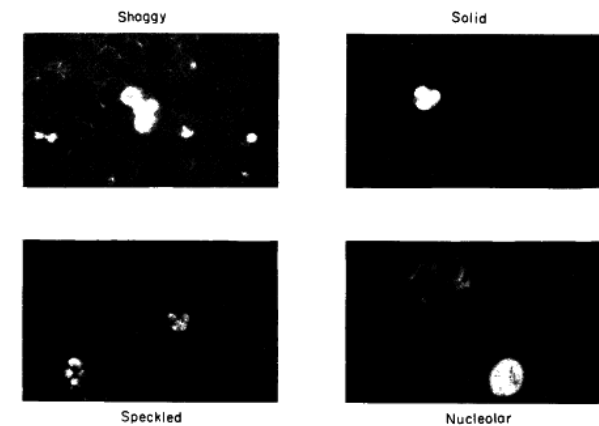


FIG. 1. Patterns of nuclear fluorescence produced by sera containing ANA. Two-layer immunofluorescence tests on human leucocyte nuclei.

Immunofluorescent antinuclear antibody tests were performed on selected sera using leucocytes and rat liver sections, and the patterns of staining compared. Antibody to DNA was detected by immunodiffusion, complement fixation and radioimmuno-electrophoresis. Specificity of reactions was determined by digestion of nuclear substrate with DNase, RNase and trypsin.

Nuclear staining patterns were correlated with the presence or absence of antibody to DNA, clinical diagnosis, immunoglobulin class and titre of antinuclear antibody and the source of nuclear antigen used.

SHAGGY
SPECKLED

SOLID
NUCLEOLAR

u nás H Z
„kométa“

M

Nu

Pattern recognition

- 1993 – Humbel – 40 obrazov na Hep2
- 1995 – Bradwell – ATLAS (nový 2003)
- 1998 – Wiik et al. KANTOR digit. obrázky
EULAR European Consensus Studies
- 1999 – Mimori MBL atlas . . .
- 2010 – WIJK et al. – konečne publikuje to,
na čom sme sa živilí najprv
- GLOSARY (Dynex, 2001) potom
- „SOP“ (2006)

Antinuclear antibodies: A contemporary nomenclature using HEp-2 cells

Allan S. Wiik^{a,*}, Mimi Høier-Madsen^a, Jan Forslid^b, Peter Charles^c, Jan Meyrowitsch^d

^a Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark

^b Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, 171 76 Stockholm, Sweden

^c Translational Research, Kennedy Institute of Rheumatology, Imperial College London, 65 Aspenlea Road, London W6 8LH, United Kingdom

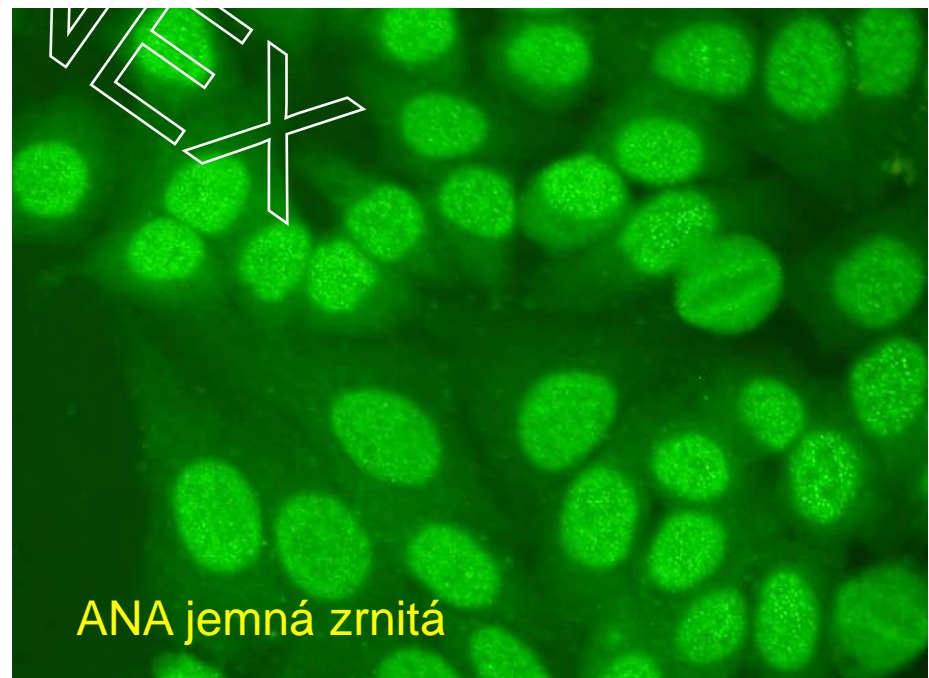
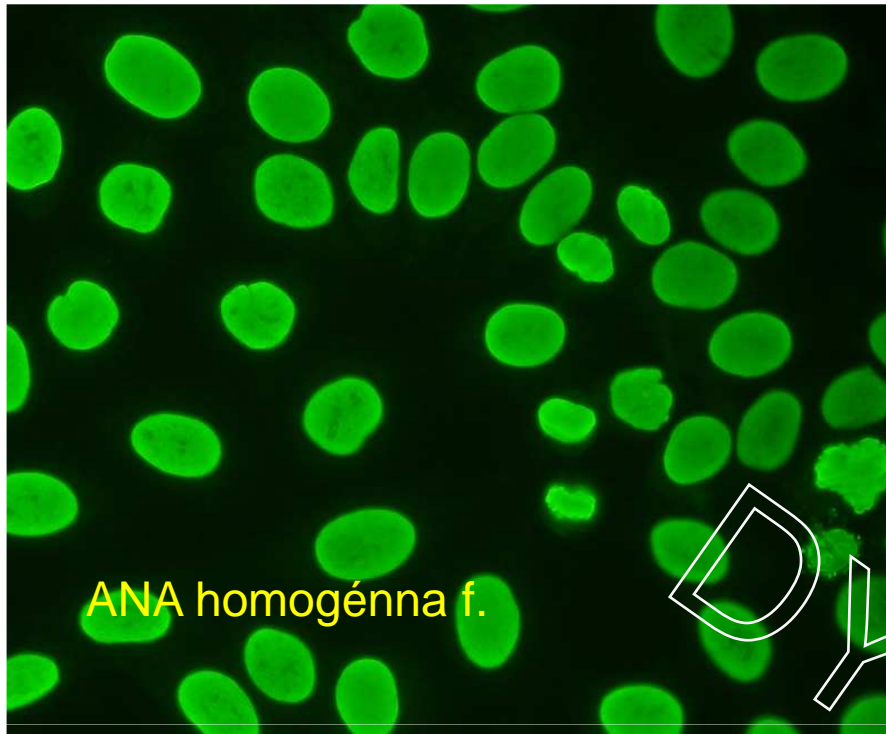
^d Percepton Ltd., Rialtovej 12, 2300 Copenhagen S, Denmark

A B S T R A C T

Keywords:
Nomenclature
Standardization
HEp-2 cells
Consensus formation
Medical training
ANA

The choice of terms used to describe indirect immunofluorescence (IIF) staining patterns of autoantibodies binding to HEp-2 cells is at present quite varied and disordered because no accurate consensus on names and descriptions exist. The aim of our study was to propose a logical and ordered IIF classification taxonomy based on 29 different selected IIF patterns. In a preliminary project carried out at Statens Serum Institut it was first shown by use of a software programme named DOORS developed by Percepton Ltd, that reading of digitized images of HEp-2 patterns on an LCD monitor could be used instead of traditional microscopy. Digitized images of HEp-2 patterns were then used in the EU supported project named CANTOR (June 1998–July 2000) aiming to reach consensus among three clinical immunology expert centres and collaborating to attain a classification version that could be used to qualitatively and quantitatively test and train image recognitions skills of laboratory technicians against expert consensus. The usability of this classification version was then tested in a course consisting of training and certification. The conclusion was that participants in the training programme clearly increased their perceptive skills using images, terms, descriptions and the graphic and statistic tools in the self-administered DOORS programme and that software-assisted training could achieve a common and accurate level of visual pattern interpretation. All results from this project were reported to the European Commission but have not previously been published in scientific literature. This communication presents the final results of agreed image classifications.

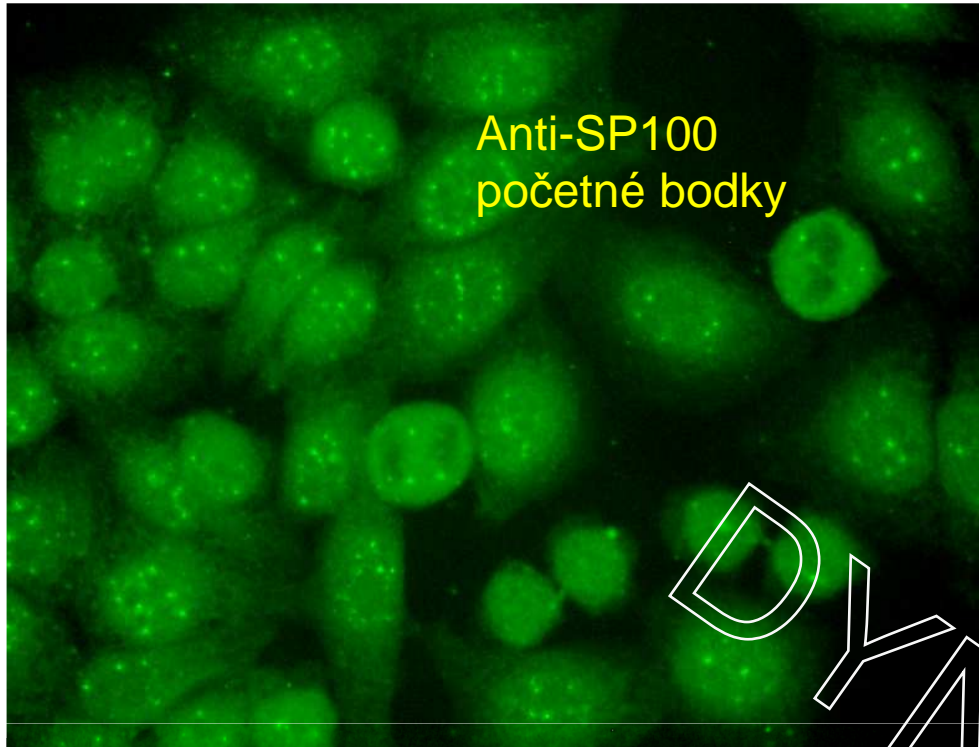
OBRAZY FLUORESCENCIE



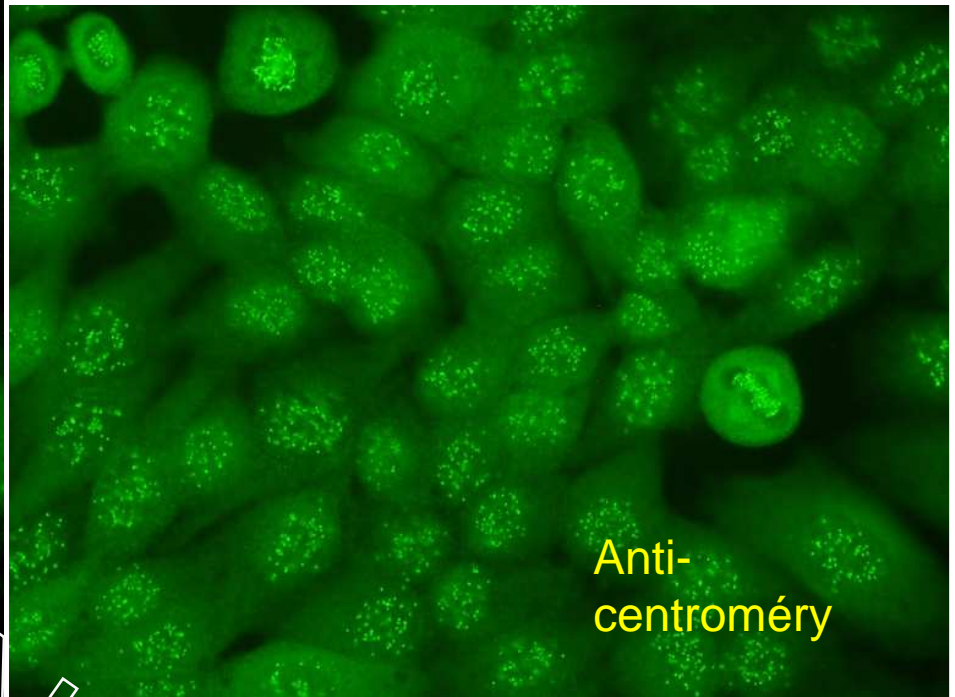
OBRAZ FLUORESCENCIE	Terčová molekula	Dg	Klin. význam	Citlivosť %
H homogénne jadrá	nukleozómy	SLE	A	85*
	DNP, chromatín	SLE	S	40 - 85*
	dsDNA	SLE	S	40 - 80*
	históny	-	N	-
	NHCP (HMG17)	DCHS	A	35 - 70

* s aktivitou choroby

OBRAZ FLUORESCENCIE	Terčová molekula	Dg	Klin. význa m	Citlivosť %
<p style="text-align: center; font-size: 2em; font-weight: bold;">Z</p> <p style="text-align: center;">zrnité jadrá</p>	nRNP	MCTD	S	90
	Sm	SLE	S	10 -30
	La (SSB)	SjS	A	80
	Ro (SSA)	SjS	A	95
	PCNA	SLE	S	5
	Ku	PM/DM	A	5 - 10
	RNAP II	SSc SLE	A	10 - 15
	Mi-2	PM/DM	S	15 - 20
	MATRIX (napr. RA33)	RA	A	20 - 60



Anti-SP100
početné bodky



Anti-
centroméry



Anti-coilin ojedinelé bodky

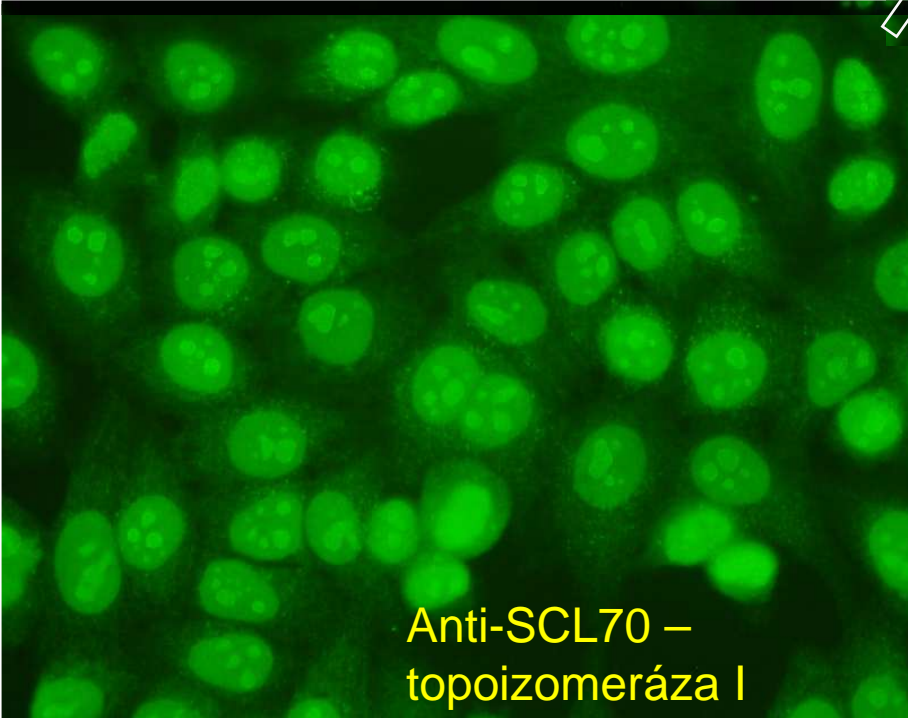
DYNEX



ANuA hrudkovité jadierka



ANuA homogénne jadierka



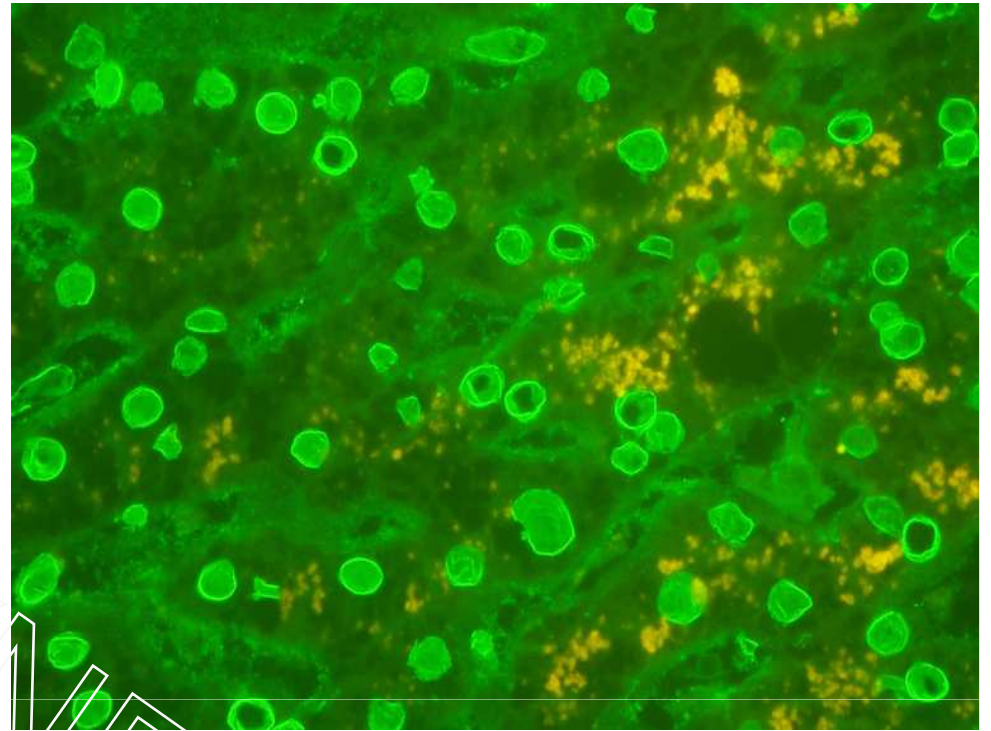
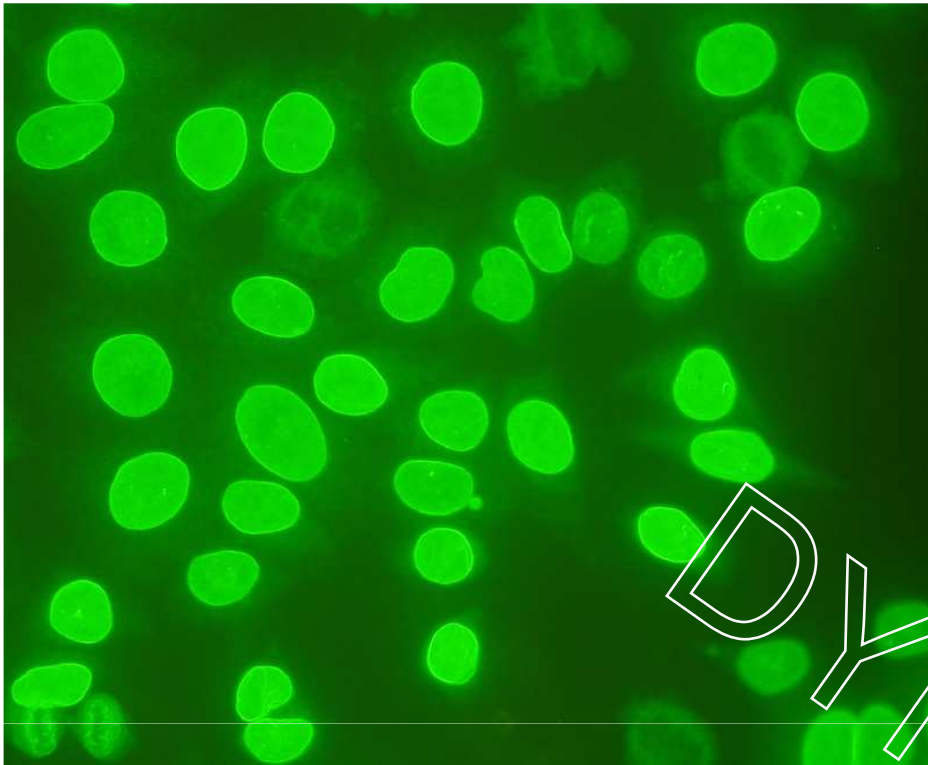
Anti-SCL70 –
topoizomeráza I



ANuA bodkované jadierka

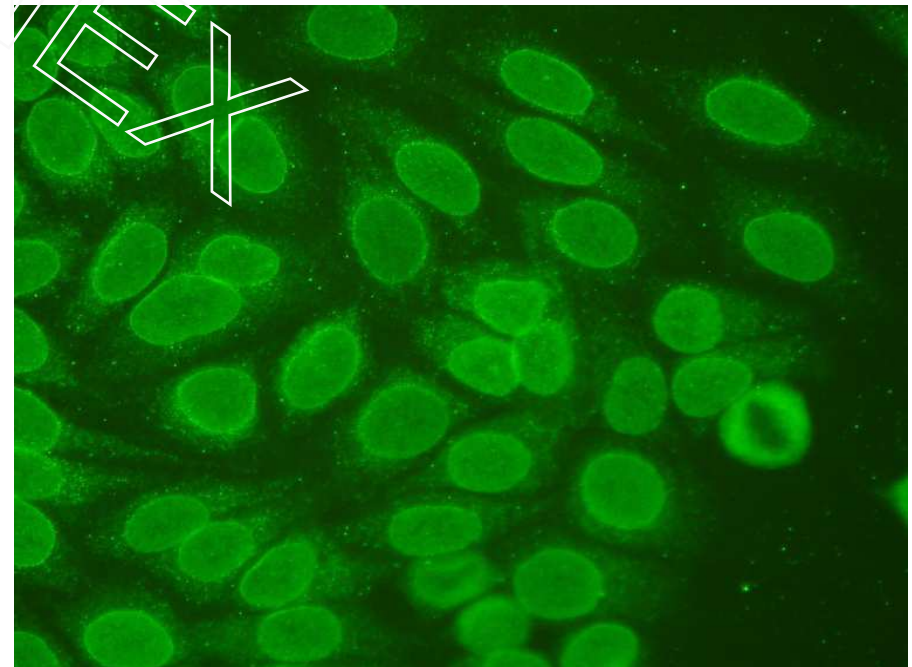
DYMEEX

OBRAZ FLUORESCENCIE	Terčová molekula	Dg	Klin. význam	Citlivosť %
Nu jadierka	SCL70	SSC	S	20 - 65
	Fibrilarín U3- RNP	SSc	S	6 - 8
	PM/SCL	PM/DM	A	5
		PS s SSc	A	25
	NOR90	SSc	S	< 2
	RNAP I a III	SSc	S	5 - 25



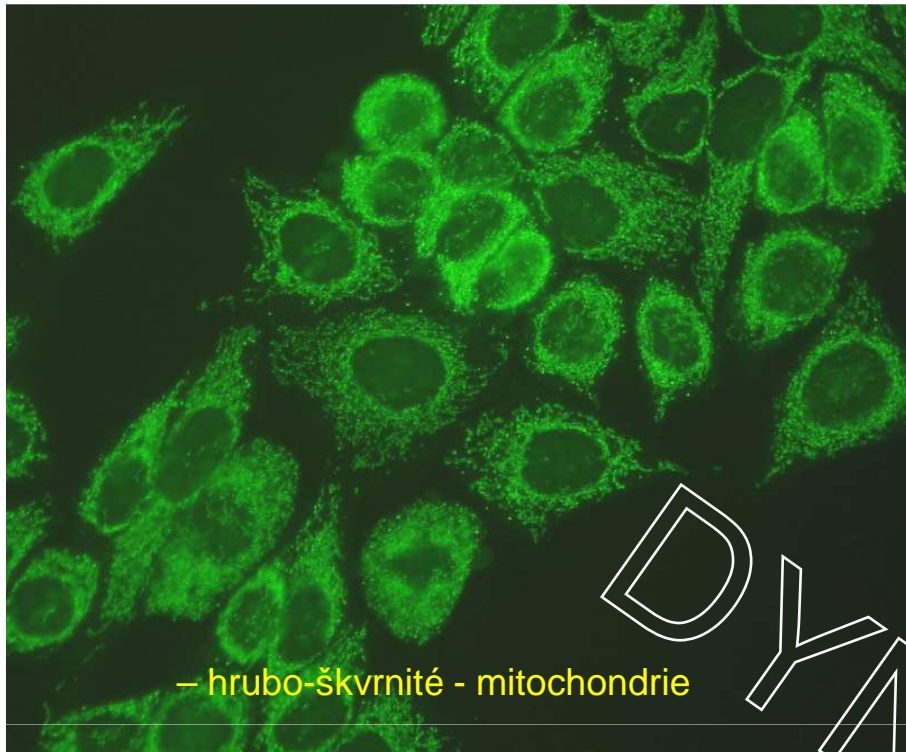
ANA jadrová membrána

hladká	
Hep2	MoLi
<hr/>	
	Hep2
	póry



OBRAZ FLUORESCENCIE	Terčová molekula	Dg	Klin. význam	Citlivosť %
<p data-bbox="369 598 577 762">M</p> <p data-bbox="360 858 586 975">Jadrová membrána</p> <p data-bbox="423 1007 524 1046"><i>RIM</i></p>	Póry jadrovej membrány	PBC	S	10*?
	Lamíny A,B,C	CAH a i.	N	

* s aktivitou choroby



Anticytoplazmatické protilátky

OBRAZ FLUORESCENCIE		Terčová molekula	Dg	Klin. význam	Citlivost' %
Cy cytoplazma	ZRNITÝ	AMA M2	PBC	S	95
		tRNA- syntetázy (Jo1 a i.)	PM/ DM	S	20 - 40
		Golgi	-	N	-
		Lyzozómy	-	N	-
	DIFÚZNY	RIB-P	SLE	S	10 - 40

1.	Používáte při hodnocení fluorescence autoprotilátek na HEp-2 SOP nebo Glossary?	ANO	38	NE	6
2.	Ak nie, radi by ste mali SOP firmy Dynex? Přejděte k bodu 8.	ANO	16	NE	5
3.	Ak áno, z jakého zdroje čerpáte? (Dynex, The Binding Site, BioSystems)	<i>vide extra</i>			
4.	Ak áno, pokladáte vámi používaný SOP za užitočný?	ANO	33	NE	
5.	Ak áno, používate terminológiu tohto SOP pri hodnotení obrazov ANA?	ANO	30	NE	3
6.	Ak áno, poskytujete hodnotenie s terminológiou tohto SOP tiež lekárovi?	ANO	24	NE	8
7.	Ak áno, prosíme o poskytnutie vášho výsledkového formulára (event. súhrn vašich nálezov) ako prílohu tohto dotazníka	ANO	8	NE	8
8.	Chcete sa zúčastniť na štandardizácii testu NIF na ANA s použitím séra s deklarovanými IU?	ANO	33	NE	9
9.	Ak áno, mohli by ste stručne uviesť váš motív? Napr. akreditácia metódy, pre pokoj svedomia apod.				

Guidelines for Clinical Use of the Antinuclear Antibody Test and Tests for Specific Autoantibodies to Nuclear Antigens

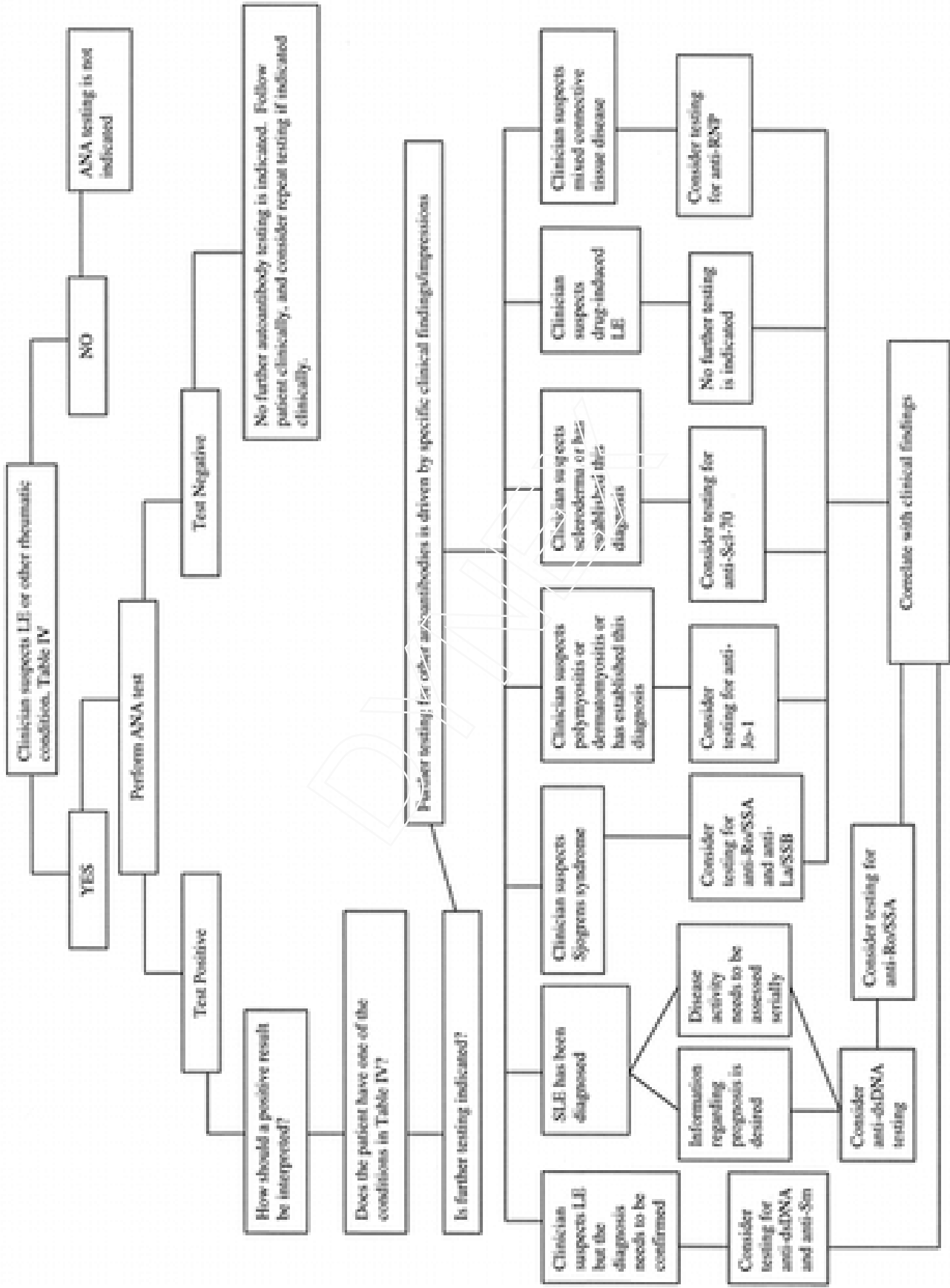
Arthur Kavanaugh, MD; Russell Tomar, MD; John Reveille, MD; Daniel H. Solomon, MD, MPH; Henry A. Homburger, MD **Arch Pathol Lab Med.** 2000;124:71–81

Table 2. Regulatory Requirements for IF-ANA and EIA-ANA Test Methods*

- Personnel: federal regulations define the minimum qualifications required for laboratory directors (doctoral degree), technical supervisors (doctoral degree, master's degree, or bachelor's degree plus experience), clinical consultants, general supervisors, and testing personnel (associate degree) engaged in “high-complexity” testing
- Competency assessment: required yearly for individuals that perform tests and includes direct observation of testing and reporting of results
- Quality control: laboratories must have an “ongoing mechanism” to identify problems and produce corrective actions
- Proficiency testing: ANA is a “regulated analyte”; acceptable performance on proficiency testing is defined by a result equal to the target value 6 2 dilutions; acceptable results must be obtained on 4 of 5 challenges in each mailing; specific ANAs are not “regulated analytes” and acceptable performance is defined by the proficiency provider

* IF indicates immunofluorescent; ANA, antinuclear antibody; and EIA, enzyme immunoassay

Po 10 rokoch prešli kompletne na automaty s EIA – problém veľkých laboratórií – referát o videokonferencii v Ljubljane 2010



Trend, ktorý počíta s rozpoznávaním obrazu

Standardisation of AAB testing by IIF remains a critical issue in and between routine laboratories and may be improved by automated interpretation systems.

The novel system provides fully automated reading of IIF images and software algorithms for the mathematical description of IIF AAB patterns. It can be used for screening and preclassification of non-organ specific AAB in routine diagnostics regarding systemic autoimmune and autoimmune liver diseases.

Autoimmun Rev. 2009 Sep;9(1):17-22. Epub 2009 Feb 24.

Challenges of automated screening and differentiation of non-organ specific autoantibodies on HEp-2 cells.

[Hiemann R](#), [Büttner T](#), [Krieger T](#), [Roggenbuck D](#), [Sack U](#), [Conrad K](#).

Department of Biology, Chemistry and Process Technology, Lausitz University of Applied Sciences, Senftenberg, Germany.

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Pattern on the Antinuclear Antibody–HEp-2 Test Is a Critical Parameter for Discriminating Antinuclear Antibody–Positive Healthy Individuals and Patients With Autoimmune Rheumatic Diseases

Henrique A. Mariz, Emília I. Sato, Silvia H. Barbosa, Silvia H. Rodrigues, Alessandra Dellavance, and Luis E. C. Andrade

Na odlišenie ANA u chorých s ARD (autoimunitné reumatické choroby) od ANA pozitívnych zdravých je dôležitý obraz ANA:

HOM, COARSE SPECKLED & CE sú výhradne pri ARD

U zdravých sa výhradne nachádza **NUCLEAR DENSE FINE SPECKLED (DFS70/LEDGF)**

Naša skúsenosť:

- Ak zvolíme podľa odporúčaní kontrolnú skupinu chorých s inými, ako ARD, dospejeme až k závažným prevalenciám pri infekciách.
- Budú sa tam vyskytovať najrozmanitejšie obrazy a znížime značne špecifickosť niektorých obrazov (jadierka a pod.) pre SSC a PM/DM
- Hep2 pozit /monkey liver neg, takmer nikdy nič na ANA 3b EuroLine

Môj e-mail detskej gastroenterolóžke

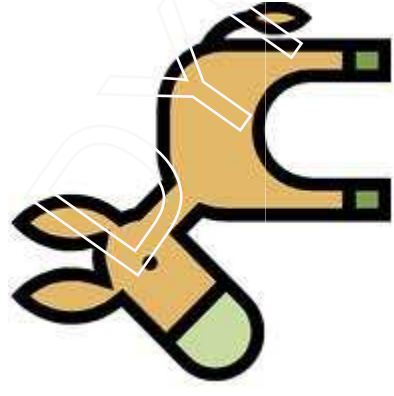
Pomerne často sa stane pri určovaní **protilátok proti endomýziu**, že vidím na bunkách fluorescenciu inú, ako anti-EM. Je to za situácie, keď to nemá veľkú váhu, lebo **sérum je riedené 1:10 a konjugát je anti-IgA**. Pre pokoj svedomia preverujem takýto nález za štandardných podmienok pre ANA (konjugát anti-IgG a riedenie séra 1:40). Nie vždy, ale predsa sa stane, že takýto nález sa potvrdí aj za týchto podmienok.

To sa stalo so sérom pac. PĎ03 – bol na všetky celiakové protilátky negatívny. 1:10 protilátky proti jadrovej membráne a anti-coilin. Sú to pomerne silno príznakové protilátky. **Anti-membránové** sa potvrdili aj s IgG pri 1:40, anti-coilin nie.

Reakcia

- Vážený pán inžinier. Ja nemám skúsenosti s tými vyšetreniami, ktoré spomínate, aj pravdu povediac by som sa nerada dostávala na "tenký ľad", **o ktorom sa v bežnej diagnostike u nás nehovorí**. Metodický list o celiakii hovorí o tkáňovej transglutamináze a k tomu ešte jedna protilátka-mali by to byť endomysiálne PL..../dve pozitívne...
- Ja skutočne tým riedeniam nerozumiem, **potrebujem doslova čo "najjednoduchšie" výsledky** a takisto sa musím vedieť pred zdravotnými poisťovňami obhájiť, keby mi prišli na kontrolu.

NUŽ



a

načo?

