Antinuclear antibodies

Xavier Bossuyt

UZ Leuven

Evidence-based guidelines for the use of immunologic tests: Antinuclear antibody testing

Daniel H. Solomon, Arthur J. Kavanaugh, Peter H. Schur

Arthritis Care & Research 2002; 47:434–444

ANA very useful for diagnosis

Systemic lupus erythematosus

Systemic sclerosis

ANA somewhat useful for diagnosis

Sjögren's syndrome

Polymyositis-dermatomyositis

ANA very useful for monitoring or prognosis

Juvenile chronic arthritis

Raynaud's phenomenon

ANA is a critical part of the diagnostic criteria

Drug-associated lupus

Mixed connective tissue disease

Autoimmune hepatitis

ANA not useful or has no proven value for diagnosis, monitoring or prognosis

Rheumatoid arthritis

Multiple sclerosis

Thyroid disease

Infectious disease

Idiopathic thrombocytopenic purpura

Fibromyalgia

- Immunofluorescence on HEp-2 cells to screen for ANA
  - Titer
  - Pattern

- More specific second line tests to identify the target antigen
  - Anti-dsDNA
  - Anti-ENA

Homogeneous pattern			Speckled pattern	
ANA titer	Anti-dsDNA		ANA titer	Anti-ENA
1:80 (n=512)	15%		1:80 (n=116)	1%
1:160 (n=414)	27%		1:160 (n=72)	12%
1:320 (n=277)	35%		1:320 (n=34)	18%
1:640 (n=192)	38%		1:640 (n=27)	33%
1:1280 (n=103)	53%		1:1280 (n=15)	60%
1:>1280 (n=64)	68%		1:>1280 (n=15)	89%

1567 consecutive samples

Bossuyt X et al. Arthritis Rheum. 2005;53:987-8

ANA pattern	Antibody to	Disease
Homogeneous	DNA Histones Scl-70	SLE DIL SSc
Speckled	U1-RNP, Sm SSA, SSB RNA-Pol III	SLE SLE and SS SSc
Centromeric	Centromeres	Limited SSc
Nucleolar	PM/Scl, U_3RNP, others	SSc, SLE, SS
Speckled cytoplasmic	Jo-1 Mitochondria	PM/DM PBC
Diffuse cytoplasmic	Ribosomes	SLE

N Engl J Med. 2009;360:711-20

Case records of the Massachusetts General Hospital. Case 5-2009. A 47-year-old woman with a rash and numbness and pain in the legs.

Kroshinsky, D, Stone JH, Bloch DB, Sepehr A

Ann Rheum Dis. 2010;69:1420-2.

ANA screening: an old test with new recommendations.

Meroni PL, Schur PH

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Ann Rheum Dis. 2014 Jan;73(1):17-23. doi: 10.1136/annrheumdis-2013-203863. Epub 2013 Oct 14.

International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies.

Agmon-Levin N[1], Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, Bossuyt X, Musset L, Cervera R, Plaza-Lopez A, Dias C, Sousa MJ, Radice A, Eriksson C, Hultgren O, Viander M, Khamashta M, Regenass S, Andrade LE, Wiik A, Tincani A, Rönnelid J, Bloch DB, Fritzler MJ, Chan EK, Garcia-De La Torre I, Konstantinov KN, Lahita R, Wilson M, Vainio O, Fabien N, Sinico RA, Meroni P, Shoenfeld Y.

ANA screening: an old test with new recommendations

Pierre Luigi Meroni, Peter H. Schur

Ann Rheum Dis. 2010;69:1420-2

Recommendations of the American College of Rheumatology (ACR) Antinuclear Antibody (ANA) Task Force

- Immunofluorescence ANA test should remain the gold standard for ANA testing.

- Hospital and commercial laboratories using bead-based multiplex platforms or other solid phase assays for detecting ANA must provide data to ordering physicians on request that their assay has the same or improved sensitivity and specificity as the immunofluorescence ANA.

- In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, etc, should be standardized according to national (eg, CDC) and/or international (eg, WHO, IUIS) standards.

- Laboratories should specify the methods used for detecting ANA when reporting their results.

Members of the ACR ANA Task Force: Peter Schur (Chair), Donald Bloch, Joe Craft, John A Goldman, Pier L Meroni, Eileen Moynihan, Morris Reichlin, Westley Reeves, Eng Tan, Dan Wallace and Mark Wener.

Antibodies to extractable nuclear antigens in antinuclear antibody-negative samples

Bossuyt X, Luyckx A.

Clin Chem. 2005;51:2426-7

2405 consecutive samples

- 565 ANA pos: anti-ENA in 102 (18%)
- 1840 ANA neg: anti-ENA in 21 (1.1%)

- sensitivity of ANA for anti-ENA: 83% (75%-89%)

Hofman et al. [Clin Chem 2002]: sensitivity: 72%

Conclusion:

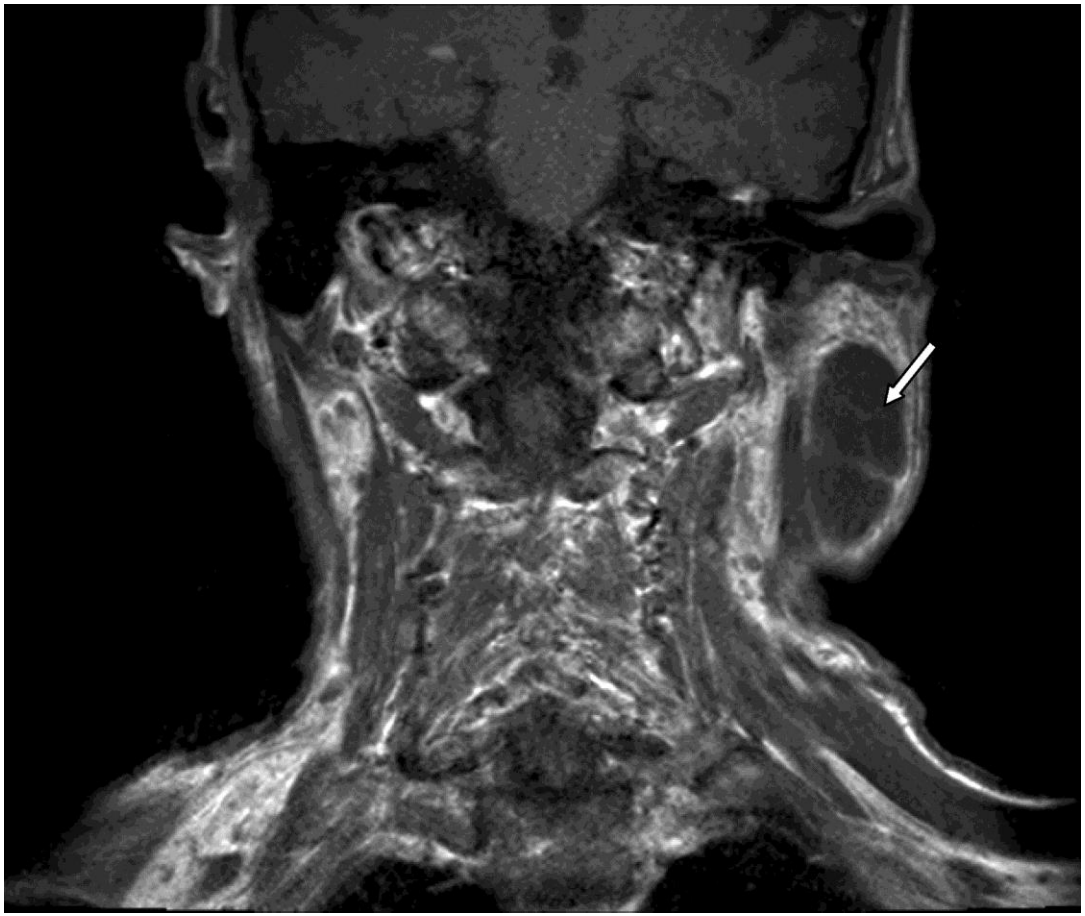
- Although ANA has high sensitivity, it may miss reactivities
- When there is a high clinical suspicion, focused testing for specific antibodies should be performed

Patient no.	Dot-blot analysis	EliA (EliA units/mL)	Clinical findings
1	SSA	SSA (13.6)	Rheumatoid arthritis
2	SSA	SSA (29.7)	Systemic lupus erythematosus
3	SSA	SSA (37.2)	No data available
4	SSA	SSA (20.7)	Sjögren syndrome, cutaneous lupus, cryogobulinemia
5	Jo-1	Jo-1 (119)	Polymyositis
6	Sm/RNP + Sm	U_1RNP (51) + RNP 70 (19) + Sm (6)	Drug-induced lupus[2]
7	Scl-70	Negative	Reactive arthritis
8	Negative	SSA (11.2)	B-CLL[3] (bone marrow transplantation)
9	Negative	SSA (38.7)	Neonatal lupus erythematosus (skin rash)
10	Negative	SSA (18.4)	Psoriatic spondylarthropathy (HLA-B27+)
11	Negative	SSA (23.5)	Chronic urticaria
12	Negative	SSA (20.3)	Pregnancy and thrombocytopenia
13	Negative	SSA (27.1)	Subacute cutaneous lupus
14	Negative	SSA (19.5)[4]	Rheumatoid arthritis
15	Negative	Sm (12.5)	Subacute sclerosing panencephalitis
16	Negative	U_1RNP (56.7)	Cervical tension

A 67-year-old woman with a systemic inflammatory syndrome and sicca

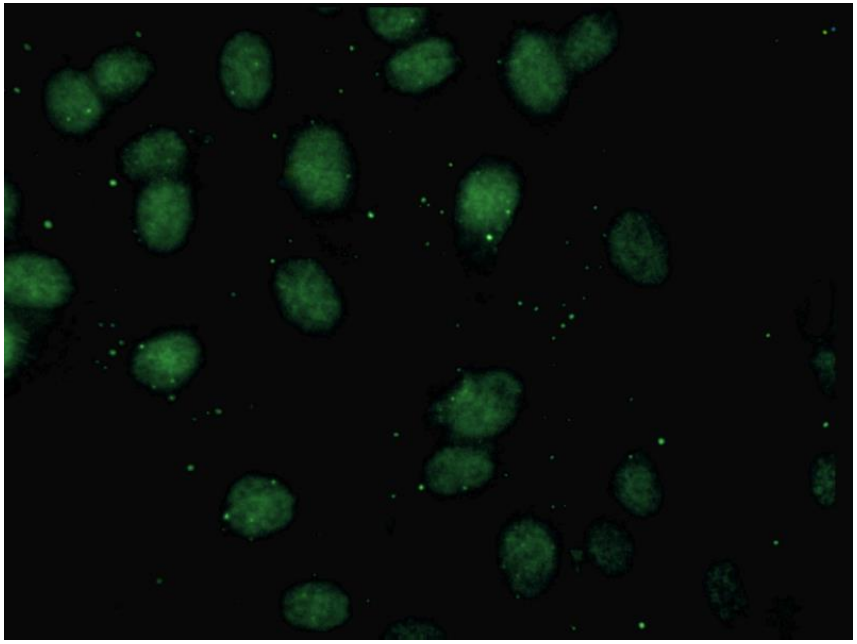
Bossuyt X, Mariën G, Vanderschueren S.

Clin Chem. 2010;56:1508-9.

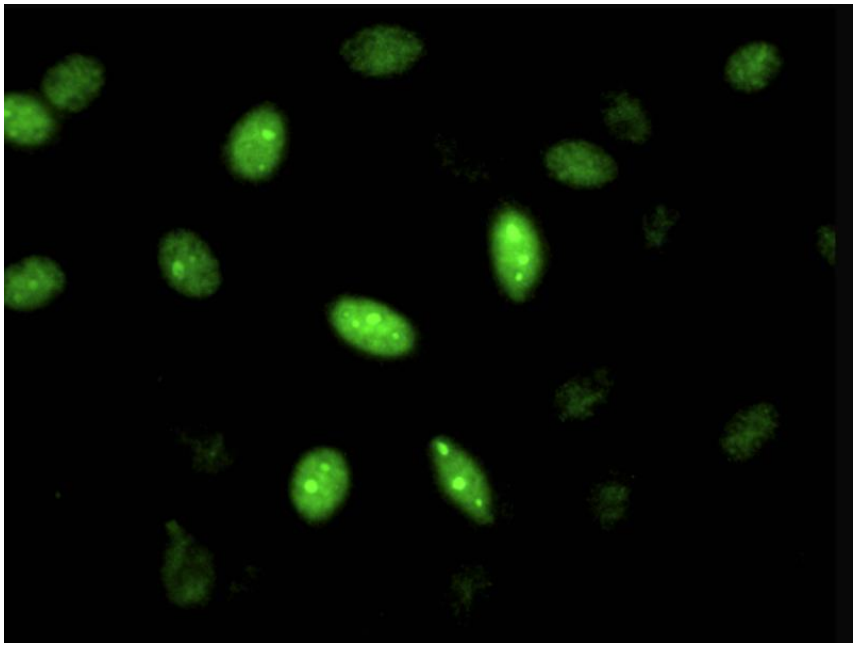


Magnetic resonance image showing cystic enlargement of the left parotid gland (arrow).

1:40



1:1280



Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay.

Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, Bossuyt X.

Autoimmun Rev. 2011;10:801-8

EVALUATION OF ANA BY IIF AND SOLID PHASE ASSAY

	n	M/F	Mean age (range)
SLE	80	10/70	36 (15-72)
SCL	10	3/7	49 (32-85)
SSc	69	25/44	53 (18-79)
MCTD	13	1/12	31 (16-66)
SS	36	5/31	50 (21-75)
PM/DM	28	11/17	54 (26-77)
Blood donors	149	75/74	44 (19-65)
CFS	139	125/114	41 (18-75)
Dis. controls	134	34/100	46 (17-81)

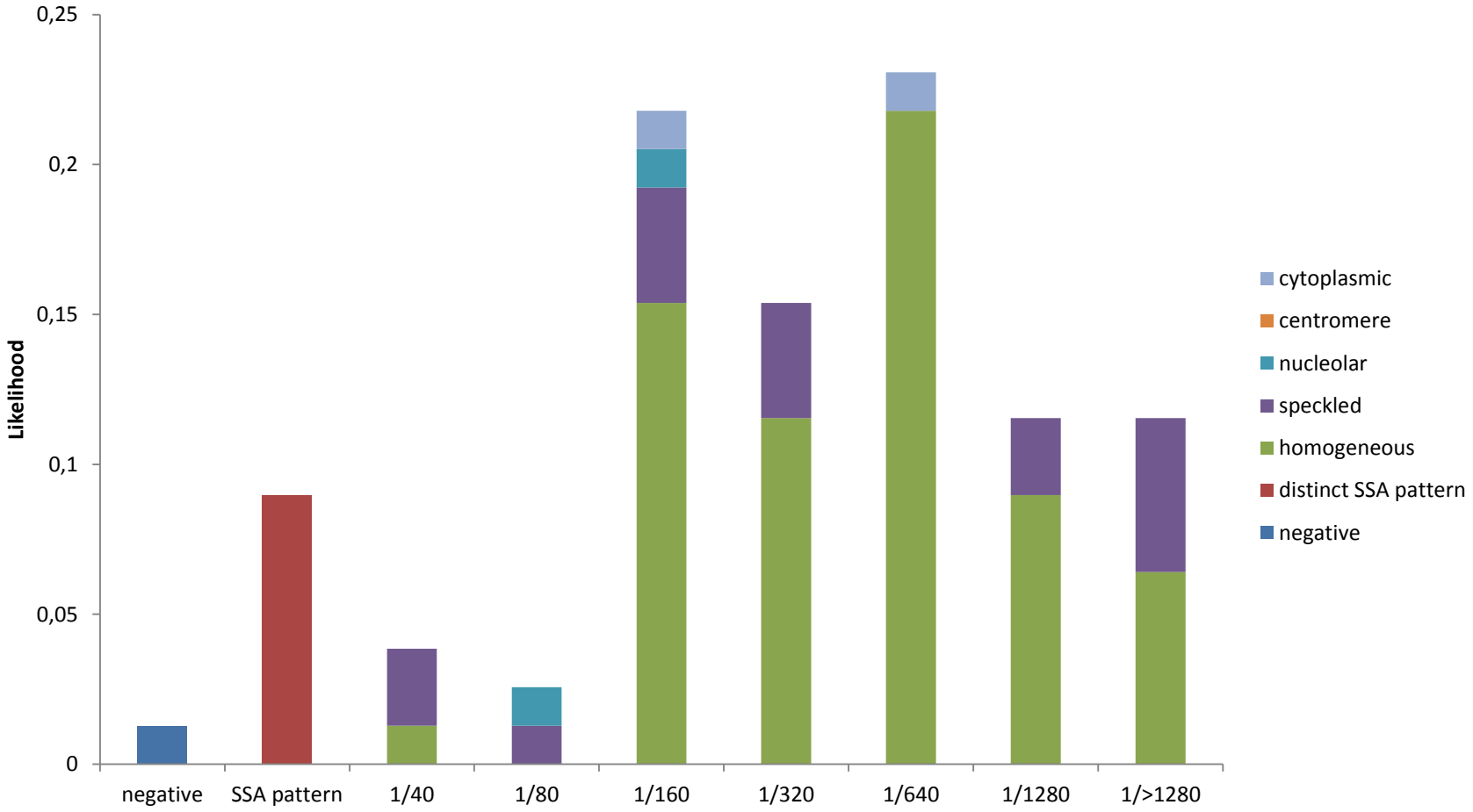
ANA: HEp-2000 Immunoconcepts
Anti-ENA, anti-dsDNA: EliA Phadia

- SSA-transfected HEp-2000™ cells (Immunoconcepts)

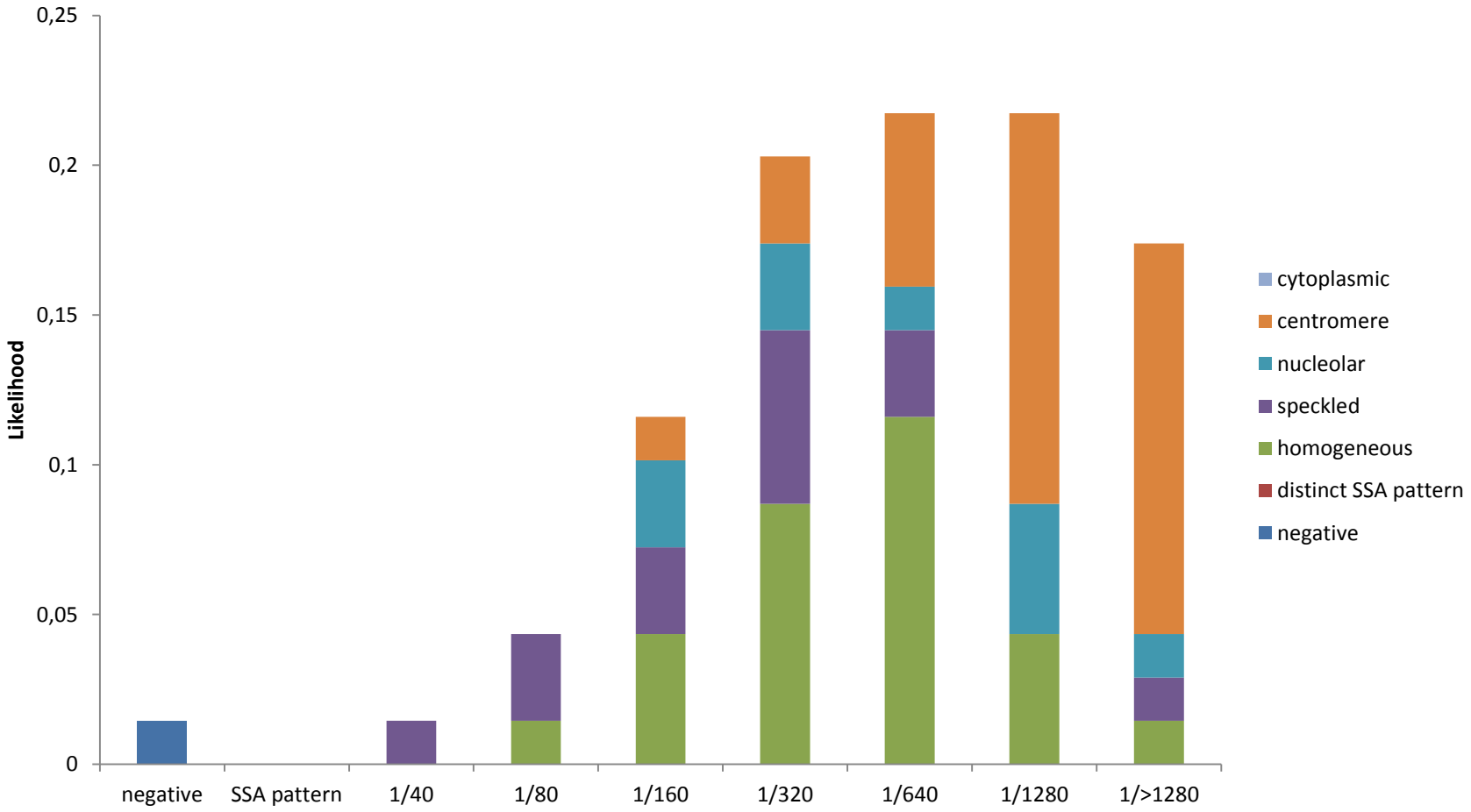
- EliA – CTD screen
  - Recombinant antigens (SSA/Ro52, SSA/Ro60, SSB, RNP-70, RNP-A, RNP-C, Sm, Centromere B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA pol III, PM-Scl, PCNA, Mi-2)
  - dsDNA: purified

Evaluation of ANA by IIF

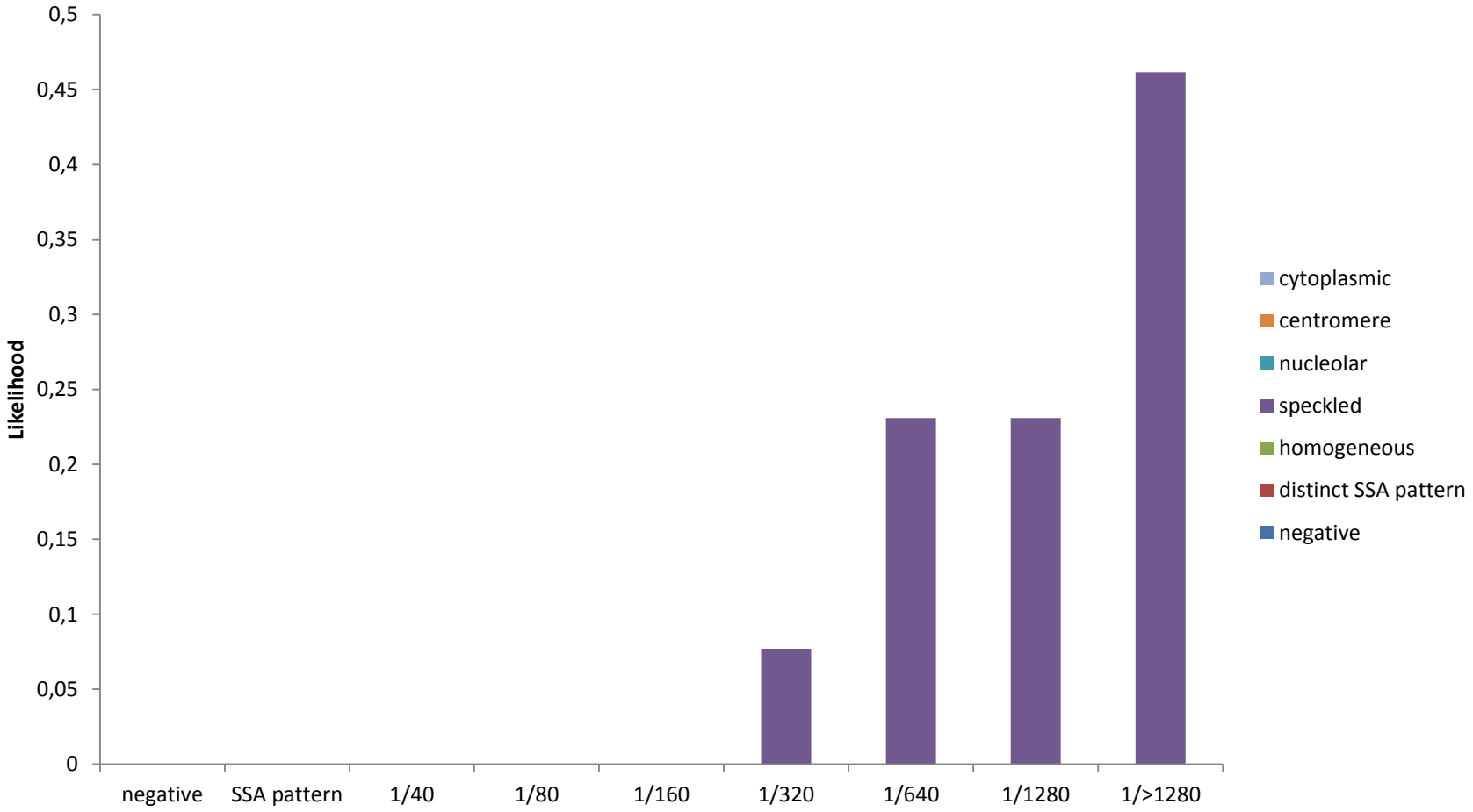
SLE


0,25
0,2
0,15
0,1
0,05
0
Likelihood
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
cytoplasmic
centromere
nucleolar
speckled
homogeneous
distinct SSA pattern
negative

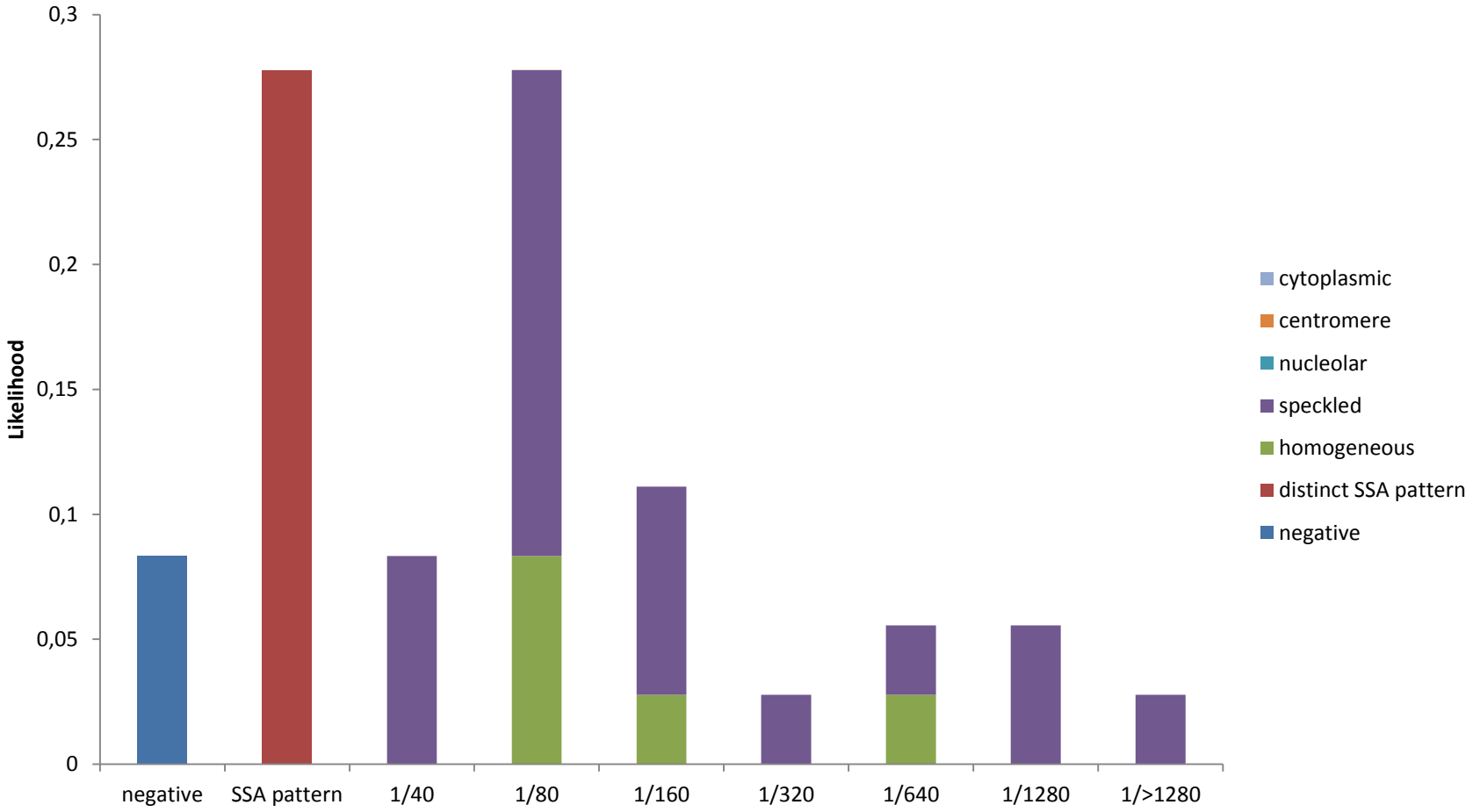
Systemic sclerosis



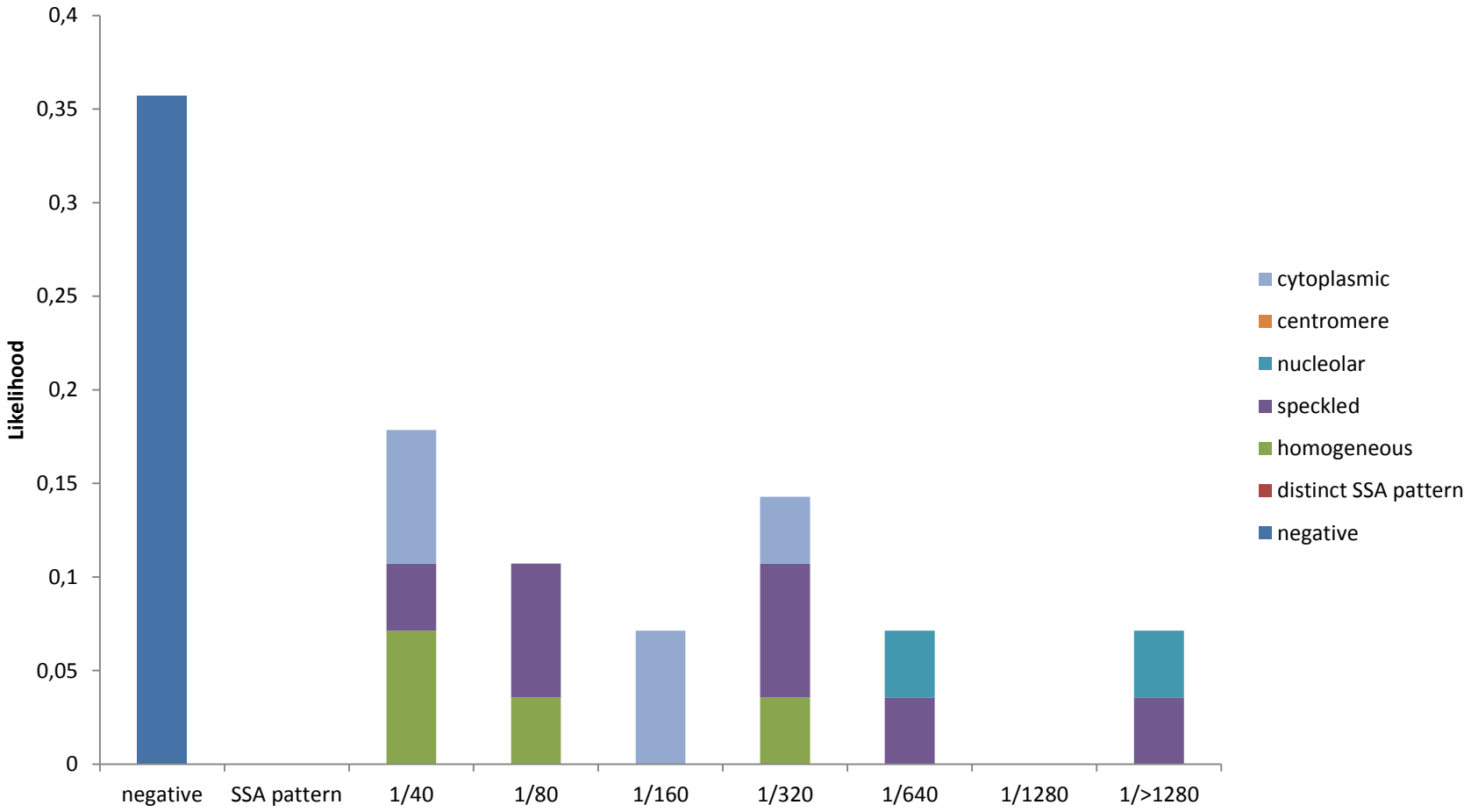
MCTD


0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
Likelihood
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
cytoplasmic
centromere
nucleolar
speckled
homogeneous
distinct SSA pattern
negative

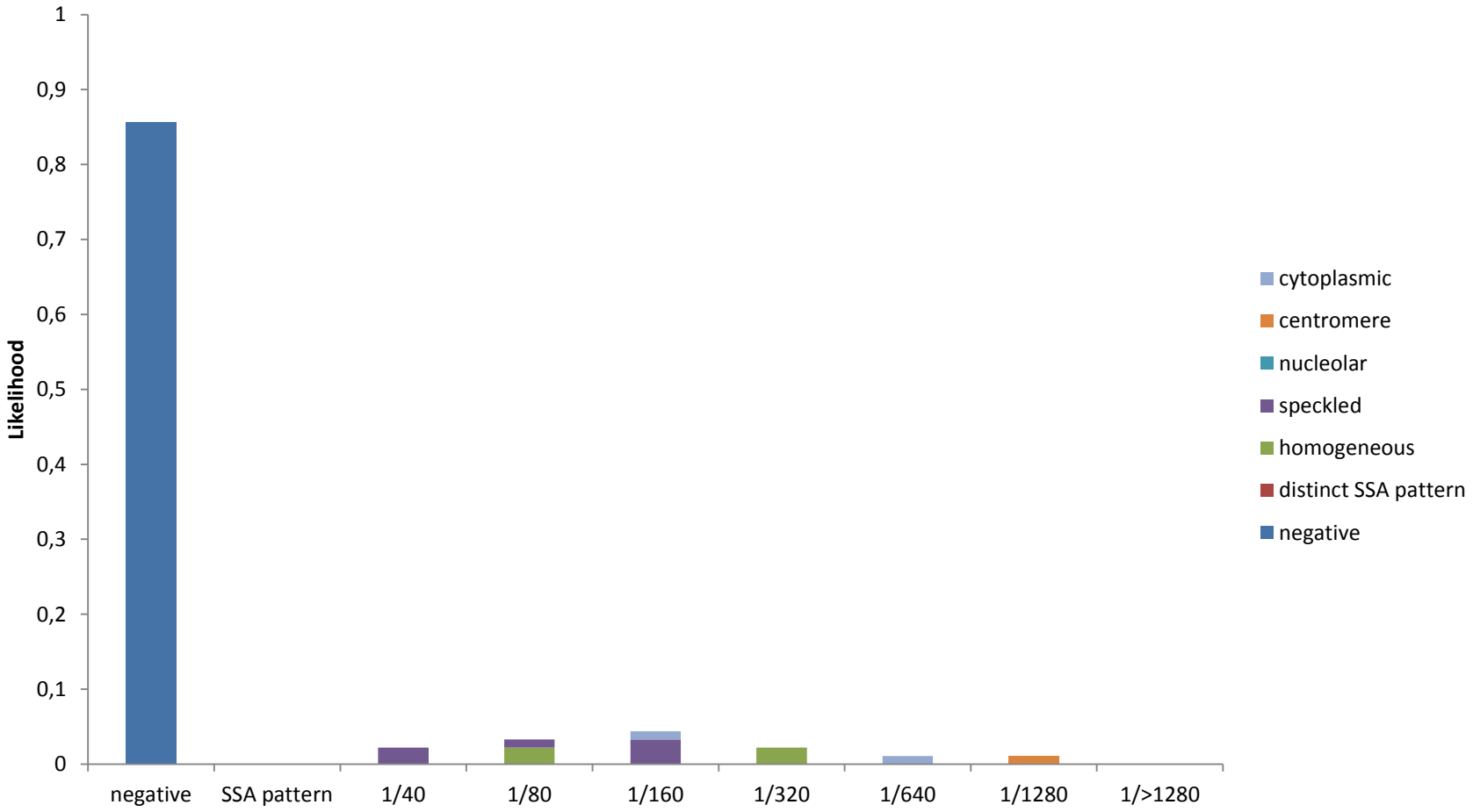
Sjögren's syndrome



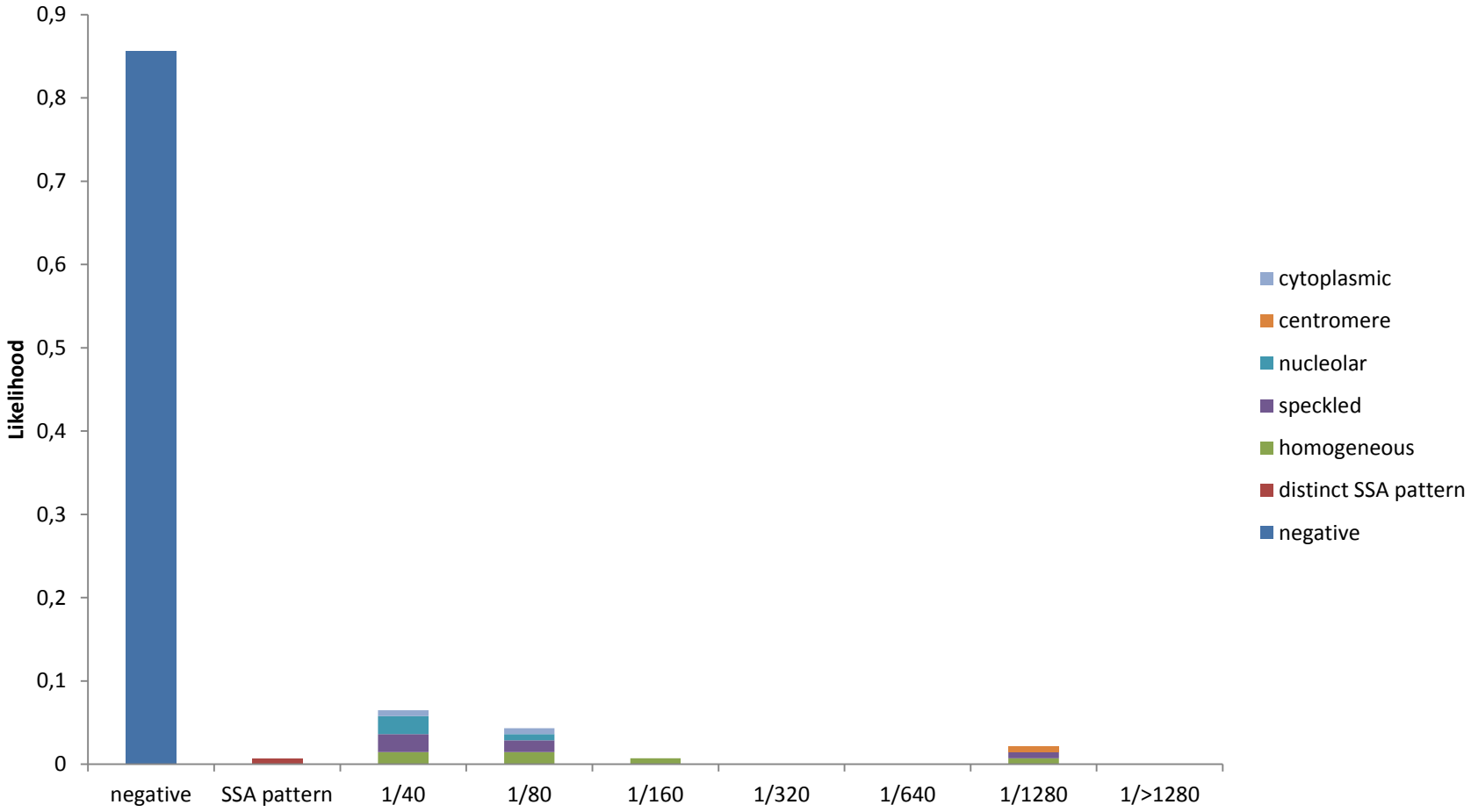
Inflammatory myopathy



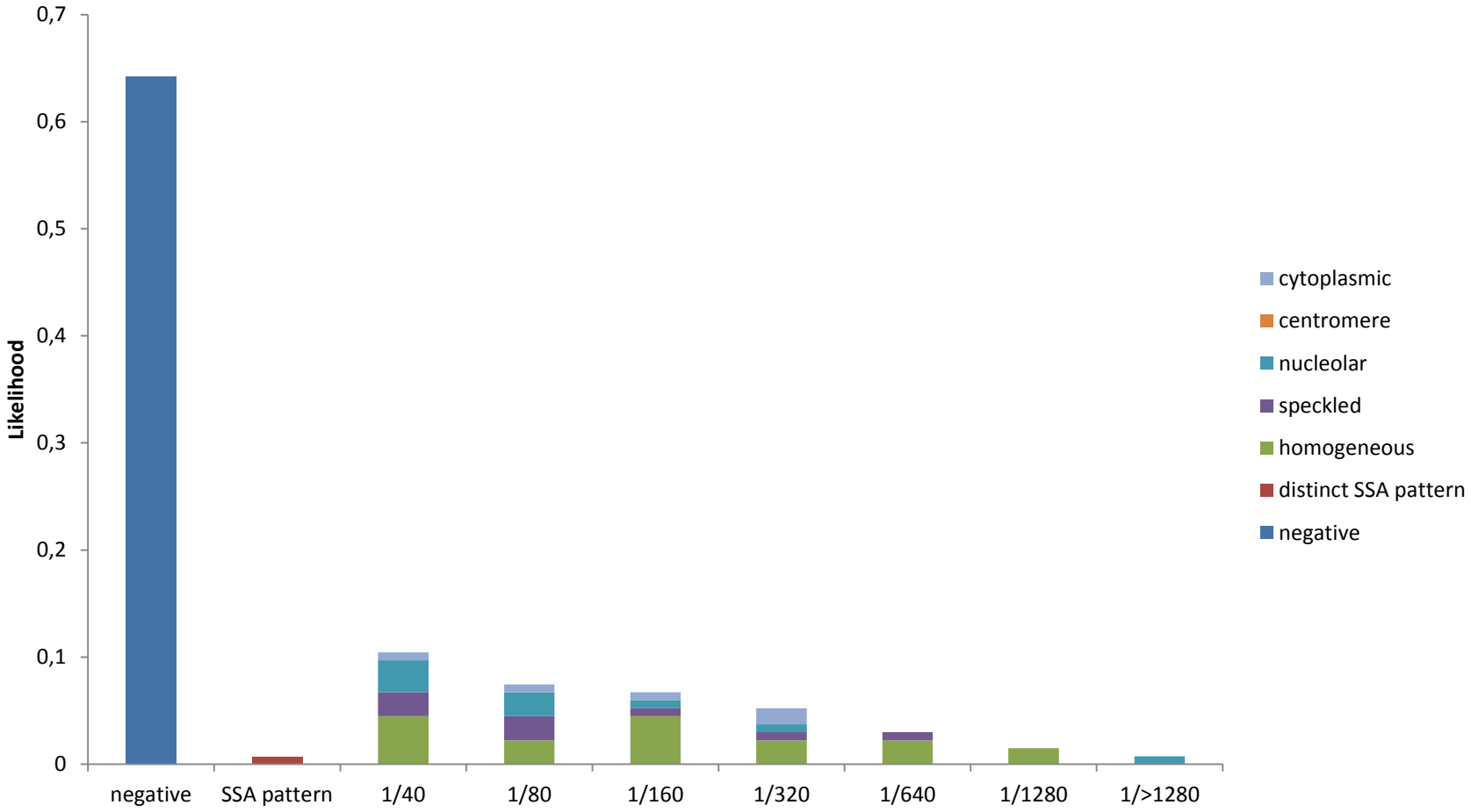
Blood donors



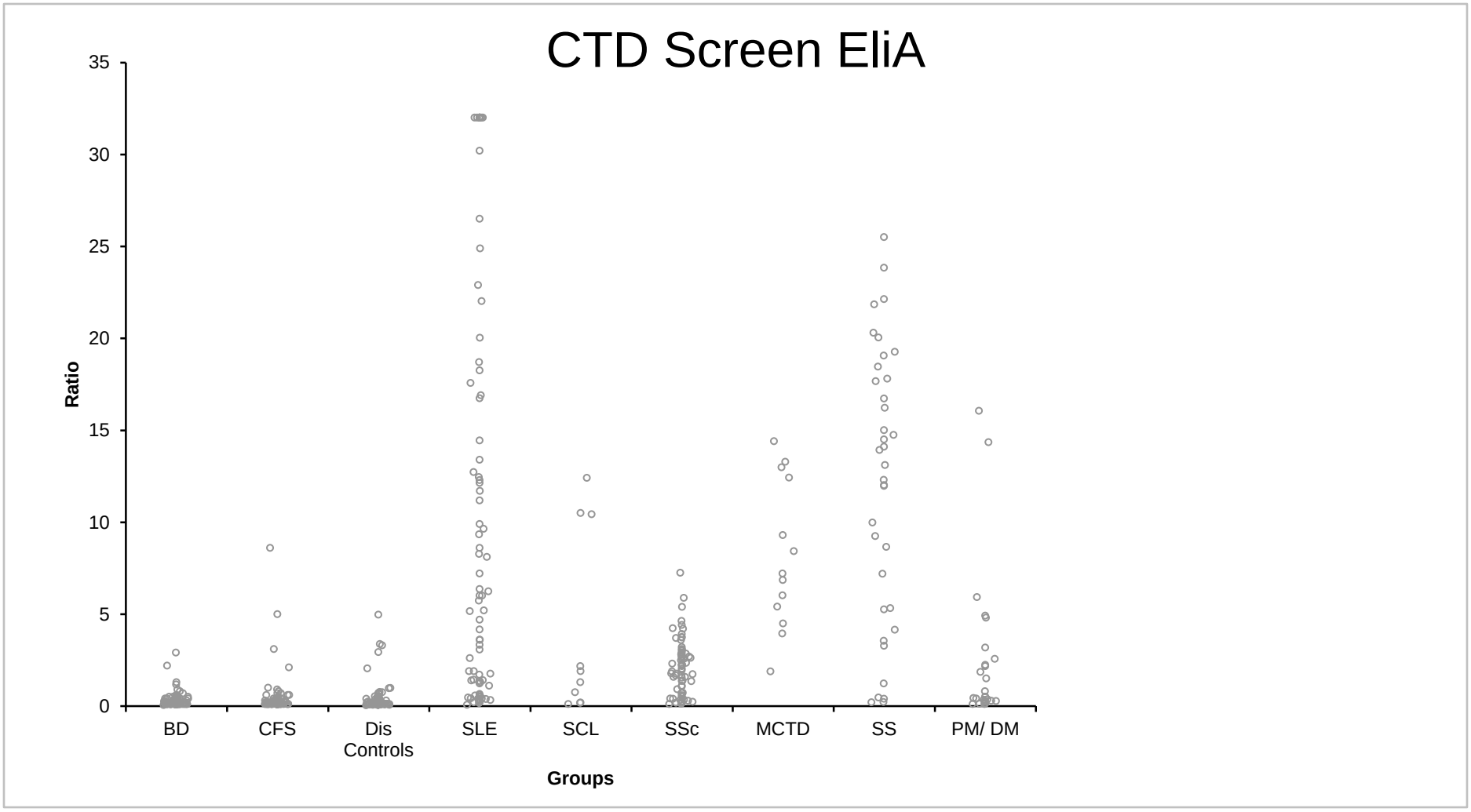
Chronic fatique syndrome



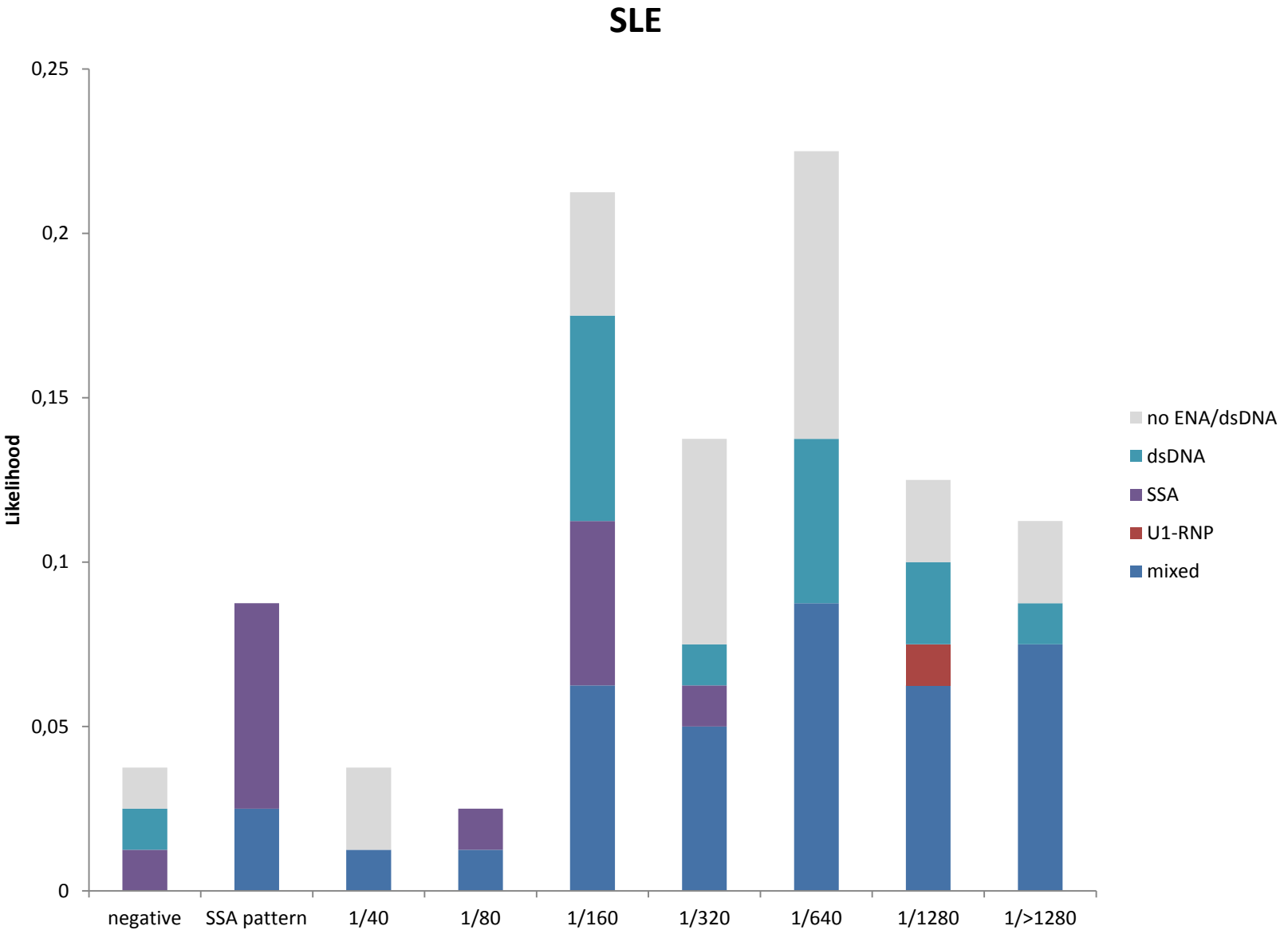
Diseased controls

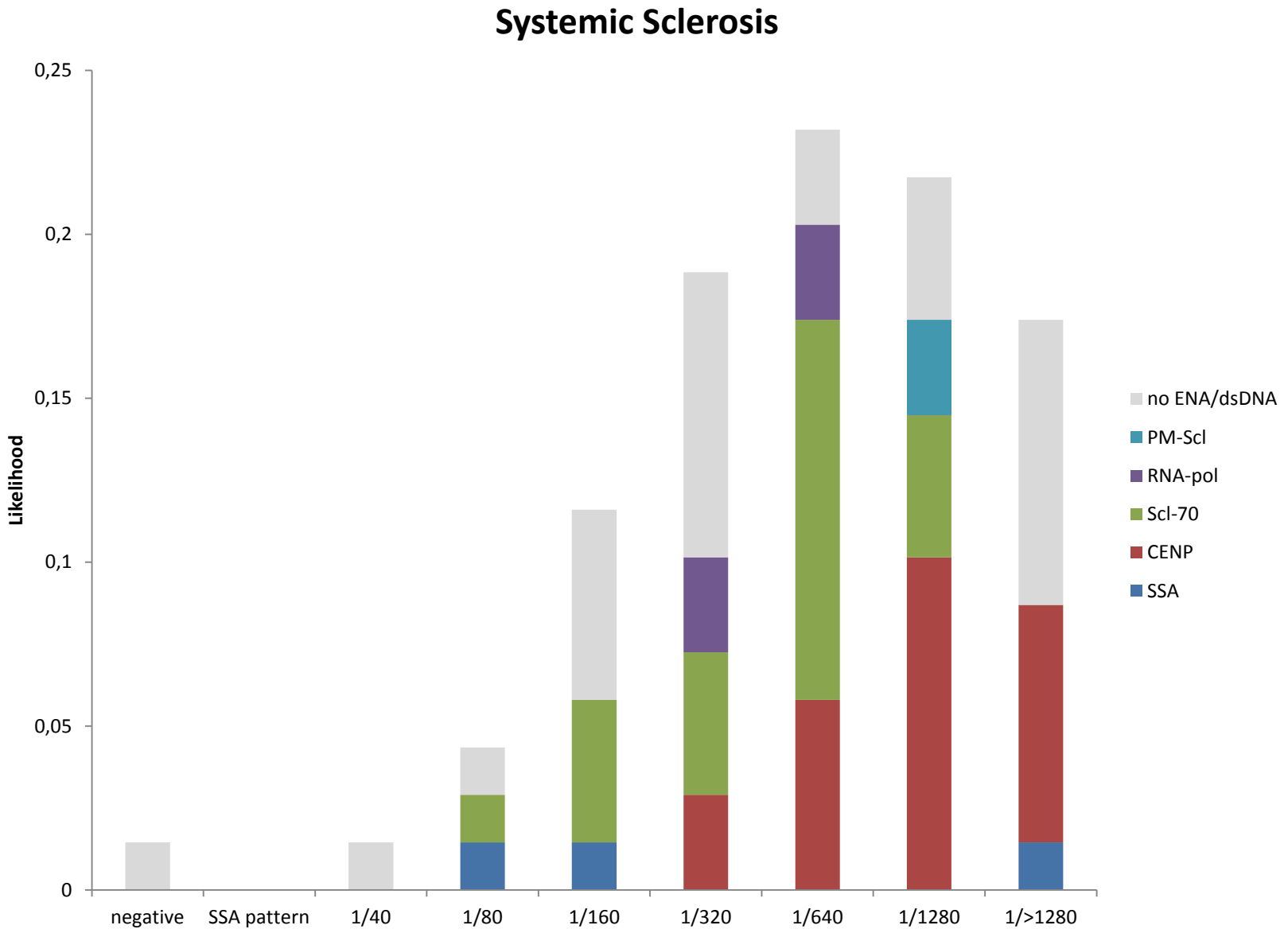


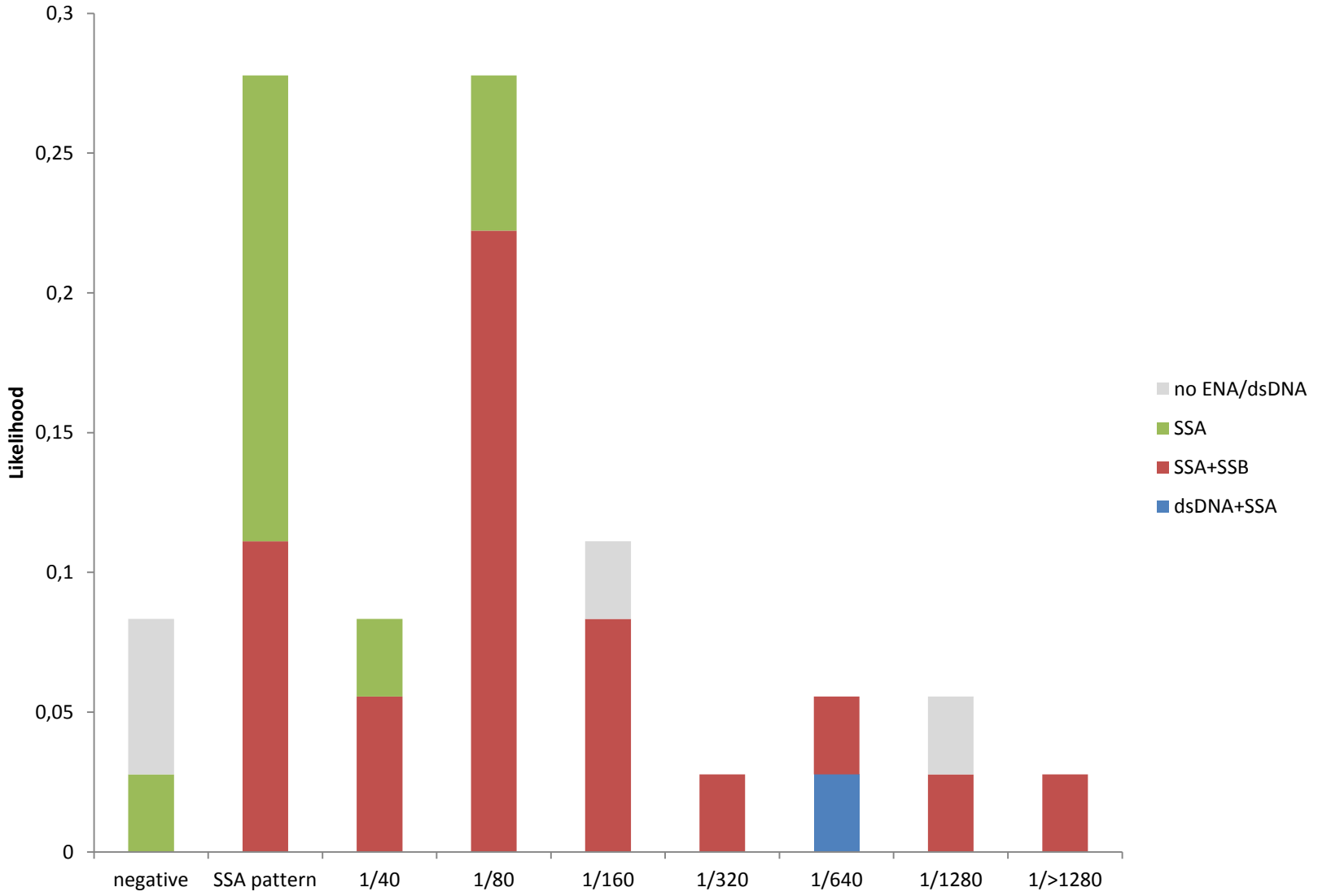
Evaluation of ANA by solid phase assay - EliA


CTD Screen EliA
Ratio
35
30
25
20
15
10
5
0
BD
CFS
Dis Controls
SLE
SCL
SSc
MCTD
SS
PM/ DM
Groups

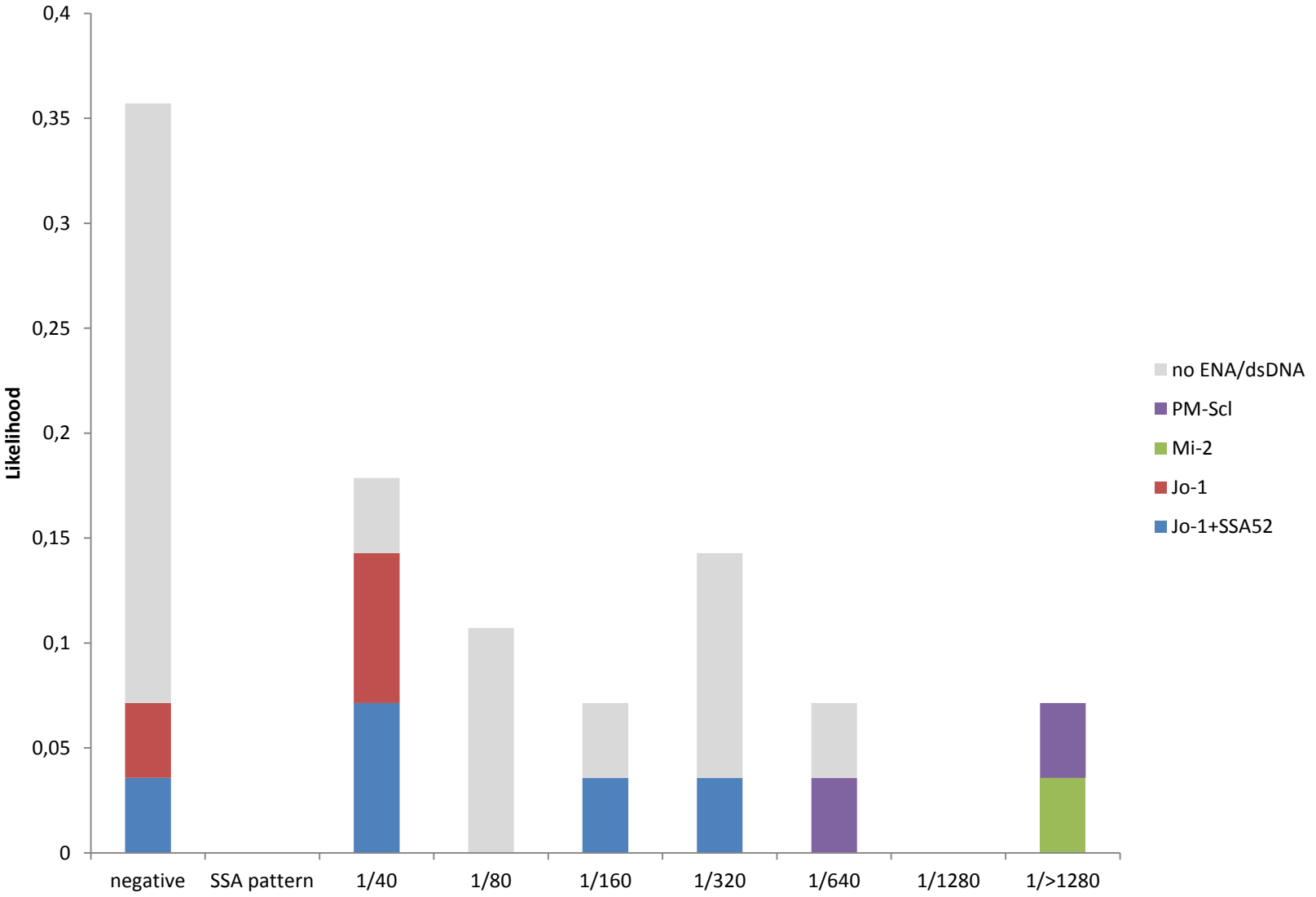
%		CTD screen		ANA 1:40 or SSA pâttern	ANA 1:80 or SSA pattern	ANA 1:160 or SSA pattern	ANA 1:320 or SSA pattern	ANA 1:640 or SSA pattern	ANA 1:1280 or SSA pattern
SLE		74		96,3	92,5	90,0	68,8	53,8	31,3
SSc		72		98,6	97,1	92,8	81,2	62,3	39,1
MCTD		100		100,0	100,0	100,0	100,0	92,3	69,2
SS		89		91,7	83,3	55,6	44,4	41,7	36,1
PM/DM		39		64,3	46,4	35,7	28,6	14,3	7,1
Blood donors		2.7		12,1	8,7	6,0	2,7	1,3	0,7
CFS		2.9		14,4	7,9	3,6	2,9	2,9	2,9
Diseased controls		3.7		35,8	25,4	17,1	11,2	6,0	3,0

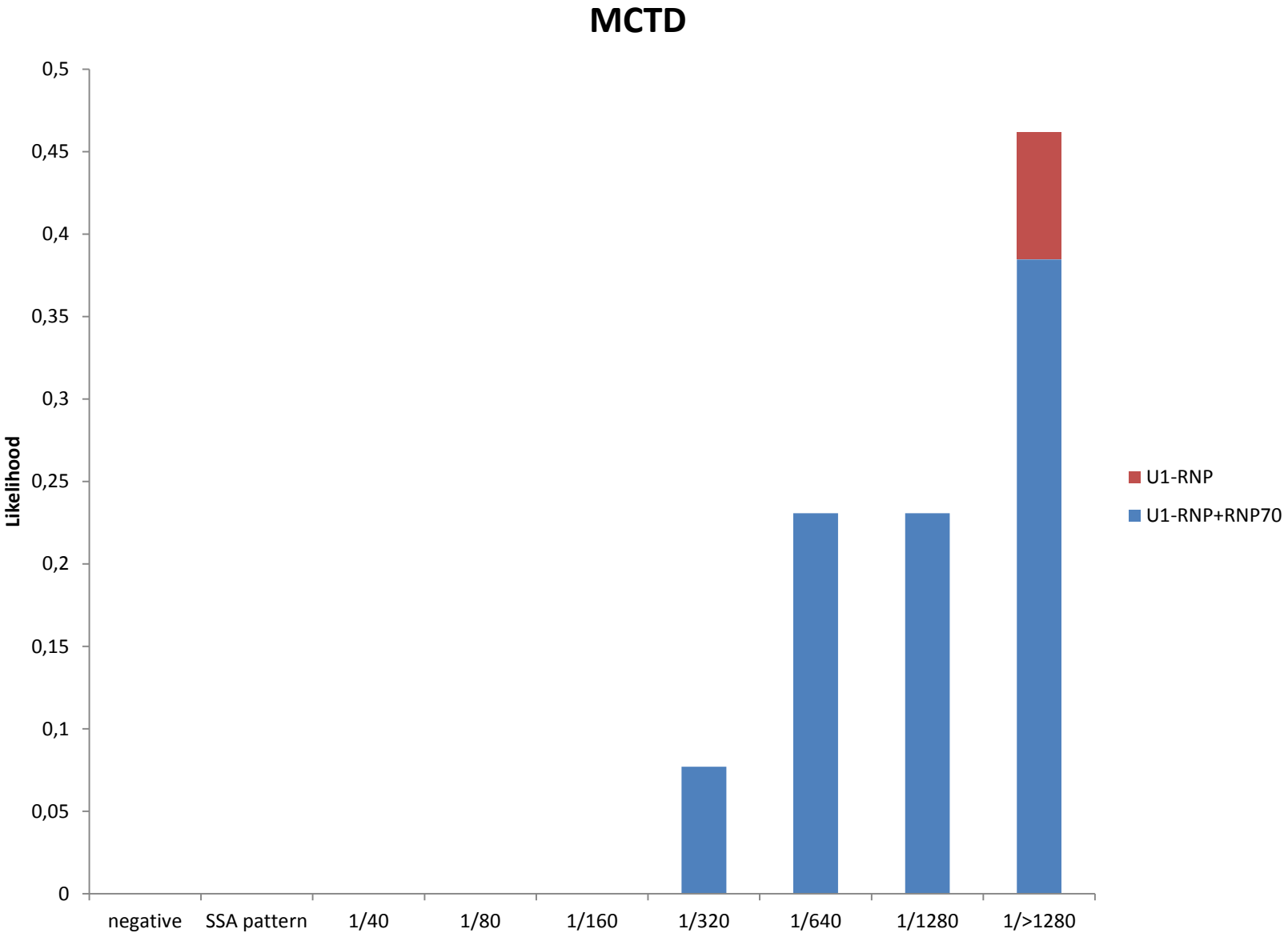

SLE
Likelihood
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
no ENA/dsDNA
dsDNA
SSA
U1-RNP
mixed


Systemic Sclerosis
Likelihood
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
no ENA/dsDNA
PM-Scl
RNA-pol
Scl-70
CENP
SSA


Sjögren's syndrome
Likelihood
0,3
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
no ENA/dsDNA
SSA
SSA+SSB
dsDNA+SSA

Inflammatory myopathy




MCTD
Likelihood
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280
U1-RNP
U1-RNP+RNP70

%		dsDNA	SSA-60	SSA-52	SSB	U1-RNP	RNP-70	Sm	Rib-P	CENP	Scl-70	Pol-III	PM-Scl	Jo-1	Mi-2	CTD screen
SLE		45	48	38	19	16	6.3	6.3	8.8							74
SCL			60	30	10											60
SSc		1.4	7.2	2.9	1.4					35	28	7.2	2.9			72
MCTD		7.7				100	92		7.7							100
SS		2.8	86	81	58											89
PM/DM				18		3.6				3.6			7.1	29	3.6	39
Blood donors		0.7								0.7						2.7
CFS		0.7	1.4	0.7						0.7						2.9
Diseased controls		0.7		2.2		0.7									0.7	3.7

%	≥1	≥2	≥3	≥4	≥5	≥6	≥7
SLE	72.5	48.8	32.5	17.5	7.5	6.3	1.3
SCL	60.0	30.0	10.0				
SSc	73.9	10.1	1.4				
MCTD	100.0	92.3	15.4				
SS	88.9	86.1	52.8				
PM/DM	39.3	17.9	7.1				
Blood donors	1.3						
CFS	2.9	0.7					
Diseased controls	4.5						

Antinuclear antibody detection: indirect immunofluorescene versus solid phase assay

Xavier Bossuyt

ANA screening: an old test with new recommendations

Pier Luigi Meroni,[1] Peter H Schur[2]

Table 1 Antinuclear antibodies (ANA) in systemic lupus erythematosus (SLE) by immunofluorescence (IFA) and solid phase immunoassay

Reference	No of SLE patients	IFA (% SLE patients positive)	Solid phase assay (% SLE patients positive)	Solid phase assay method
9	55	91% (1:80)*	87% 89% 78%	Radim SpA EIA Zeus EIA VarElisa ReCombi (Pharmacia)
11	53	91% (>1:50)	49%	Athena Multilyte
16	71 (including SLE, DLE, drug-induced)	98% (1:40)	91%	RADIAS (Bio-Rad)
17	34	76% (>1:160)	62%	ELIA Pharmacia
19	202	87%	75%	VarElisa ELISA
30	50	84% at 1:50 80% at 1:100 76% at 1:200	40% 56%	AntiNucleosomes GmbH QUANTA Lite (INOVA)
28	38	92%	79%	QUANTA Lite (INOVA)
32	192	99% (81%)†	75.5%	Bioplex
33	35	97% (>1:160)	100% 94% 100% 60% 62%	Quanta Life Bio-Rad Relisa VarElisa UniCap

*Indicates the dilution used if reported in the paper.
†Two different percentages are reported in the paper.

Annals Rheum Dis 2010;69:1420-22

ORIGINAL INVESTIGATION

Diagnostic Accuracy for Lupus and Other Systemic Autoimmune Diseases in the Community Setting

Sonali Narain, MD, MPH; Hanno B. Richards, MD; Minoru Satoh, MD, PhD; Marlene Sarmiento, BSN; Richard Davidson, MD, MPH; Jonathan Shuster, PhD; Eric Sobel, MD, PhD; Paulette Hahn, MD; Westley H. Reeves, MD

Background: Most individuals with autoimmune and other immune disorders undergo initial evaluation in the community setting. Since misdiagnosis of systemic autoimmune diseases can have serious consequences, we evaluated community physicians' accuracy in diagnosing autoimmune diseases and the consequences of misdiagnosis.

Methods: We studied the patients referred to our Autoimmune Disease Center for 13 months (n=476). We estimated the degree of agreement with the final diagnosis (κ statistic) and the accuracy indexes (sensitivity, specificity, and predictive values) of the referring physicians' diagnoses.

Results: We found a 49% agreement between the referring and final diagnoses (κ=0.36). Of 263 patients referred with a presumptive diagnosis of systemic lupus erythematosus (SLE), 125 received a diagnosis of other conditions (κ=0.34). Of those referred with SLE, 76 (29%) were seropositive for antinuclear antibodies but did not have autoimmune disease. The degree of agreement for referring rheumatologists (κ=0.55) was better than that for nonrheumatologists (κ=0.32). Stepwise logistic regression indicated that rheumatologists were 4 times more likely to make an accurate diagnosis of SLE than were nonrheumatologists ($P<.003$). Thirty-nine patients who were seropositive for antinuclear antibodies but had no autoimmune disease had been treated with corticosteroids at dosages as high as 60 mg/d.

Conclusions: Many patients with a positive antinuclear antibody test are incorrectly given a diagnosis of SLE and sometimes treated with toxic medications. The data support the importance of continuing medical education for community physicians in screening for autoimmune diseases and identifying patients who may benefit from early referral to a specialist.

Arch Intern Med. 2004;164:2435-2441

Aim

To evaluate the diagnostic performance of
IIF, solid phase assay, & the combination of both for
Systemic lupus erythematosus (SLE)
Systemic slcerosis (SSc),
pSjogren's syndrome (SS)

1

Autoimmunity Reviews 10 (2011) 801–808

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay

Katrijn Op De Beeck [a,1], Pieter Vermeersch [b,1], Patrick Verschueren [c], René Westhovens [c], Godelieve Mariën [b], Daniel Blockmans [d], Xavier Bossuyt [a,b,*]

IIF: HEp-2000 (Immunoconcepts)

EliA CTD screen (Thermo Fisher)
SSA/Ro 52, SSA/Ro 60, SSB/La, U1-RNP (RNP-70, A, C), Sm, centromere B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA, and Mi-2, dsDNA

2

Autoimmunity Reviews 12 (2012) 137–143

Contents lists available at SciVerse ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Antinuclear antibody detection by automated multiplex immunoassay in untreated patients at the time of diagnosis

Katrijn Op De Beéck [a], Pieter Vermeersch [b], Patrick Verschueren [c], René Westhovens [c], Godelieve Mariën [b], Daniel Blockmans [d], Xavier Bossuyt [a,b,*]

[a] Experimental Laboratory Immunology, Catholic University Leuven, Leuven, Belgium
[b] Department of Laboratory Medicine, Immunology, University Hospitals Leuven, Leuven, Belgium
[c] Department of Rheumatology, University Hospitals Leuven, Leuven, Belgium
[d] Department of General Internal Medicine, University Hospitals Leuven, Leuven, Belgium

BioPlex 2200
chromatin, Sm, ribosomal protein, SSA-60, SSB
RNP-A and RNP-68, SSA-52, Scl-70, Jo-1, centromere B
dsDNA

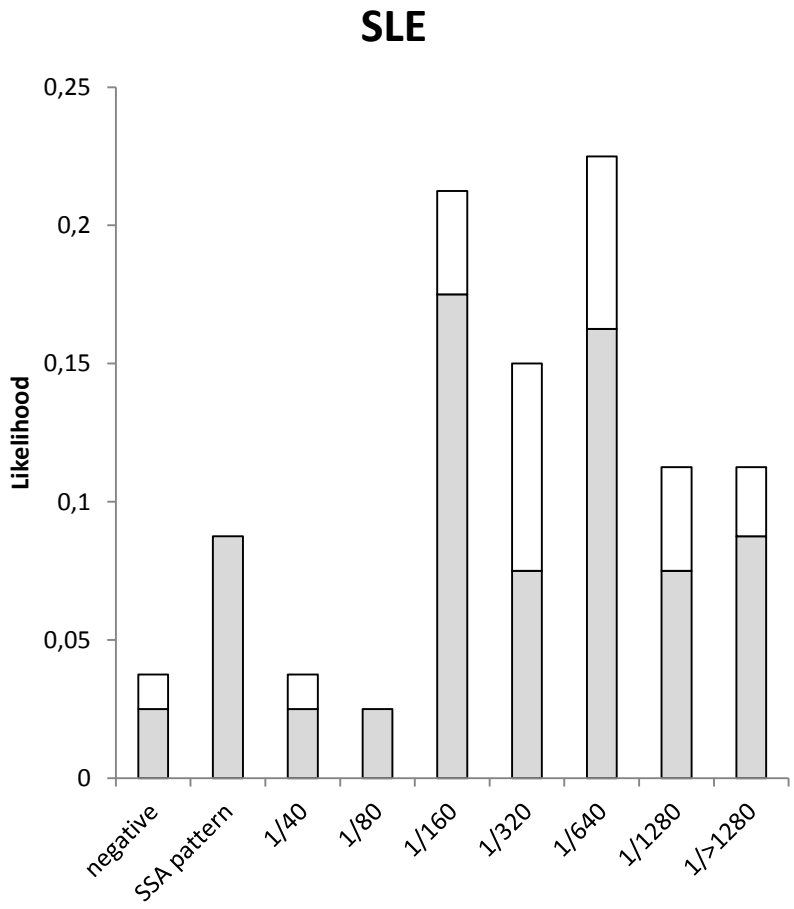
3

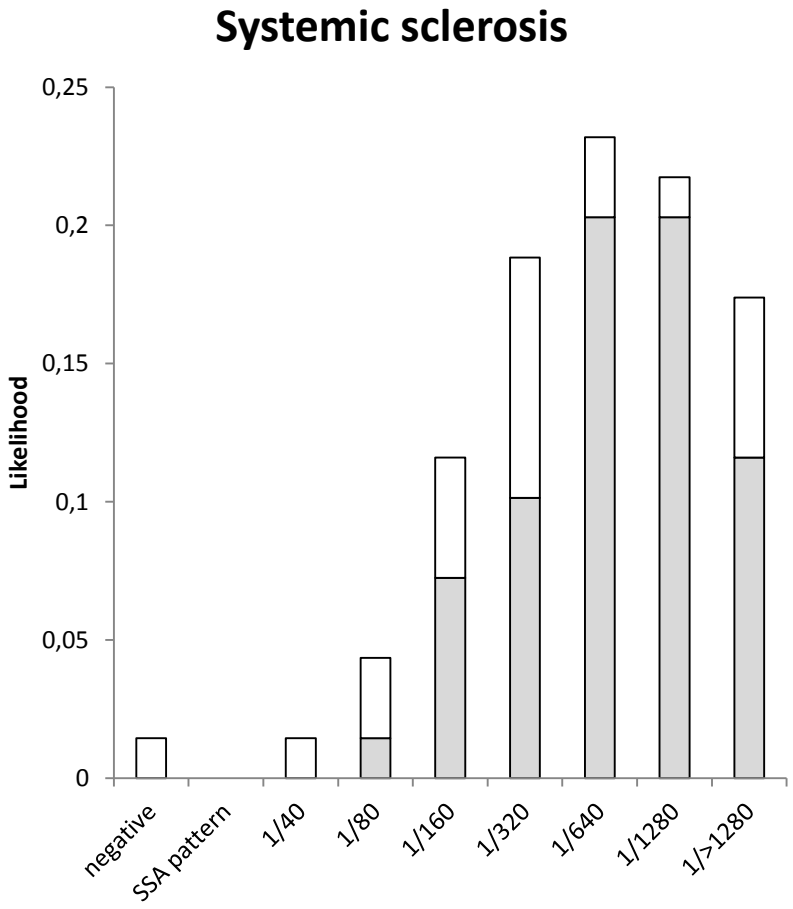
ARTHRITIS & RHEUMATISM
Vol. 40, No. 9, September 1997, pp 1601–1611

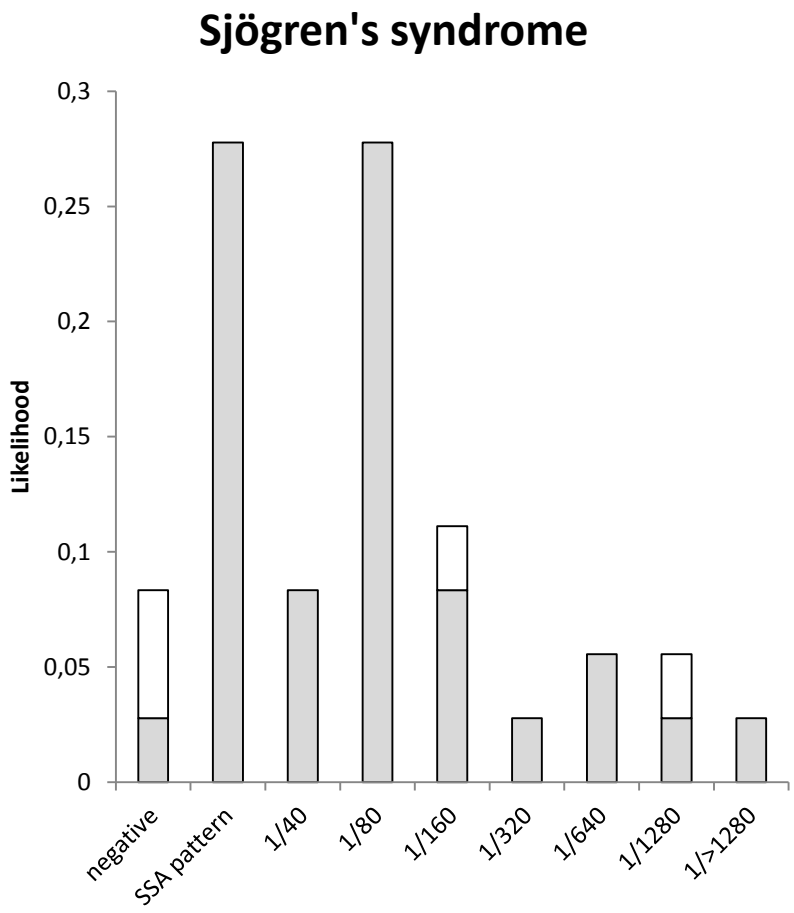
RANGE OF ANTINUCLEAR ANTIBODIES IN "HEALTHY" INDIVIDUALS

E. M. TAN, T. E. W. FELTKAMP, J. S. SMOLEN, B. BUTCHER, R. DAWKINS, M. J. FRITZLER, T. GORDON, J. A. HARDIN, J. R. KALDEN, R. G. LAHITA, R. N. MAINI, J. S. McDOUGAL, N. F. ROTHFIELD, R. J. SMEENK, Y. TAKASAKI, A. WIIK, M. R. WILSON, and J. A. KOZIOL

	IIF 1:80	IIF 1:160	EliA CTD screen	BioPlex 2200
Sensitivity				
Systemic lupus erythematosus				
Reference 1, 2 (n=80)	92.5 (84-97)	90 (81-95)	74 (63-82)	79 (69-86)
Reference 3 (n=41)	97 (87-99)	95 (83-99)		
Systemic sclerosis				
Reference 1, 2 (n=69)	97 (90-99)	93 (84-97)	72.5 (61-82)	72 (61-82)
Reference 3 (n=37)	95 (82-98)	86.5 (72-94)		
Sjögren's syndrome				
Reference 1, 2 (n=36)	83 (68-92)	56 (40-70)	89 (75-96)	89 (75-96)
Reference 3 (n=40)	76 (62-88)	74 (57-83)		
Specificity				
Healthy controls (n=125) (Ref. 3)	87 (80-92)	95 (90-98)		
Blood donors (n=149) (Ref.1, 2)	91 (85-95)	94 (89-97)	97 (93-99)	95 (90-97)
Chronic fatigue syndrome (n=139) (Ref.1, 2)	92 (86-96)	96 (92-98)	97 (92-99)	93 (87-96)
Diseased controls (n=134) (Ref.1, 2)	75 (67-81)	82 (75-88)	96 (91-98)	89 (82-93)
All controls (n=422) (Ref.1, 2)	*86 (83-89)*	*91 (88-93)*	*97 (95-98)*	*92 (89-94)*


SLE
Likelihood
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280


Systemic sclerosis
Likelihood
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280

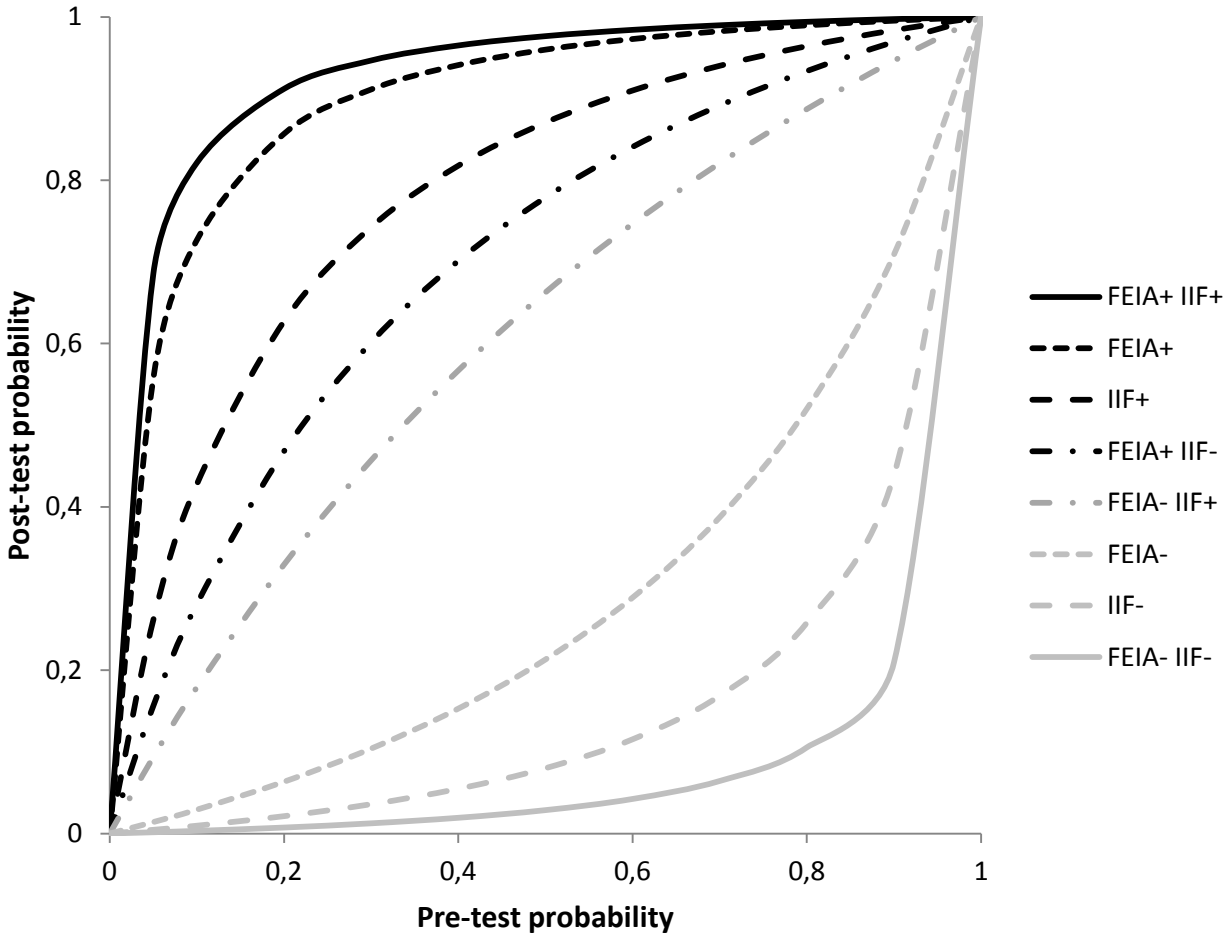

Sjögren's syndrome
Likelihood
0,3
0,25
0,2
0,15
0,1
0,05
0
negative
SSA pattern
1/40
1/80
1/160
1/320
1/640
1/1280
1/>1280

LR	Systemic lupus erythematosus	Systemic sclerosis	Sjögren's syndrome
IIF(+) (Ref. 1, 2)	6.7 (5.3-8.6)	7.1 (5.5-9.0)	6.1 (4.6-8.0)
IIF(+) (Ref. 3)	7.1 (4.6-11.2)	6.4 (4.0-10.1)0	5.7 (3.5-5.9)
IIF(-) (Ref. 1, 2)	0.08 (0.04-0.19)	0.03 (0.01-0.13)	0.19 (0.09-0.40)
IIF(-) (Ref. 3)	0.03 (0.004-0.20)	0.16 (0.03-0.35)	0.26 (0.15-0.46)
EliA CTD screen (+)	23.9 (13.8-41.5)	23.5 (13.5-40.9)	28.8 (16.7-49.9)
EliA CTD screen (-)	0.27 (0.19-0.39)	0.28 (0.19-0.42)	0.11 (0.05-0.29)
IIF (+) EliA CTD screen (+)	41.4 (19.6-87.7)	43.6 (20.7-46.8)	46.8 (22.0-99.8)
IIF (+) EliA CTD screen (-)	2.0 (1.2-3.1)	2.0 (1.3-3.3)	0.45 (0.12-1.8)
IIF (-) EliA CTD screen (+)	3.5 (1.0-12.2)		7.8 (2.3-26.4)
IIF (-) EliA CTD screen (-)	0.03 (0.007-0.12)	0.03 (0.009-0.13)	0.06 (0.02-0.25)

LR	Systemic lupus erythematosus	Systemic sclerosis	Sjögren's syndrome
IIF(+) (Ref. 1, 2)	6.7 (5.3-8.6)	7.1 (5.5-9.0)	6.1 (4.6-8.0)
IIF(+) (Ref. 3)	7.1 (4.6-11.2)	6.4 (4.0-10.1)0	5.7 (3.5-5.9)
IIF(-) (Ref. 1, 2)	0.08 (0.04-0.19)	0.03 (0.01-0.13)	0.19 (0.09-0.40)
IIF(-) (Ref. 3)	0.03 (0.004-0.20)	0.16 (0.03-0.35)	0.26 (0.15-0.46)
EliA CTD screen (+)	23.9 (13.8-41.5)	23.5 (13.5-40.9)	28.8 (16.7-49.9)
EliA CTD screen (-)	0.27 (0.19-0.39)	0.28 (0.19-0.42)	0.11 (0.05-0.29)
IIF (+) EliA CTD screen (+)	41.4 (19.6-87.7)	43.6 (20.7-46.8)	46.8 (22.0-99.8)
IIF (+) EliA CTD screen (-)	2.0 (1.2-3.1)	2.0 (1.3-3.3)	0.45 (0.12-1.8)
IIF (-) EliA CTD screen (+)	3.5 (1.0-12.2)		7.8 (2.3-26.4)
IIF (-) EliA CTD screen (-)	0.03 (0.007-0.12)	0.03 (0.009-0.13)	0.06 (0.02-0.25)

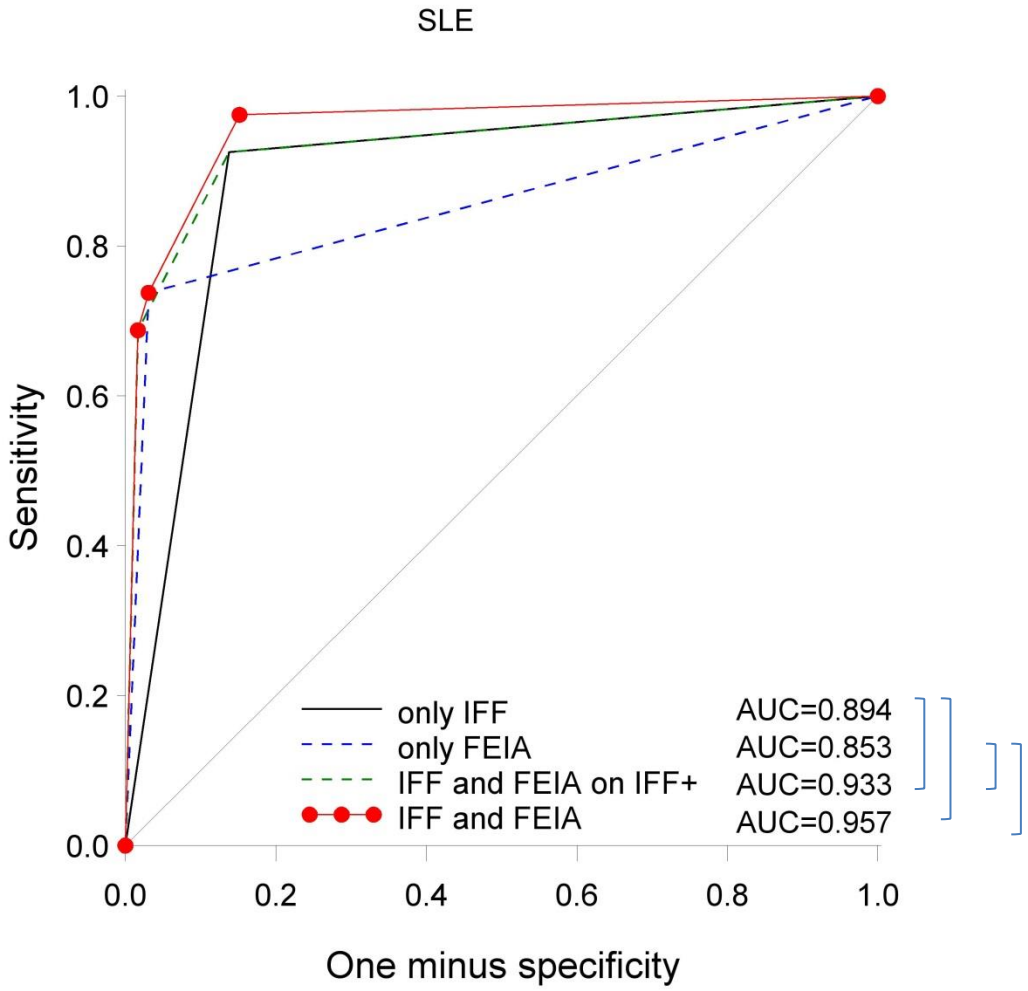
Prevalence	Systemic lupus erythematosus	Systemic sclerosis	Sjögren's syndrome	Controls
IIF (+) EliA CTD screen (+)	69%	72%	78%	1,6%
IIF (+) EliA CTD screen (-)	24%	25%	5,5%	12%
IIF (-) EliA CTD screen (+)	5%		11%	1,4%
IIF (-) EliA CTD screen (-)	2%	3%	5,5%	85%

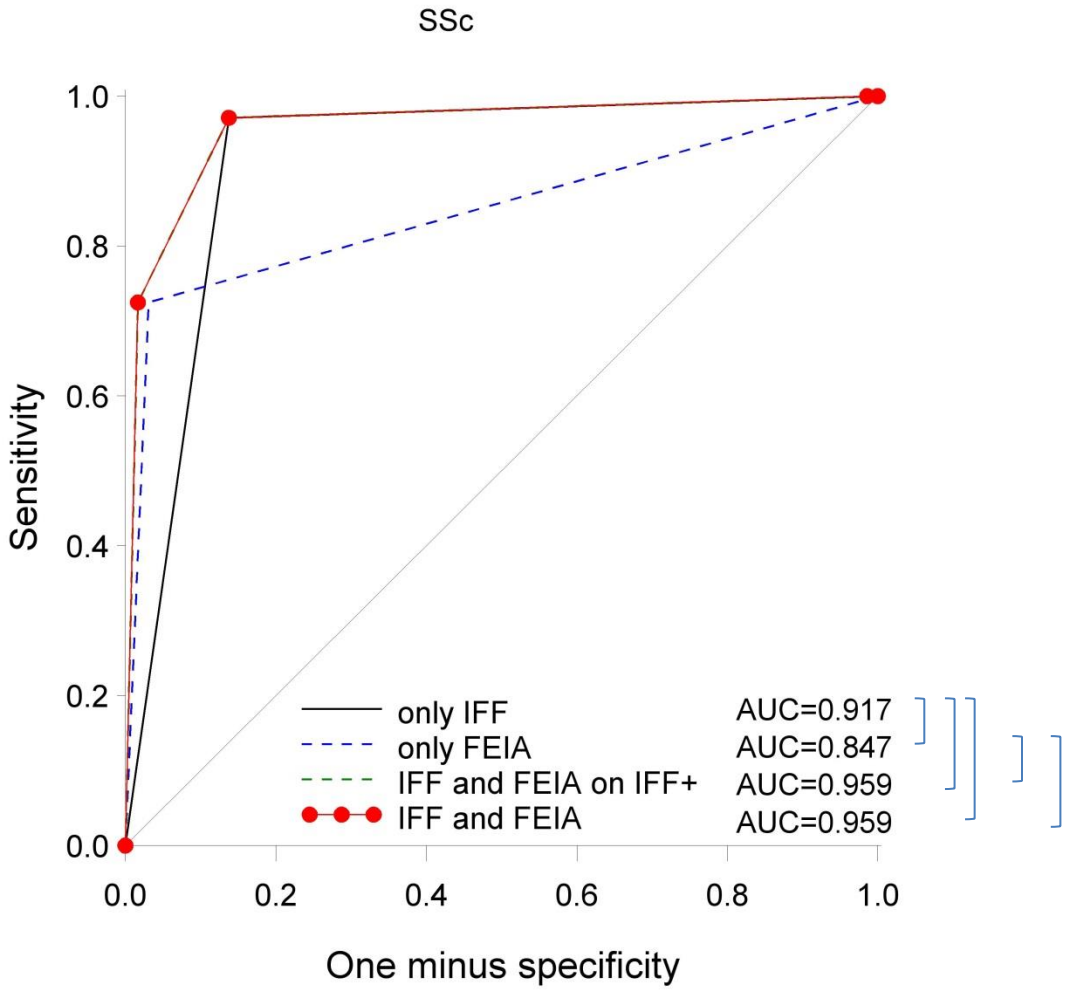
SLE

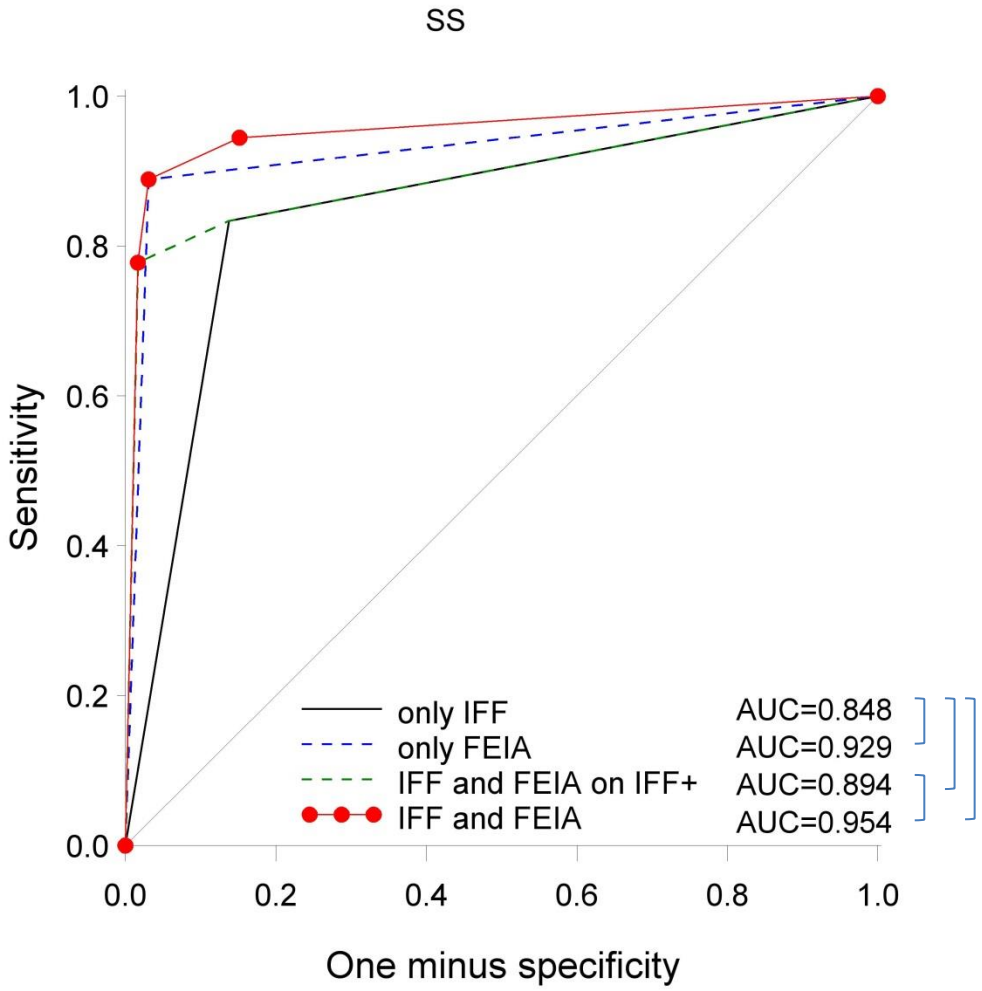

1
0,8
0,6
0,4
0,2
0
Post-test probability
0
0,2
0,4
0,6
0,8
1
Pre-test probability
FEIA+ IIF+
FEIA+
IIF+
FEIA+ IIF-
FEIA- IIF+
FEIA-
IIF-
FEIA- IIF-

Comparative analysis of different strategies

1. IIF and FEIA on all samples
2. IIF
   - If IIF positive: FEIA
   - If IIF negative: stop
3. FEIA
4. IIF


SLE
Sensitivity
One minus specificity
0.0
0.2
0.4
0.6
0.8
1.0
only IFF
only FEIA
IFF and FEIA on IFF+
IFF and FEIA
AUC=0.894
AUC=0.853
AUC=0.933
AUC=0.957


SSc
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
One minus specificity
only IFF
only FEIA
IFF and FEIA on IFF+
IFF and FEIA
AUC=0.917
AUC=0.847
AUC=0.959
AUC=0.959


SS
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
One minus specificity
only IFF
only FEIA
IFF and FEIA on IFF+
IFF and FEIA
AUC=0.848
AUC=0.929
AUC=0.894
AUC=0.954

Conclusion

Integrated interpretation
of IIF and solid phase assay results
adds value

Optimal strategy
depends on the pathology

Bossuyt X, Fieuws S.
Ann Rheum Dis. 2014 Mar;73(3)

Antinuclear antibodies by automated indirect immunofluorescence. Opportunities for value added reporting.

Xavier Bossuyt
Leuven, Belgium

Instrument	NOVA View	AKLIDES	EUROPattern	Image Navigator	Helios	ZENIT G Sight
Manufacturer	INOVA	Medipan	EUROPattern	Immuno Concepts	Aesku	Menarini
LIMS connection (software)	Yes (QUANTA Link)	Yes	Yes (EUROLabOffice)	Yes (direct)	Yes (direct)	Yes (ZenIT)
Slide identification via barcode	Yes by handheld scanner	Yes by handheld scanner	Yes by integrated scanner	Yes	Yes by integrated scanner	Yes by integrated scanner
Loading capacity	5 slides (up to 60 wells)	5 slides (up to 60 wells)	50 slides (up to 500 wells)	4 slides (up to 84 wells)	20 slides (up to 240 wells)	5 slides (up to 70 wells)
Image acquisition speed	~45s/well for 3 images	~40s/well	<20s/well	~25s/well for 4 images	10s/picture Customizable from 1 to 10 images	>60s/well pictures: 5 (small scan), 50 (medium scan), 220 (full scan)
100% QC for substrate and process integrity / counterstaining	Yes / DAPI	Yes / DAPI	Yes / Propidium iodide	None / None	None / None	None / None
Automatic pos./neg. discrimination incl. presorting of images	Yes	Yes	Yes	Yes	Yes	Yes
Batchwise verification of negative samples	Yes	Yes	Yes	Yes	Yes	Yes
Automatic pattern recognition	Yes	Yes	Yes	No	No	Yes
Pattern Analysis method	Pattern recognition by mathematical algorithm	Pattern recognition by mathematical algorithm	Pattern recognition by mathematical algorithm	No pattern matching capabilities	No pattern matching capabilities	Pattern recognition by mathematical algorithm
No. of recognizable ANA staining pattern list out	6 Homogeneous Speckled Centromere Nucleolar Nuclear dot Cytoplasm Negative Positive unrecognized	10 Homogeneous Speckled Centromere Nucleolar Nuclear dot Cytoplasm Negative Positive unrecognized	8 Homogeneous Speckled Centromere Nucleolar Nuclear dot Cytoplasm Nuclear rim Mitotic, negative	None	None	5 Homogeneous Speckled Centromere Nucleolar Cytoplasm Negative
Instrument calibration to minimize variability	Yes	Yes	Yes	Yes	Yes	No
Integration with slide processing in 1 instrument	No	No	No	No	Yes	No

Mahler et al. J. Immunol Research in press

Automated fluorescence microscopy is increasingly used for image acquisition, quantitative analysis, and pattern recognition of anti-nuclear antibody testing.

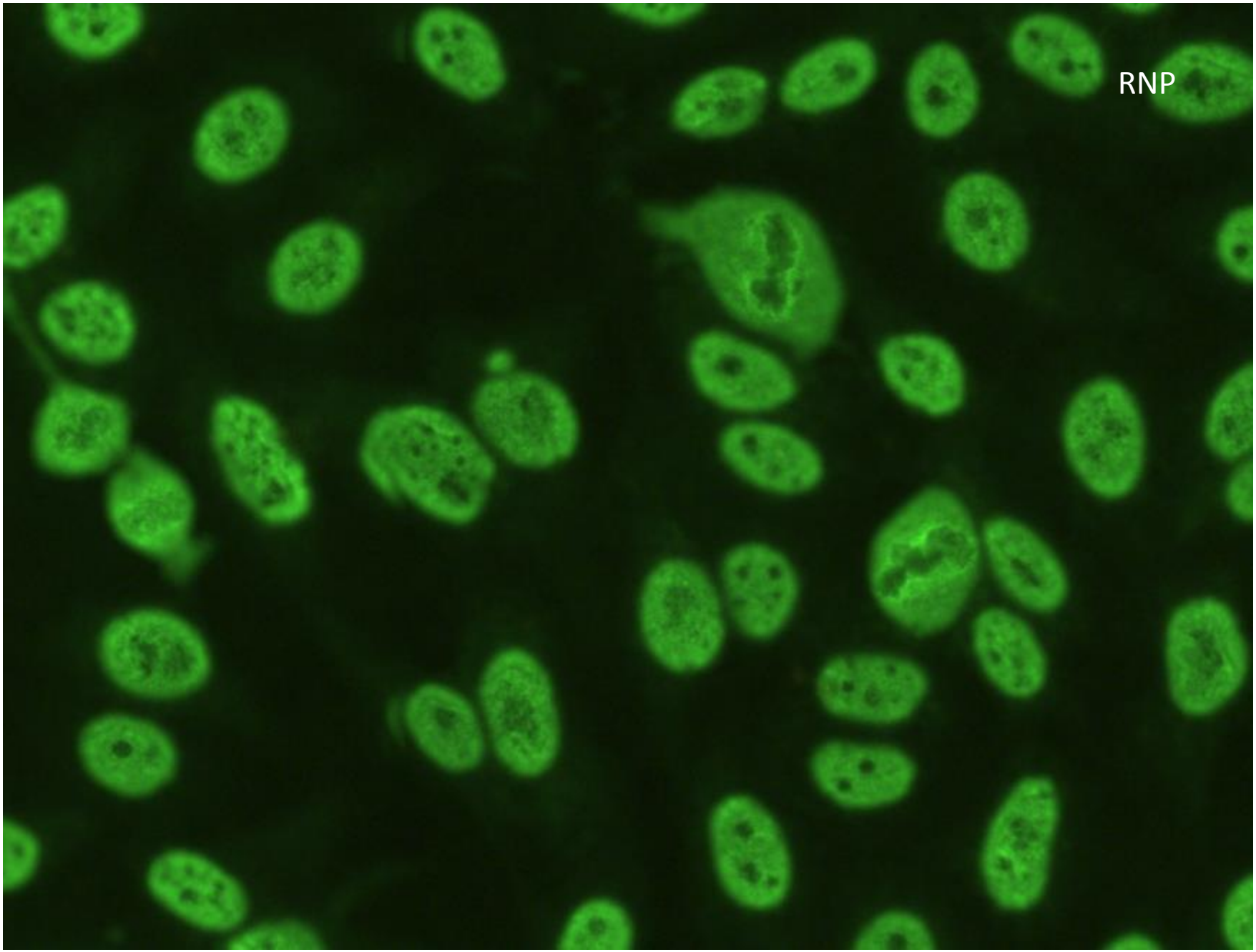
Positive/negative discrimination

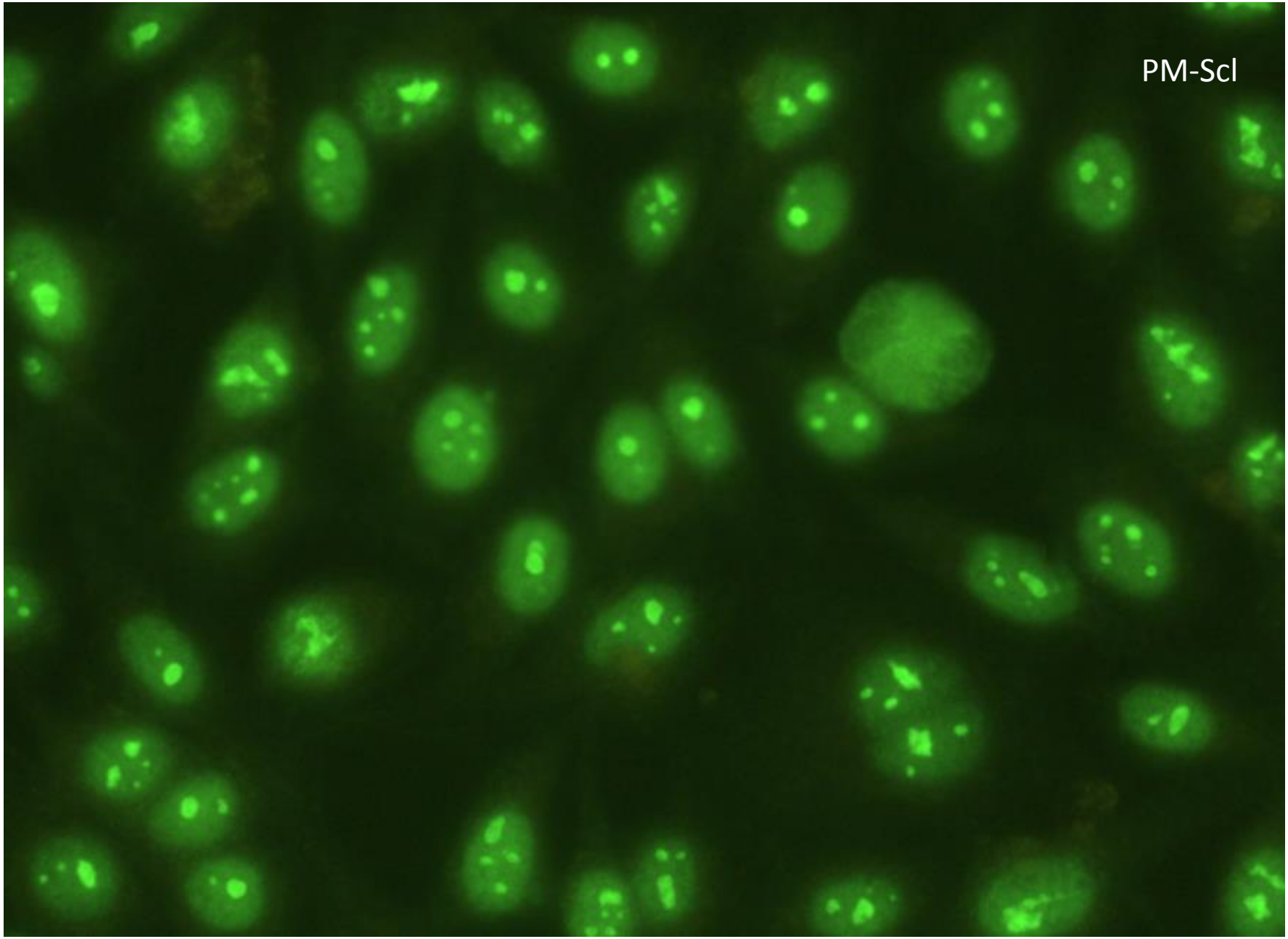
- Exposure time is inversely proportional to intensity of fluorescence
- Probability value is given to samples

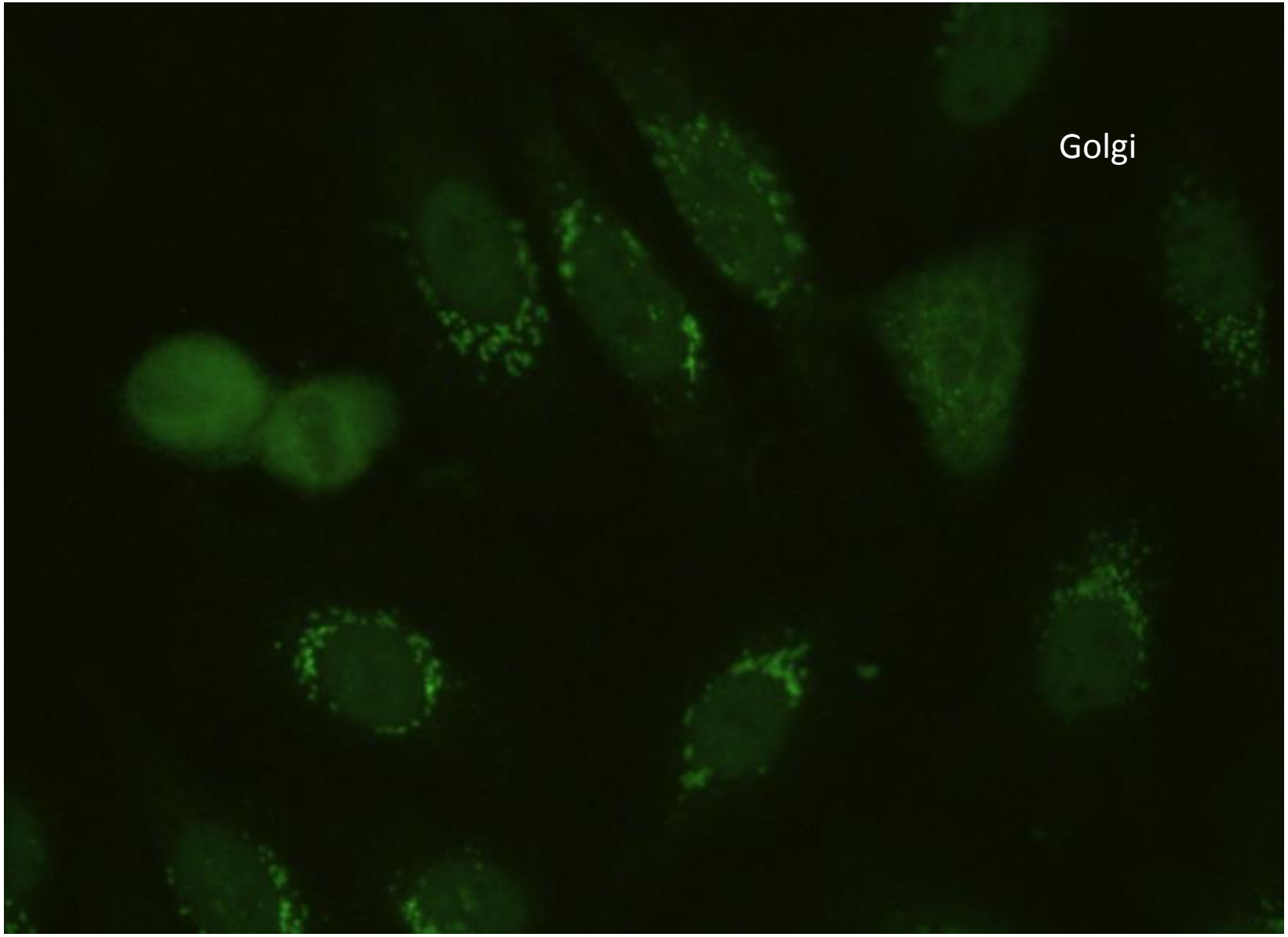
Pattern recognition: ANA

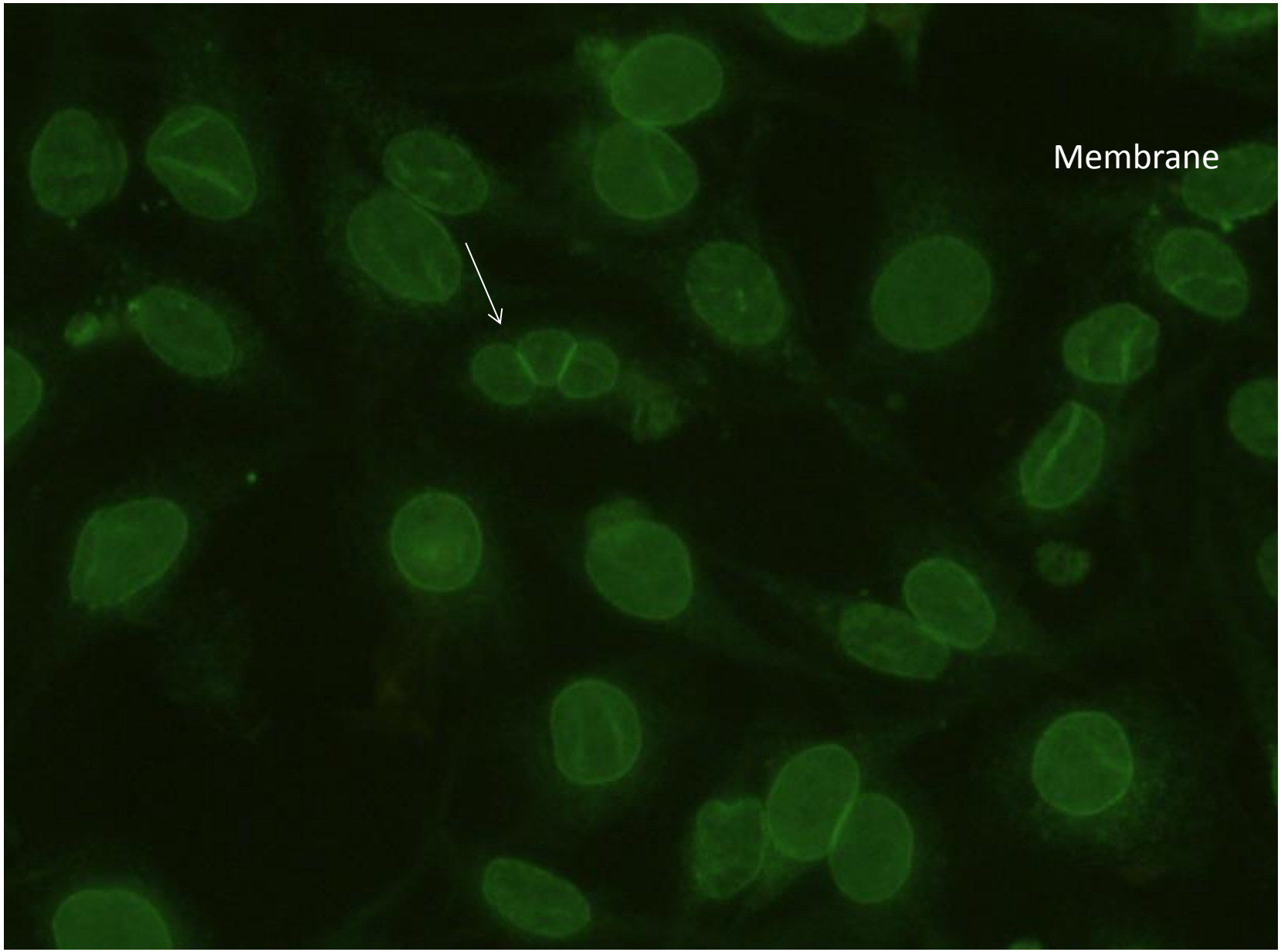
- five patterns:
  - Homogeneous
  - Speckled
  - Centromere
  - Nucleolar
  - Mitochondrial
- Based on statistical, morphological and geometric features

IMAGE ACQUISITION


RNP


PM-Scl


Golgi


Membrane

DE GRUYTER DOI 10.1515/cclm-2013-0016 — Clin Chem Lab Med 2013; 51(9): 1771–1779

Carolien Bonroy*, Charlotte Verfaillie, Vanessa Smith, Lies Persijn, Evy De Witte, Filip De Keyser and Katrien Devreese

Automated indirect immunofluorescence antinuclear antibody analysis is a standardized alternative for visual microscope interpretation

Clinica Chimica Acta 415 (2013) 101–106

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Detection of antinuclear antibodies by automated indirect immunofluorescence analysis

Xavier Bossuyt [a,b,*], Sarah Cooreman [b], Heidi De Baere [c], Patrick Verschueren [d], René Westhovens [d], Daniel Blockmans [e], Godelieve Mariën [b]

Review of digital images

↔

manual approach (pos/neg)

- 90% identical interpretation (n=332) (*Bonroy et al. CCLM 2013*)
- 88% identical interpretation (n=268) (*Bossuyt et al. CCA 2013*)

Table 1 Within- and between-run imprecision determined on the probability index (PI) obtained on Zenit G-sight. A selection of routine serum samples with a minimal fluorescence intensity of 2+ was used for the analysis.

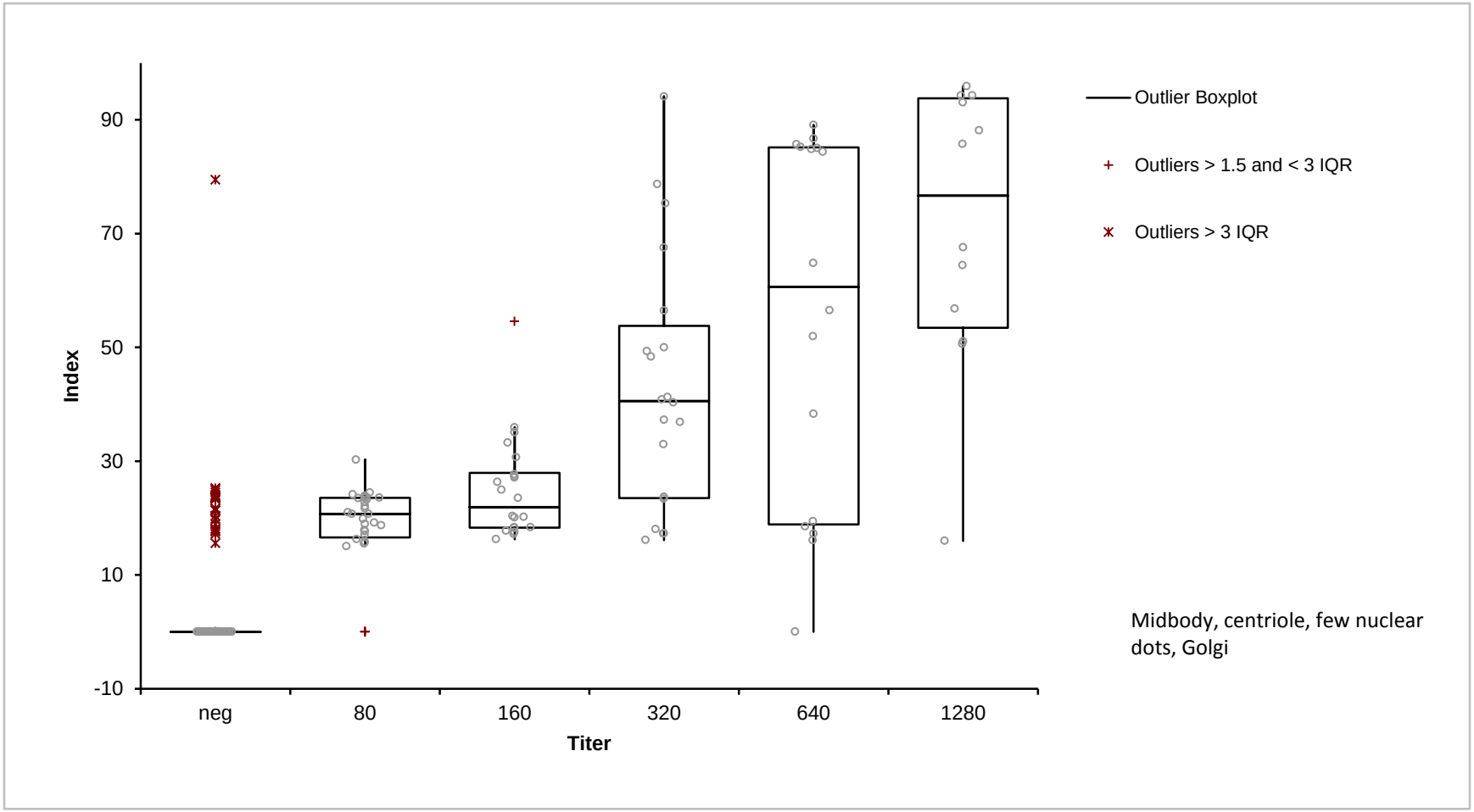
Pattern	n	Mean PI	Range PI	SD	CV%
Within-run imprecision					
Speckled	18	89.2	87–91	1.2	1.4
Homogeneous	18	77.8	57–86	8.5	10.9
Centromere	18	75.1	45–87	11.7	15.6
Nucleolar	18	86.1	71–90	4.7	5.4
SSa	18	83.8	41–90	12.5	14.9
Between-run imprecision					
Homogeneous (positive control)	12	81.3	42–87	12.8	15.8
Negative control[a]	12	7.2	1–11	15.8	48.0

[a]5 out of the 12 PI measurements was below the negative cut-off value (PI<8). CV%, coefficient of variation; n, number of PI measurements (each sample was applied on 6 different wells and analyzed in triplet); PI, probability index; SD, standard deviation.

Bonroy et al. CCLM 2013

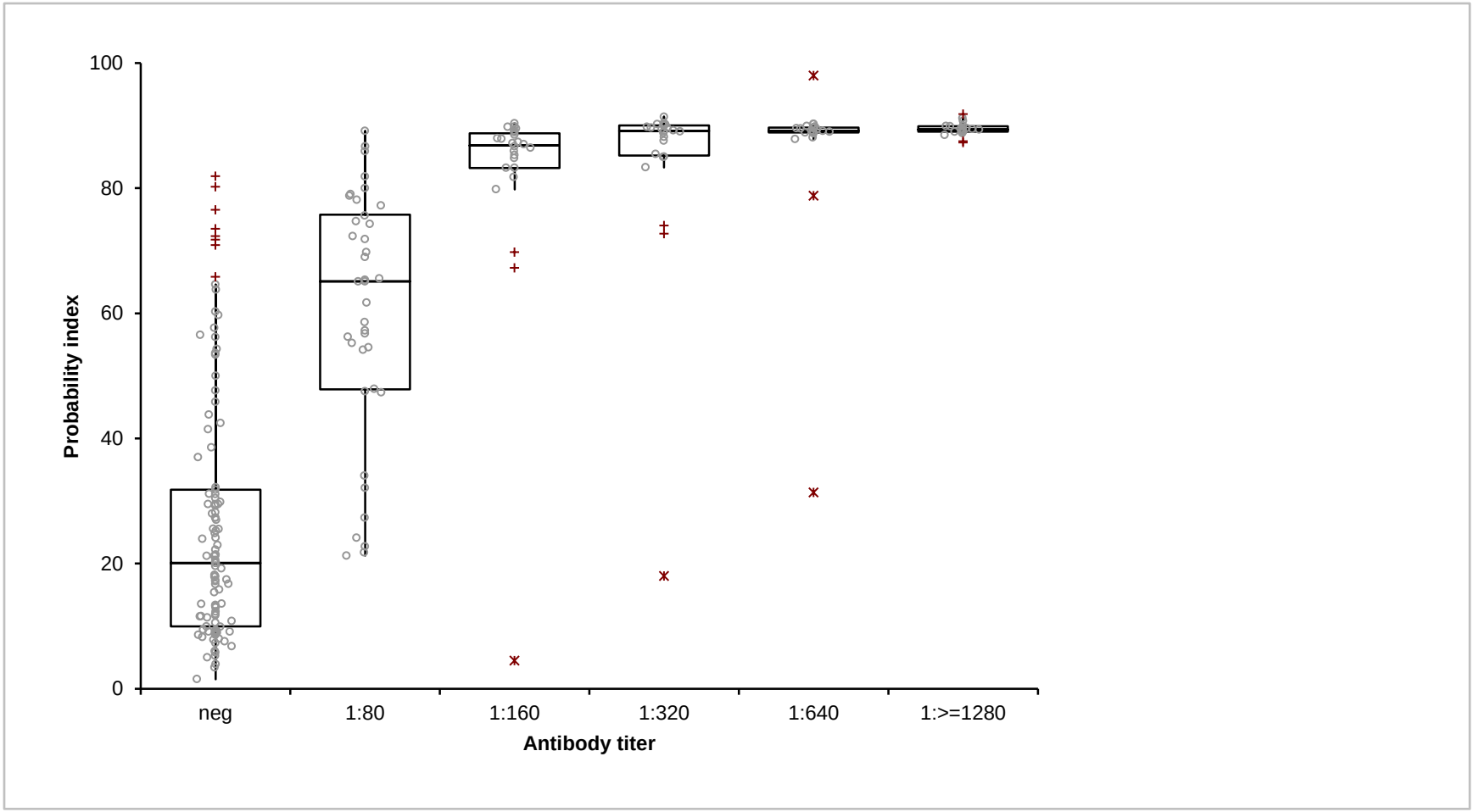
FLUORESENCE INTENSITY VERSUS ANTIBODY TITER

HEp-2



Bossuyt et al. CCA 2013

G-Sight HEp-2000



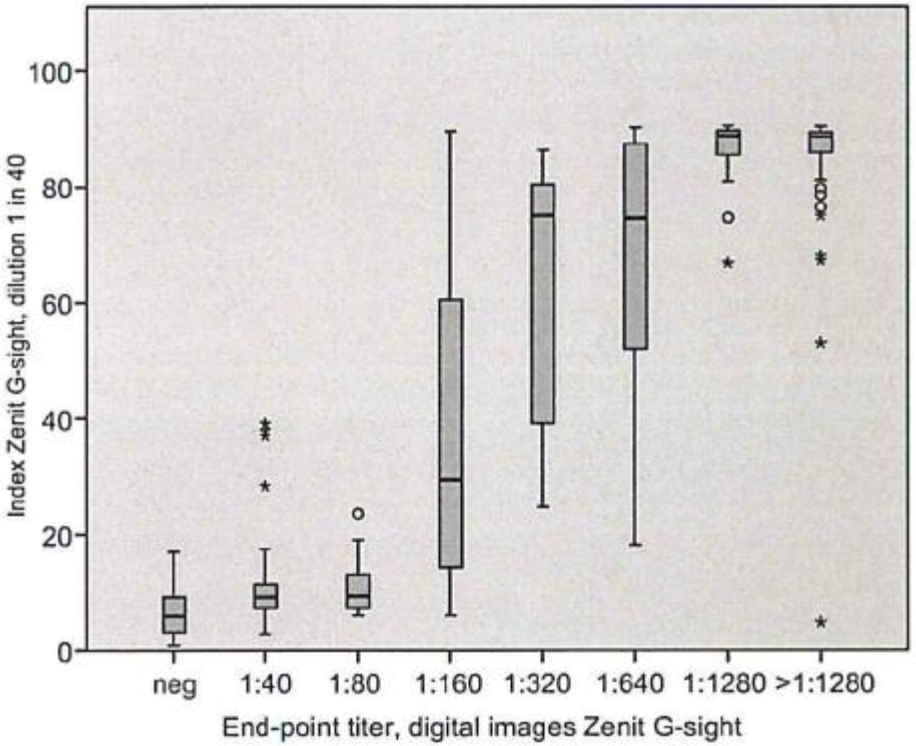
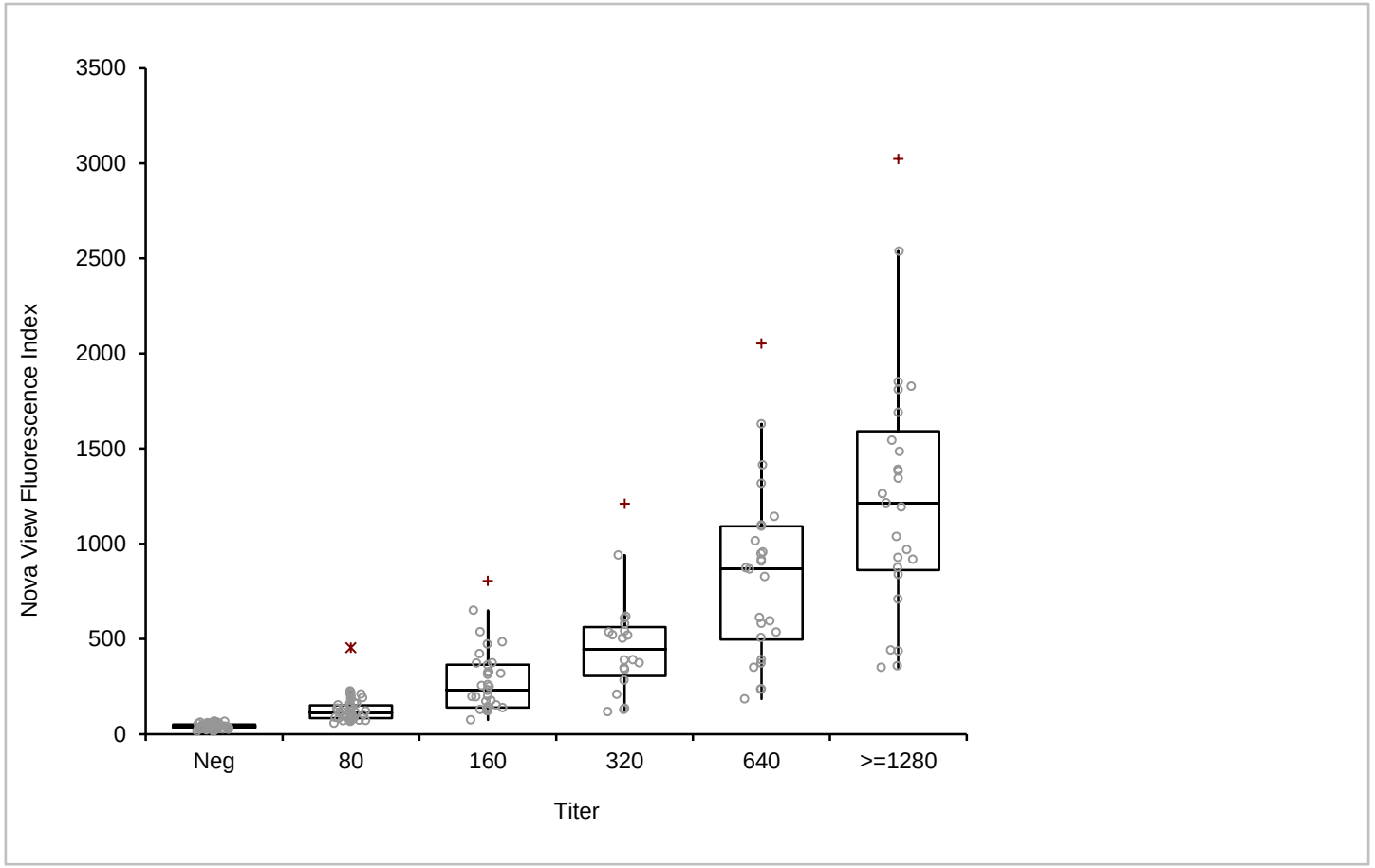


Figure 2 G-sight probability indexes as a function of the antibody titer.

Probability indexes were determined on the one in 40 serum dilution. End-point titers (highest dilution if more than one pattern present) were determined by expert reading of the digital images. In total, 303 samples were included in the analysis (systemic sclerosis patients, disease controls and healthy donors). The figure shows median values and boxplots from the first to the third quartile. The whiskers extend to the furthest observations within ±1.5 interquartile range (IQR) of the first and third quartile. Observations outside the 1.5 IQRs are marked with (o), and those outside the 3.0 IQRs are marked as (*).

Bonroy et al. 2013

NOVA View



Schouwers et al. Clin Chem Lab Med. 2014;52:547-51

Pattern recognition

Visual inspection	Correct assignment by G-Sight HEp-2	Correct assignment by G-sight HEp-2000
Homogeneous (n=42)	68%	92%
Speckled (n=34)	71%	56%
Centromere (n=6)	83%	42%

Pattern recognition (*Bonroy et al. 2013*)

Pattern assignment was incorrect in 29% of 132 pos samples

No pattern assigned in 45% of samples

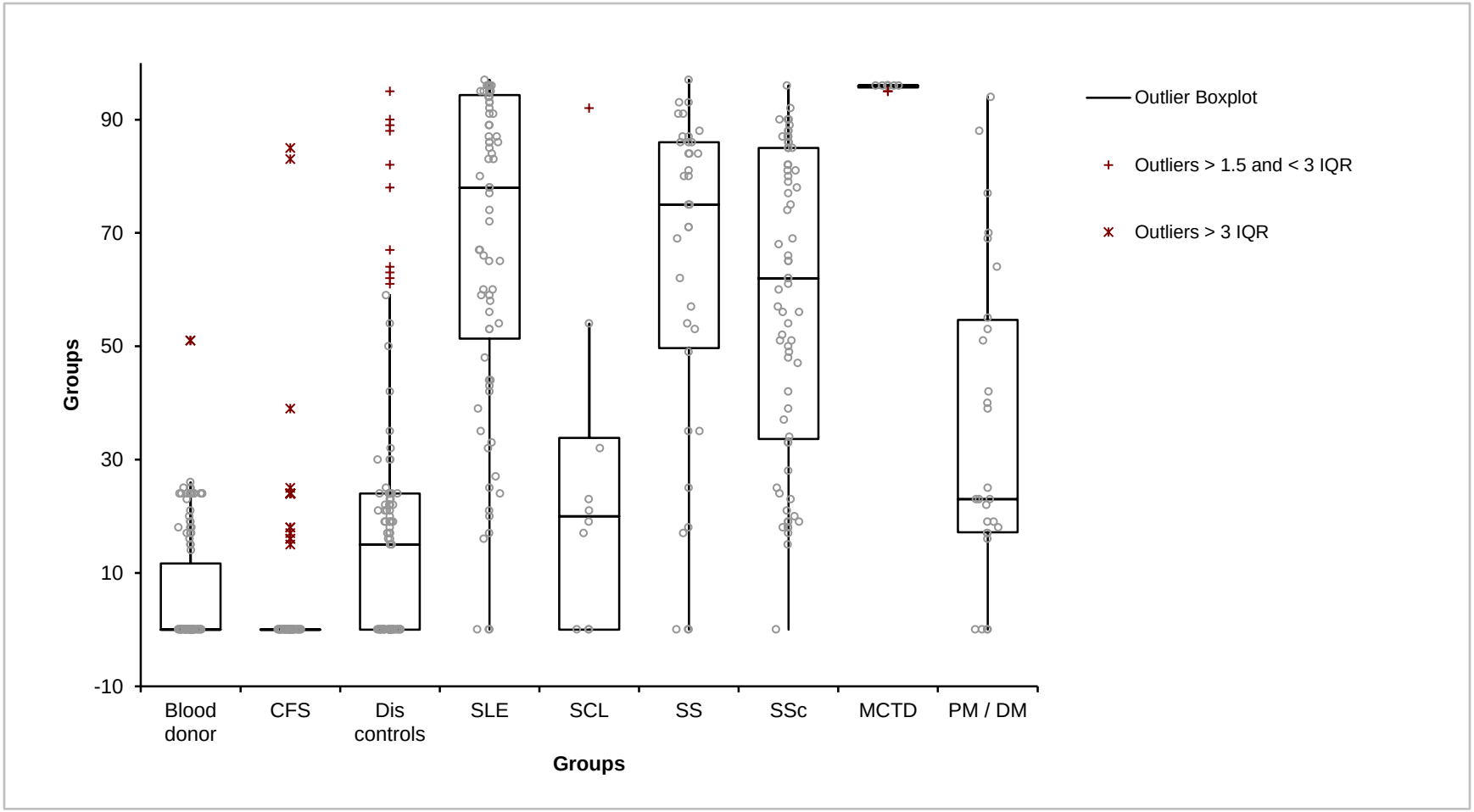
Global accuracy of pattern assignment: 26%

FLUORESCENCE INTENSITY IN WELL-DEFINED PATIENT COHORTS

Study Population

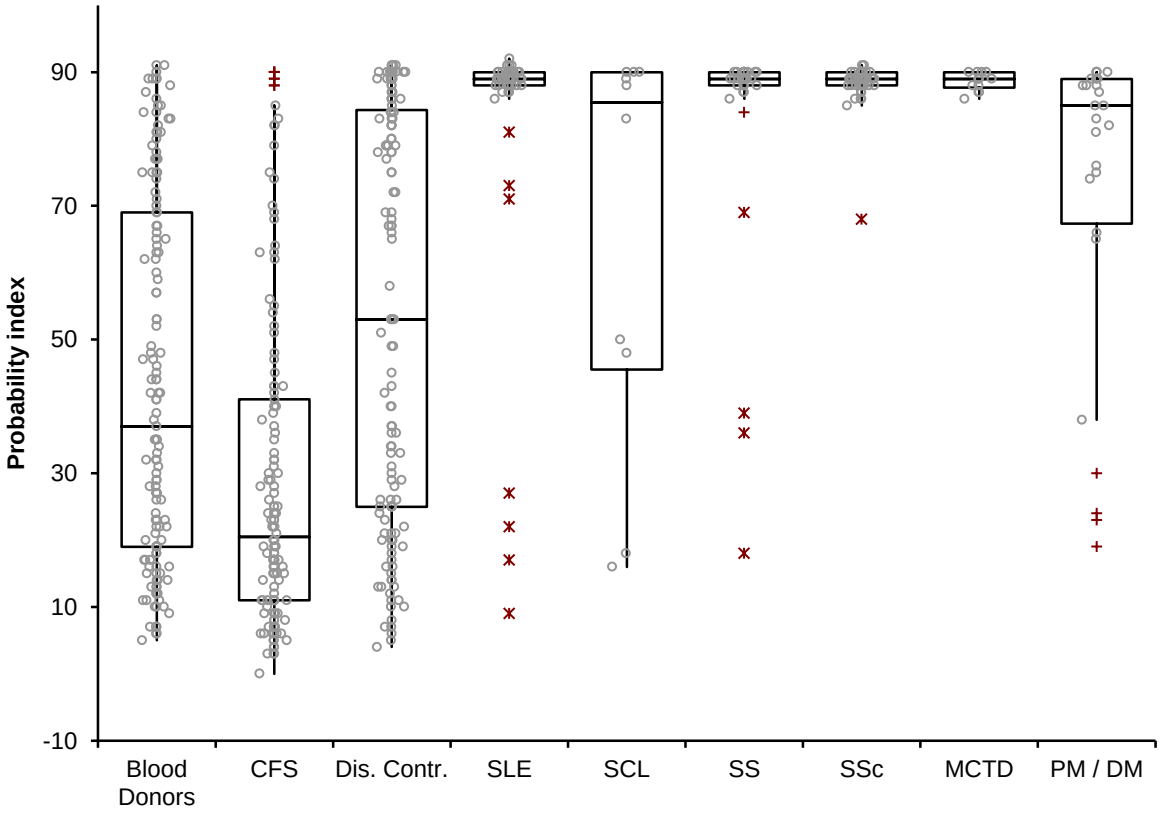
	n	M(%)/F(%)	Median age (range)
Blood donors	108	51/49	45 (19-65)
Chronic Fatigue Syndrome	150	19/81	41 (16-75)
Diseased Controls	134	25/75	45 (17-81)
	392		
Systemic Lupus Erythematosus	85	12/88	35,5 (15-72)
Sjögren's Syndrome	36	14/86	56 (21-75)
Systemic Sclerosis	76	34/66	53 (18-80)
Polymyositis / Dermatomyositis	32	44/56	50 (24-77)
Mixed Connective Tissue Disease	16	6/94	31,5 (16-66)
	245		

HEp2



Bossuyt et al. CCA 2013

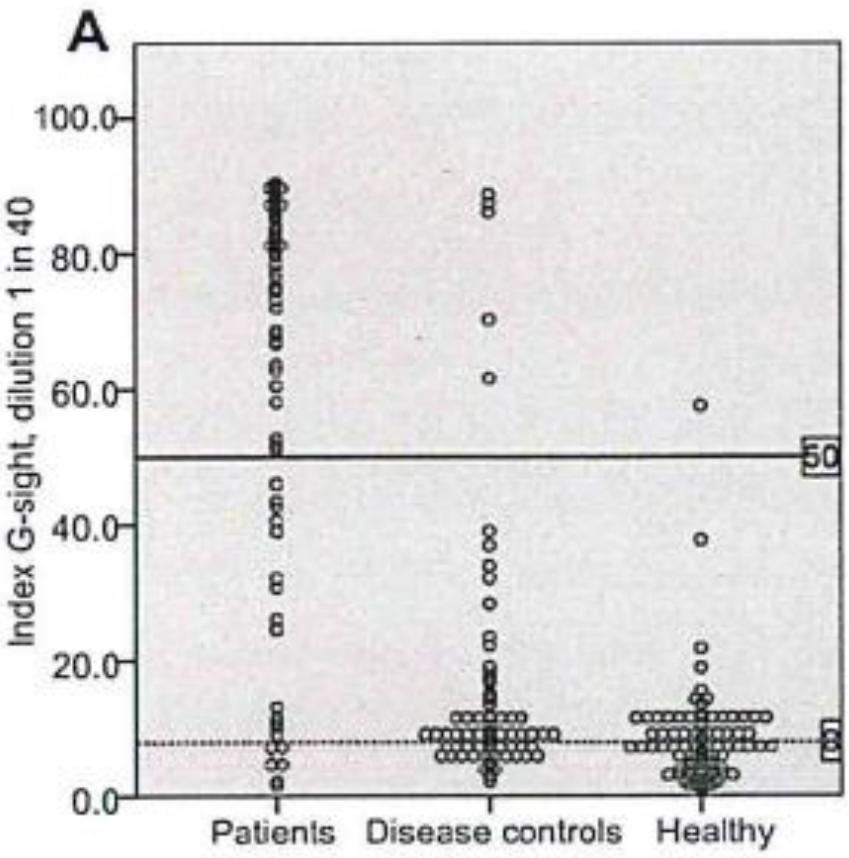
G-Sight HEp-2000



G-Sight HEp-2

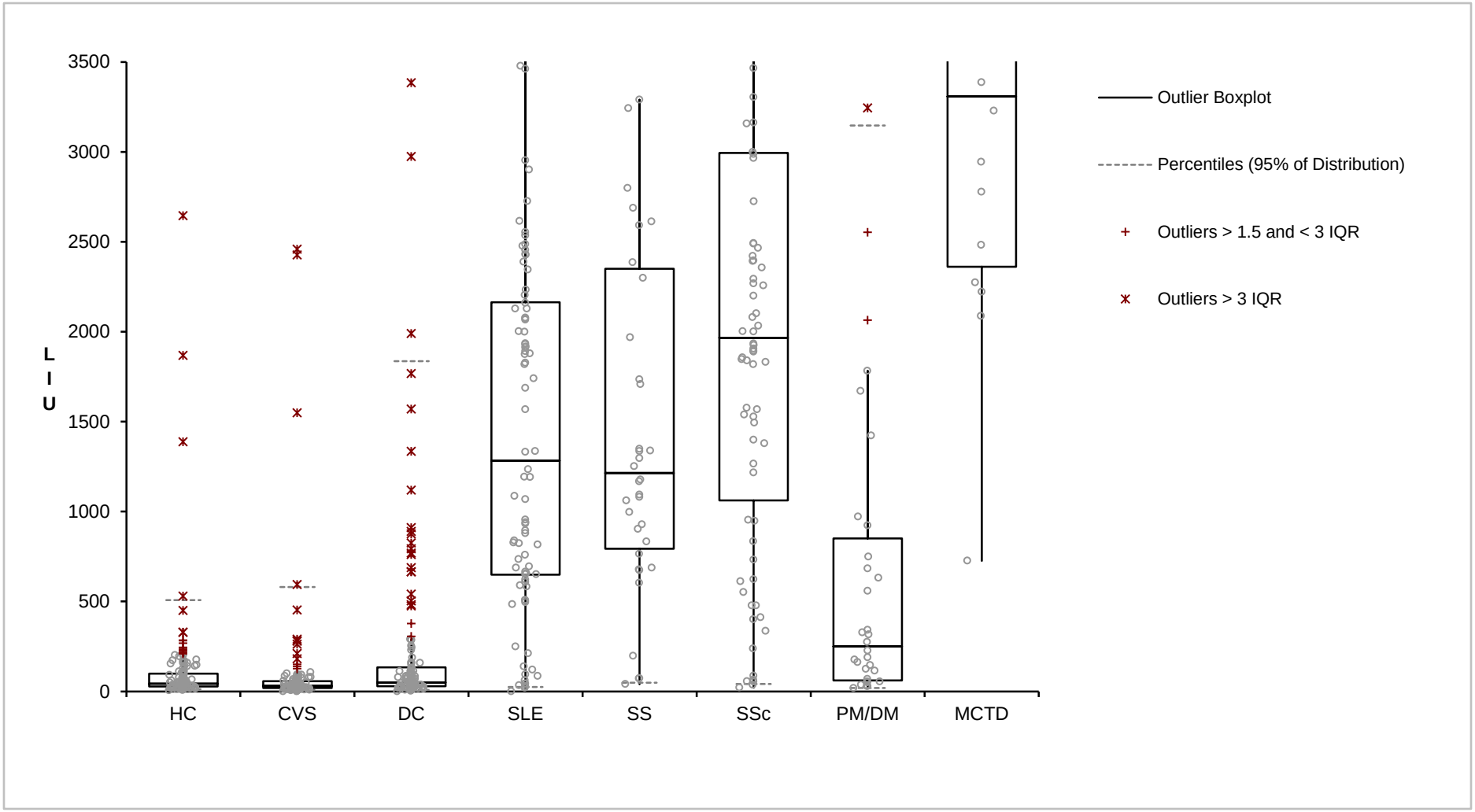
Probability index	Blood donor	CFS	Diseased controls	SLE	SS	SSc	MCTD	PM/DM
≤10	**0.75**	**0.84**	**0.50**	0.04	0.09	0.01	0.00	0.15
11-≤30	**0.24**	**0.13**	**0.38**	0.09	0.09	0.20	0.00	**0.41**
31-≤50	0.01	0.01	0.03	0.12	0.09	0.14	0.00	0.11
51-≤85	0.00	0.01	0.07	**0.32**	**0.43**	**0.42**	0.00	**0.26**
>85	0.00	0.00	0.03	**0.43**	**0.31**	**0.22**	**1.00**	0.07

Probability index	SLE	SS	SSc	MCTD	PM/DM
≤10	0.06 (0.02-0.2)	0.1 (0.04-0.4)	0.02 (0.003-0.1)		0.2 (0.09-0.5)
11 ≤30	0.4 (0.2-0.8)	0.3 (0.1-1.0)	0.8 (0.5-1.3)		1.6 (1.0-2.7)
31 ≤50	6.8 (2.6-17.8)	5.0 (1.4-18.6)	8.5 (3.3-21.5)		6.5 (1.8-23.8)
51 ≤85	**12.1** (6.2-23.6)	**16.0** (7.9-32.2)	**15.7** (8.2-29.9)		**9.7** (4.0-22.9)
>85	**43.9** (16.0-120.4)	**32.2** (10.8-95.9)	**22.3** (7.6-65.1)	**102.5** (38.7-271.8)	7.6 (1.4-39.6)



Bonroy et al. CCLM 2013

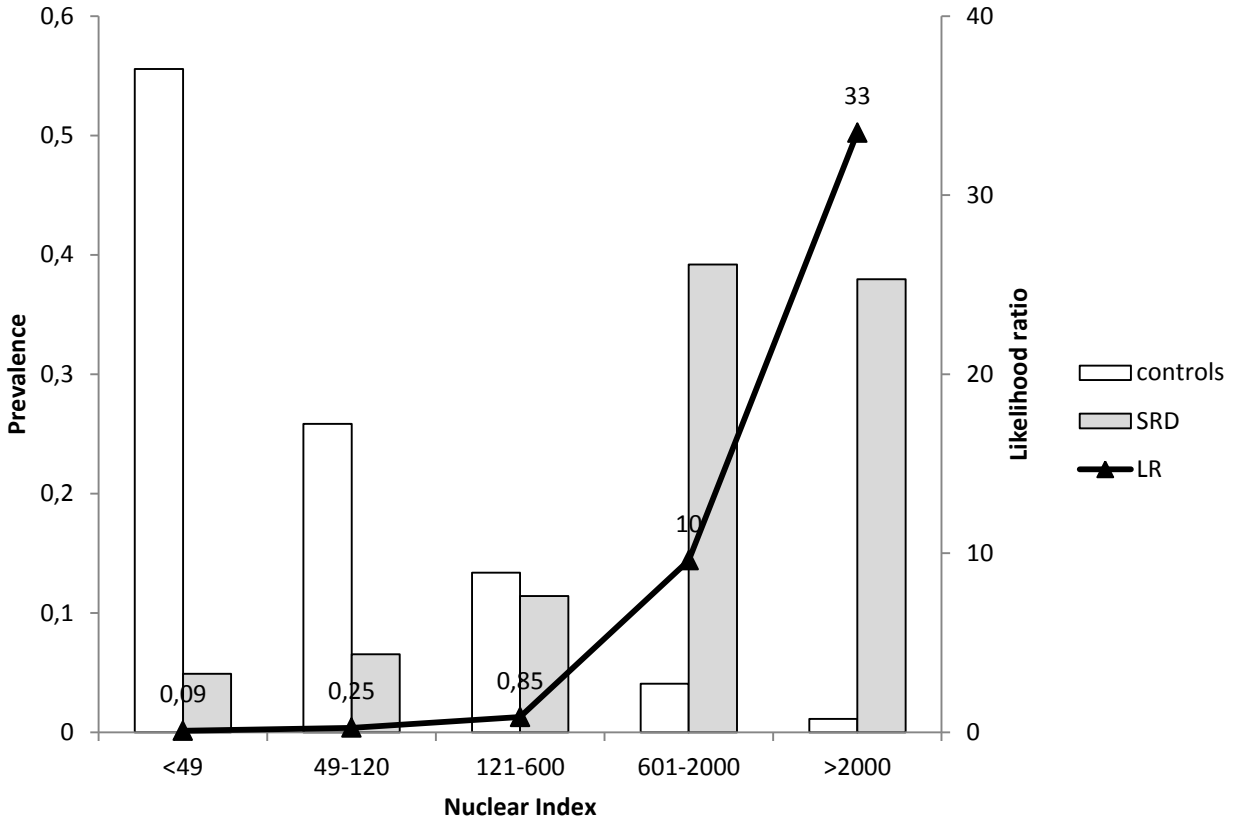
NOVA View


3500
3000
2500
2000
1500
1000
500
0
L
I
U
HC
CVS
DC
SLE
SS
SSc
PM/DM
MCTD
Outlier Boxplot
Percentiles (95% of Distribution)
Outliers > 1.5 and < 3 IQR
Outliers > 3 IQR

NOVA View LR

LIU	Controls	SLE	SS	SSc	PM/DM	MCTD	SARD
Prevalence							
<49	***0,56***	*0,06*	*0,03*	*0,03*	*0,13*		*0,05*
49-120	***0,26***	*0,05*	*0,06*	*0,05*	*0,19*		*0,07*
121-600	*0,13*	*0,11*	*0,03*	*0,09*	***0,34***		*0,11*
601-2000	*0,04*	***0,46***	***0,61***	***0,34***	***0,25***	*0,06*	***0,39***
>2000	*0,01*	***0,33***	***0,28***	***0,49***	*0,09*	***0,94***	***0,38***
LR							
<49		0,11	**0,05**	**0,05**	0,23		**0,09**
49-120		0,18	0,21	0,20	0,73		0,25
121-600		0,79	0,21	0,69	2,57		0,85
601-2000		**11,2**	**15,0**	8,4	6,1	1,5	9,6
>2000		**29,1**	**24,5**	**42,9**	8,3	82,7	**33,5**

NOVA View


0,6
0,5
0,4
0,3
0,2
0,1
0
Prevalence
40
30
20
10
0
Likelihood ratio
33
10
0,09
0,25
0,85
<49
49-120
121-600
601-2000
>2000
Nuclear Index
controls
SRD
LR

LR		
1		no clinical value
>10	<0.1	clinically important difference
5 – 10	0.1 – 0.2	modest, but substantial difference
2 – 5	0.2 – 0.5	small difference that may be relevant

Post-test odds

=

Pre-test odds x LR

probability → odds

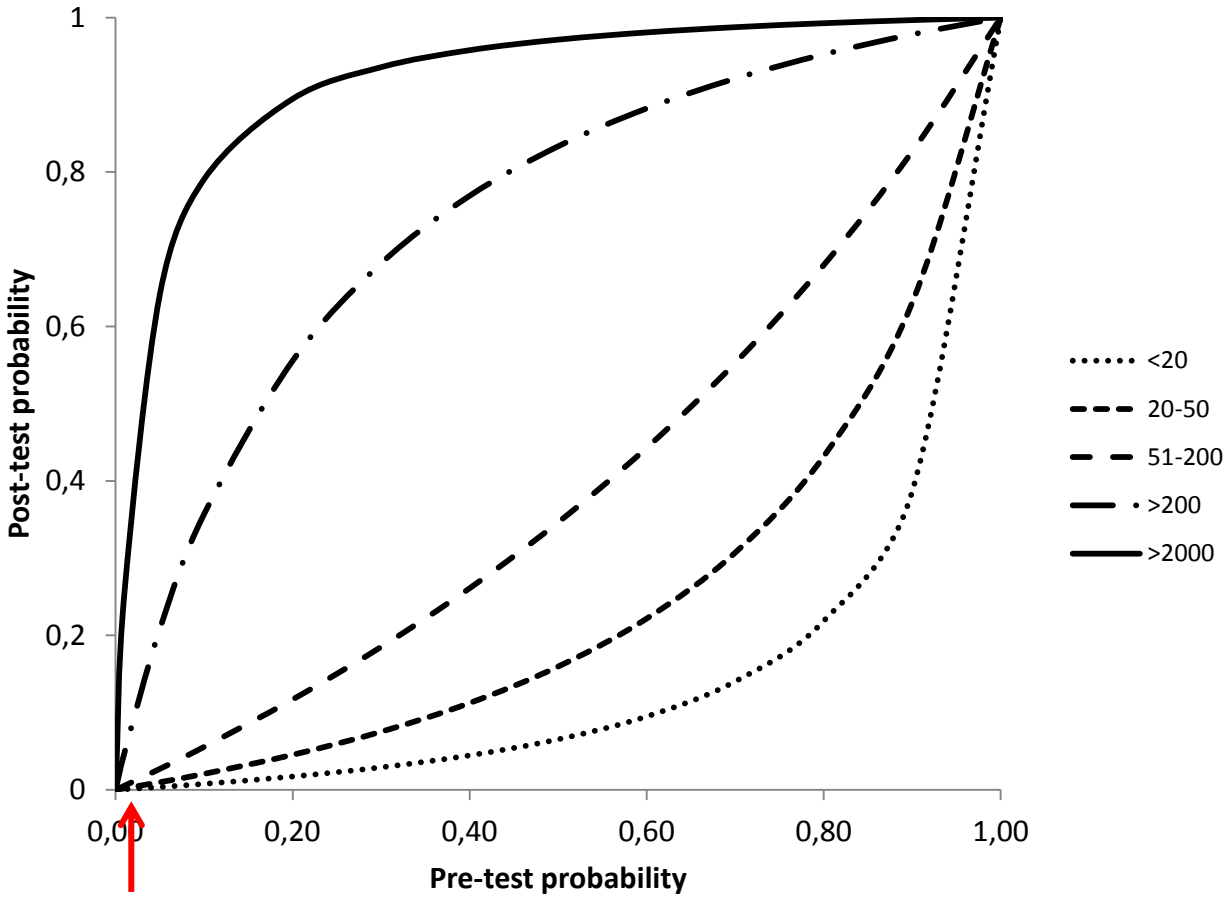
0.2 → 0.2 / (1 – 0.2)

0.25

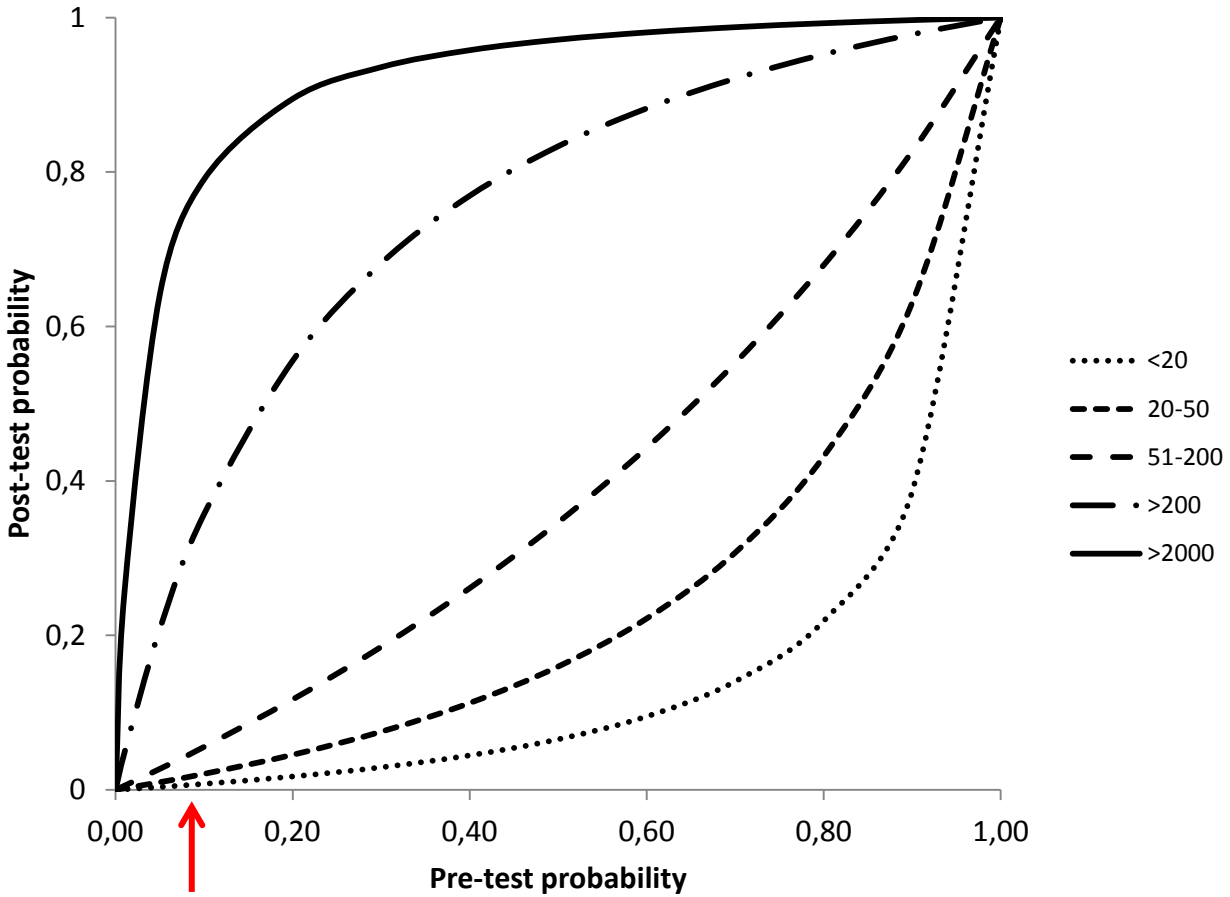
odds → probability

4 → 4 / (1 + 4)

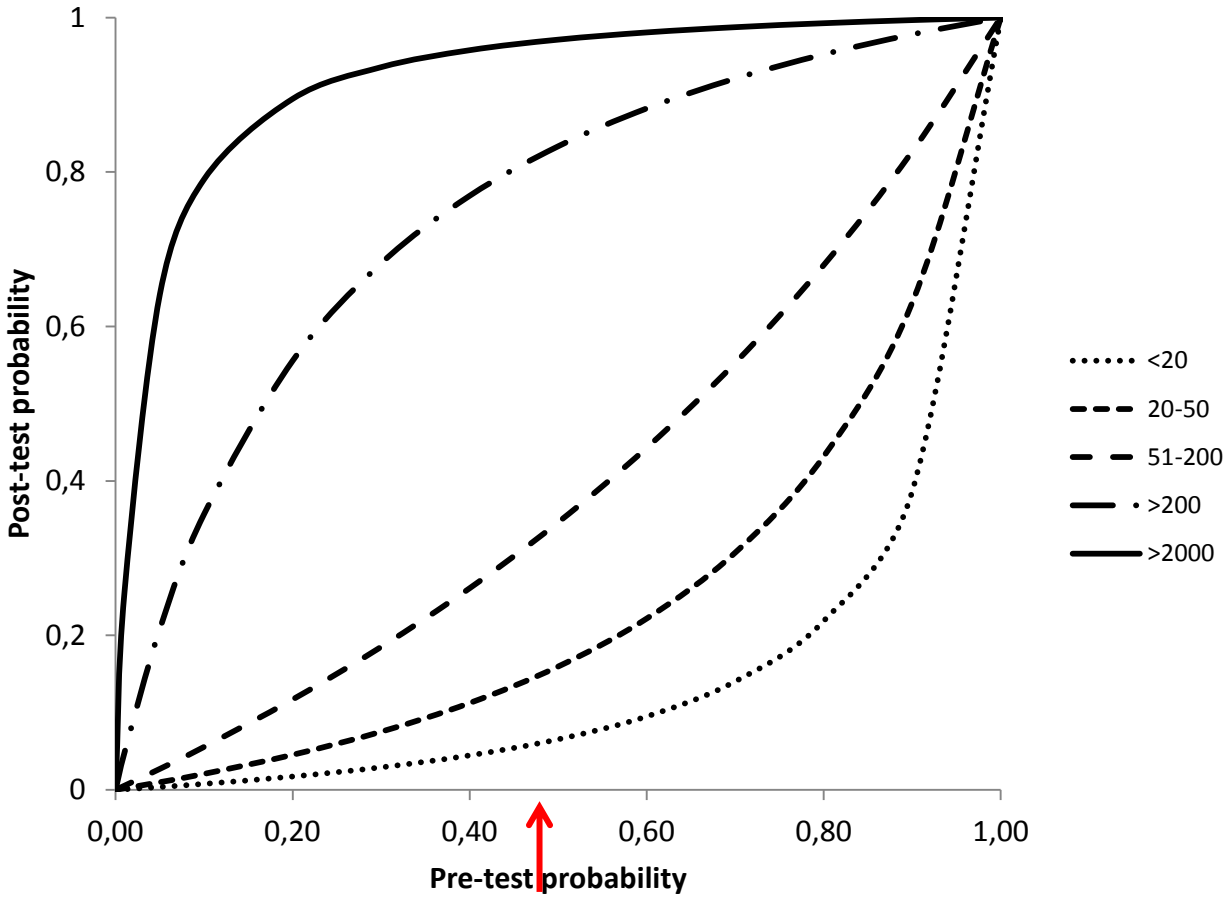
0.8



Pre-test probability	Post-test probability			
	<49	120-600	600-2000	>2000
1%: a young women with hair loss and polyarthralgia	0.07%	0,5%	5%	25%



Pre-test probability	Post-test probability			
	<49	120-600	600-2000	>2000
10%: a young women with photosensitivity and mild leucopenia (3000-3500/mm^3)	0,7%	5,5%	36%	79%



Pre-test probability	Post-test probability			
	<49	120-600	600-2000	>2000
50%: a young women with photosensitivity , malar rash and symmetrical polyarthritis	6,5%	35%	83%	97%

G-Sight HEp-2

Probability index	Blood donor	CFS	Diseased controls	SLE	SS	SSc	MCTD	PM/DM
≤10	**0.75**	**0.84**	**0.50**	0.04	0.09	0.01	0.00	0.15
11-≤30	**0.24**	**0.13**	**0.38**	0.09	0.09	0.20	0.00	**0.41**
31-≤50	0.01	0.01	0.03	0.12	0.09	0.14	0.00	0.11
51-≤85	0.00	0.01	0.07	**0.32**	**0.43**	**0.42**	0.00	**0.26**
>85	0.00	0.00	0.03	**0.43**	**0.31**	**0.22**	**1.00**	0.07

Probability index	SLE	SS	SSc	MCTD	PM/DM
≤10	0.06 (0.02-0.2)	0.1 (0.04-0.4)	0.02 (0.003-0.1)		0.2 (0.09-0.5)
11 ≤30	0.4 (0.2-0.8)	0.3 (0.1-1.0)	0.8 (0.5-1.3)		1.6 (1.0-2.7)
31 ≤50	6.8 (2.6-17.8)	5.0 (1.4-18.6)	8.5 (3.3-21.5)		6.5 (1.8-23.8)
51 ≤85	**12.1** (6.2-23.6)	**16.0** (7.9-32.2)	**15.7** (8.2-29.9)		**9.7** (4.0-22.9)
>85	**43.9** (16.0-120.4)	**32.2** (10.8-95.9)	**22.3** (7.6-65.1)	**102.5** (38.7-271.8)	7.6 (1.4-39.6)

Table 3 Distribution of the test results of the three ANA screening tests [probability indexes (PI) for Zenit G-sight and ratios for Symphony and CTD screen] in systemic sclerosis patients (n=141), disease controls (n=78) and healthy donors (n=100). For each cohort, the fraction (likelihood) of patients/controls within a specific result interval is shown.

	Zenit G-sight			Symphony			CTD screen		
	PI<8	PI 8–50	PI>50	Ratio<1	Ratio 1–10	Ratio>10	Ratio<1	Ratio 1–7	Ratio>7
SSc	5.3%	11.3%	83.5%	32.3%	37.6%	30.1%	23.3%	63.9%	12.8%
DC	38.7%	54.7%	6.7%	97.3%	1.3%	1.3%	96.0%	2.7%	1.3%
HD	60.6%	38.3%	1.1%	97.9%	2.1%	0.0%	94.7%	5.3%	0.0%

In total, 17 samples were excluded from the analysis as they showed visual artefacts on the digital images. DC, diseased controls; HD, healthy donors; PI, probability index; SSc, systemic sclerosis patients.

Table 4 Likelihood ratios for systemic sclerosis of the three ANA screening tests [probability indexes (PI) for G-sight, ratios for Symphony and CTD screen].

	Zenit G-sight			Symphony			CTD screen		
Result intervals	PI<8	PI 8–50	PI>50	Ratio<1	Ratio 1–10	Ratio>10	Ratio<1	Ratio 1–7	Ratio>7
Likelihood ratios	0.10	0.25	23.50	0.33	21.18	50.83	0.24	15.43	21.60

PI, probability index.

DE GRUYTER DOI 10.1515/cclm-2013-0912 — Clin Chem Lab Med 2014; aop

Thomas M. Maenhout[a,*], Carolien Bonroy[a], Charlotte Verfaillie, Veronique Stove and Katrien Devreese

Automated indirect immunofluorescence microscopy enables the implementation of a quantitative internal quality control system for anti-nuclear antibody (ANA) analysis

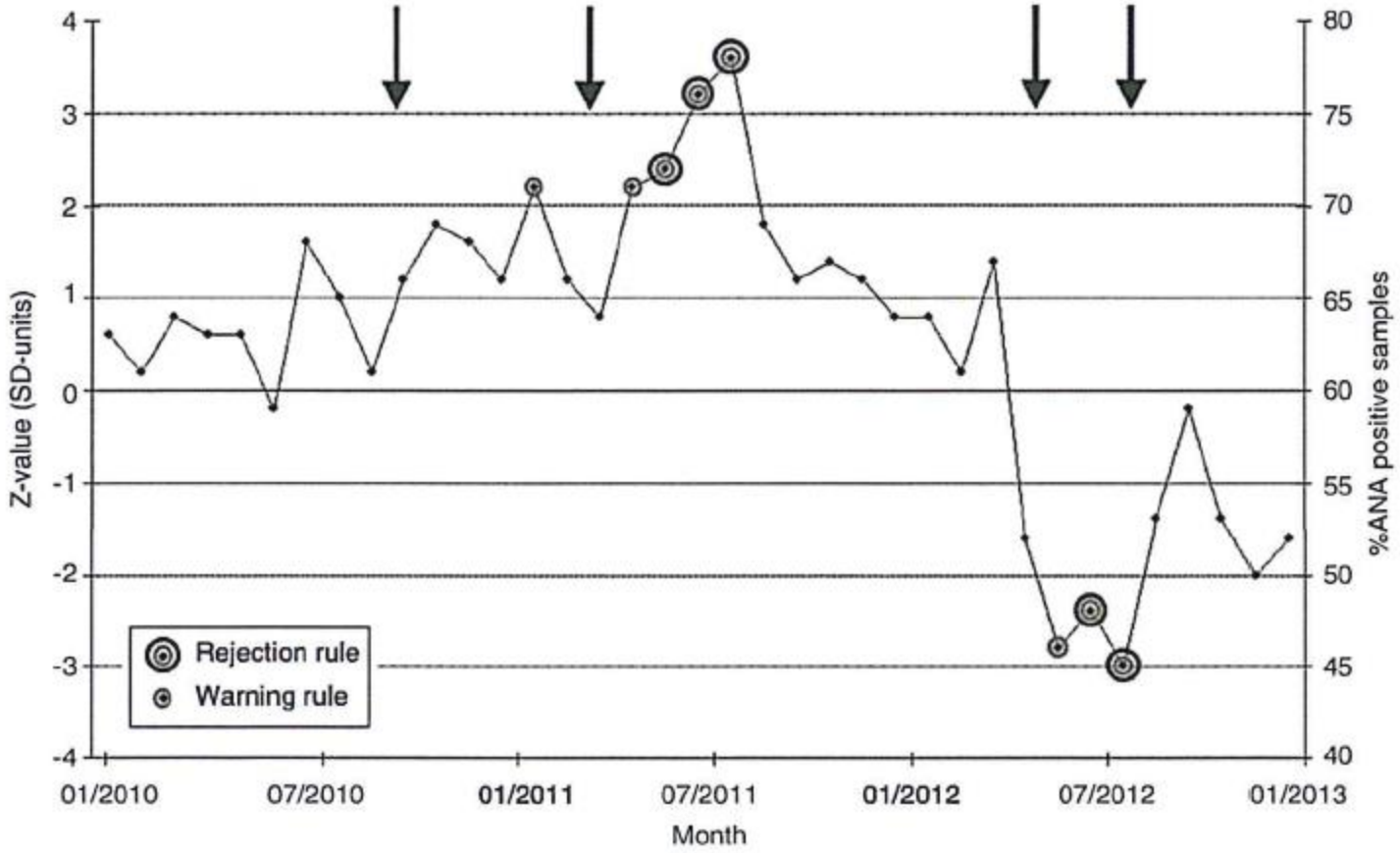
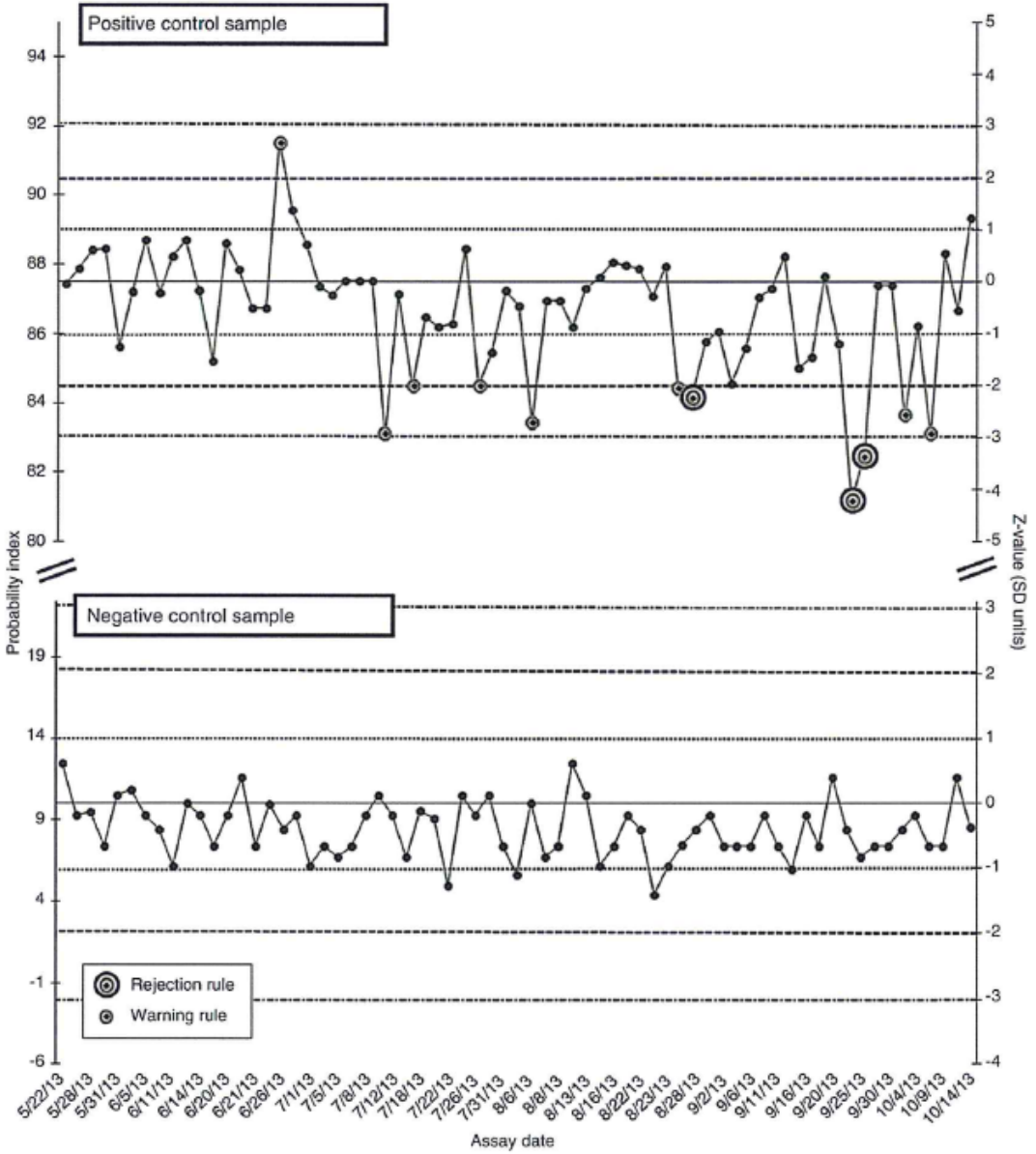


Figure 1 Levey-Jennings chart of the % anti-nuclear antibody (ANA) positives per month (n=19,079).
Left y-axis indicates the Z-score against the target; right y-axis indicates the %ANA positives per month; the x-axis indicates time of year. Single open circles indicate a warning rule; double open circles indicate rejection rules. Horizontal lines indicate target mean and standard deviation (SD). Black arrows indicate the introduction of new reagent lot.



Maenhout et al. 2014

Figure 2 Levey-Jennings chart of the probability index (PI) of the positive and negative control samples.
The left y-axis indicates the PI; the right y-axis indicates the Z-score against the target value. Single open circles indicate a 1_{2S} violation (warning rule); double open circles indicate a 2_{2S}, 1_{3S} or R_{4S} violation (rejection rule). Horizontal lines indicate the target mean and standard deviation (SD) limits.

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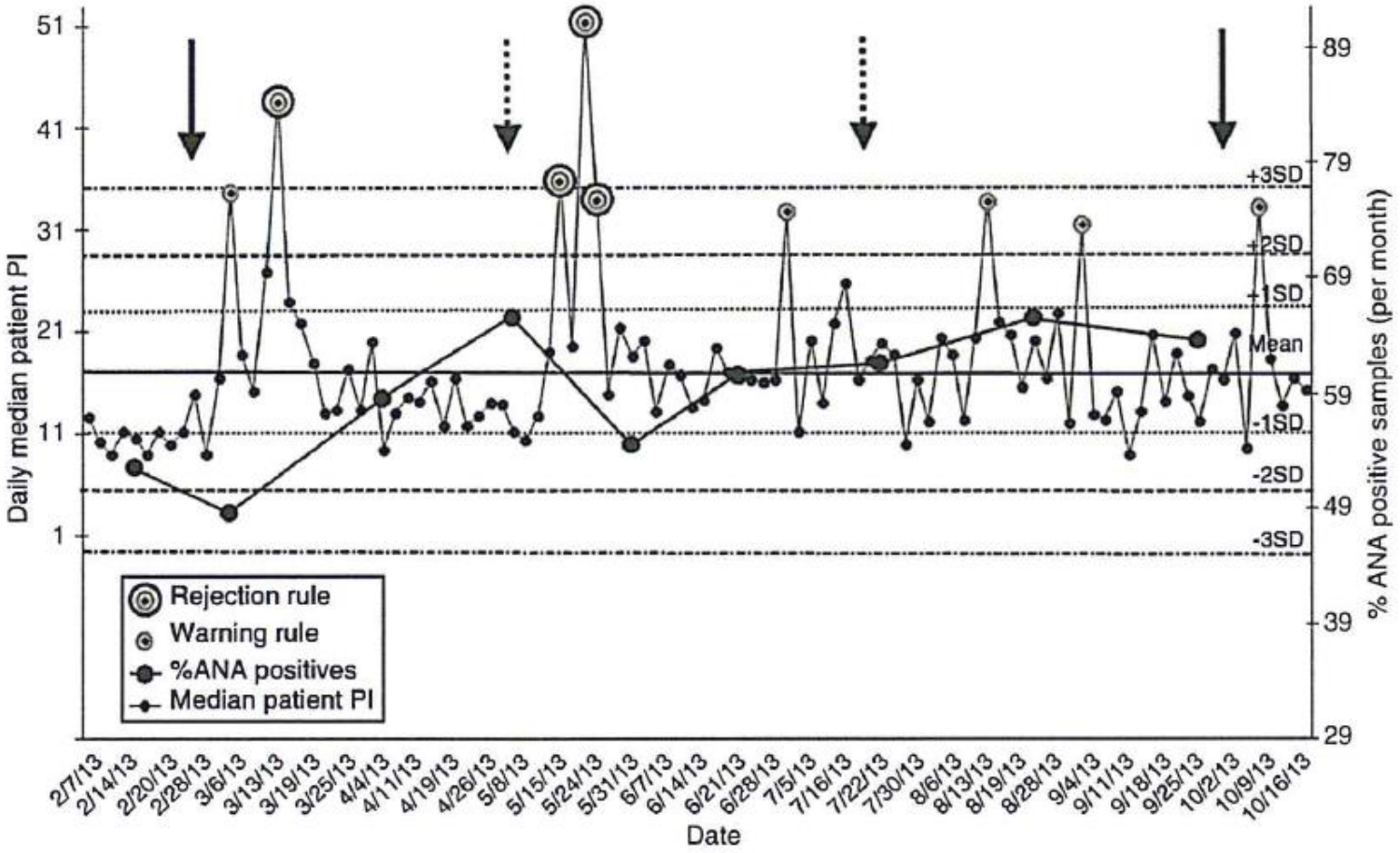


Figure 3 Levey-Jennings chart of the daily median patient probability index (PI) (small dots) and the % anti-nuclear antibody (ANA) positives per month (large dots) from February until mid October 2013 (n=3735).
Single open circles indicate warning rules; double open circles a rejection rule. Horizontal lines indicate overall mean and standard deviation (SD). Black arrows indicate the introduction of new reagent lot; dashed arrows indicate introduction of a reagent vials.

Conclusions

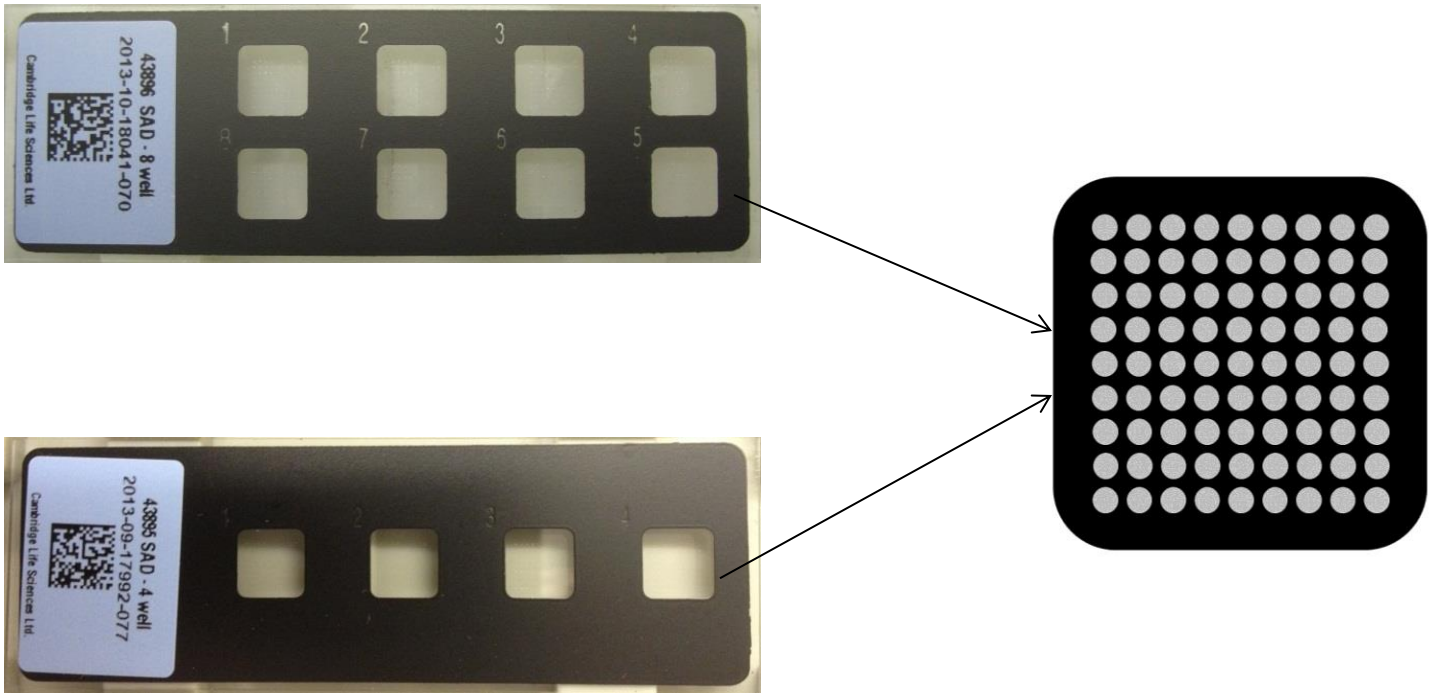
- High quality image acquisition
- Significant correlation between intensity and antibody titer.
- Fluorescence intensity provides clinically useful information
- Enables implementation of a quantitative internal quality control system
- Accuracy of pattern recognition is limited

ZENIT•AMiDot

a microdot array based immunoassay developed to allow simultaneous detection of multiple autoantibodies on a single patient.

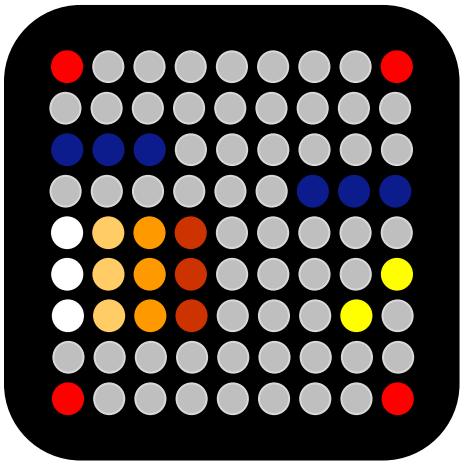
ZENIT•AMiDot

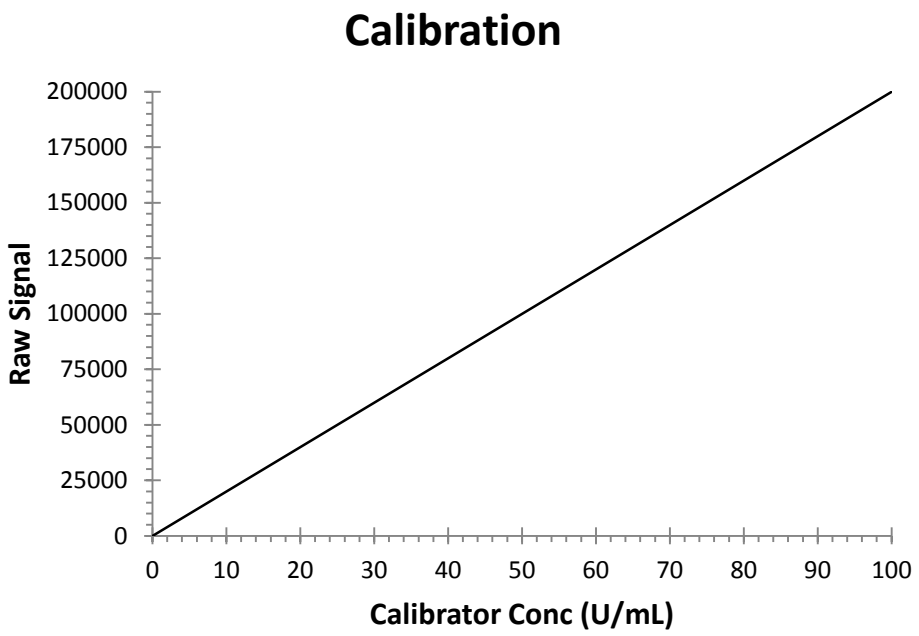
- One patient sample per well – 9 x 9 microarray (81 dots)



ZENIT•AMiDot

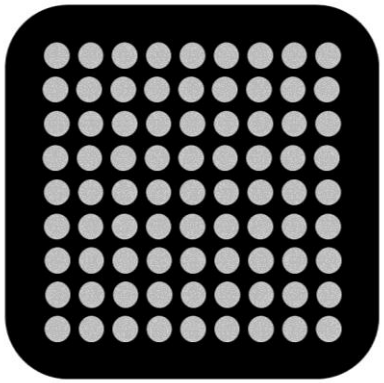
- Each 9 x 9 microarray contains:
- Multiple autoimmune antigen dots
- Calibration dots
- Control dots
- Registration dots





ZENIT•AMiDot

- Each 9 x 9 microarray is:
- Printed on to an activated glass surface.
- Each dot is ~20µm in diameter
- Each autoimmune antigen, calibrator and control is printed in duplicate or triplicate.
- Automated analysis
- Image taken by Reader (G-Sight or AMiDot reader)



Software receives the image, analyses the image, and produces a patient report

ZENIT•AMiDot

Antigen	Recombinant/Native	Antigen	Recombinant/Native
Ro60/SS-A	recombinant	PL7/PL12	recombinant
Ro52/SS-A	recombinant	MPO	native
La/SS-B	recombinant	PR3	native
Sm	native	GBM	native
U1-snRNP (68kD/A/C)	recombinant	Cathepsin	native
Jo-1	recombinant	SRP-54	recombinant
Scl70	native	BPI	native
PMScl100	recombinant	ASCA	native
dsDNA	plasmid	tTG	recombinant
Histones	Native	deaminated Gliadin	recombinant
Mi-2	recombinant	TPO	recombinant
Centromere B	recombinant	Intrinsic Factor	recombinant
Ku	recombinant	Gastric Parietal Cell	native
PCNA	recombinant	M2	recombinant
Ribosomal P0	recombinant	LKM-1	recombinant
Elastase	native	LC1	recombinant

Conclusion AMiDot

- Allows multiplex detection of autoantibodies
- Overall a good concordance with results obtained by EliA.

- Multiplex ANA testing is an efficient way to simultaneously test for specific antibodies.
- Small sample volumes can be used.
- Further studies are warranted to prove the reliability of this technique.

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