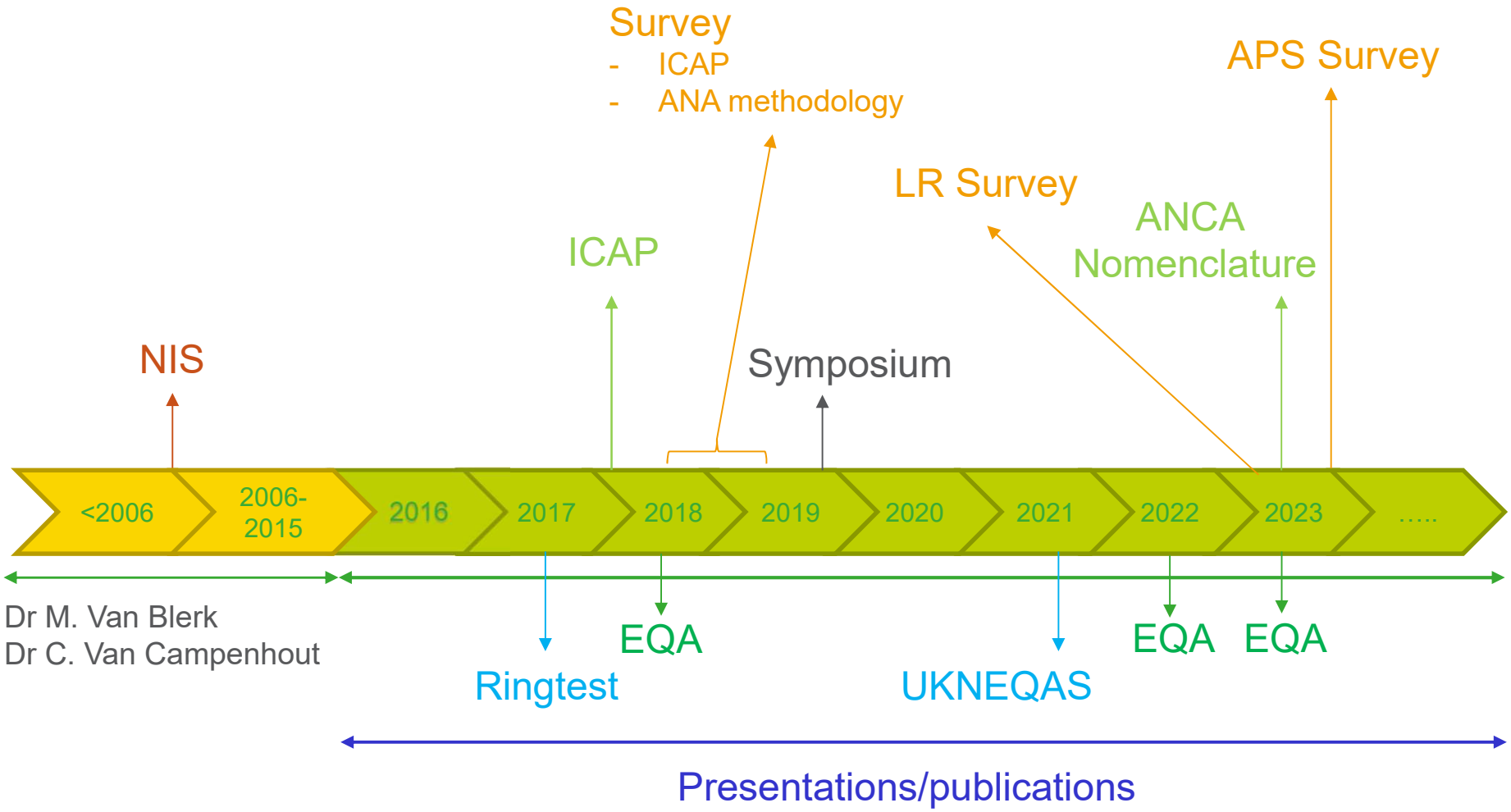
NIS EQA: AN OVERVIEW AND PERSPECTIVES

Broeders Sylvia
Sciensano

Symposium Non-infectious serology 08/03/2024

Timeline



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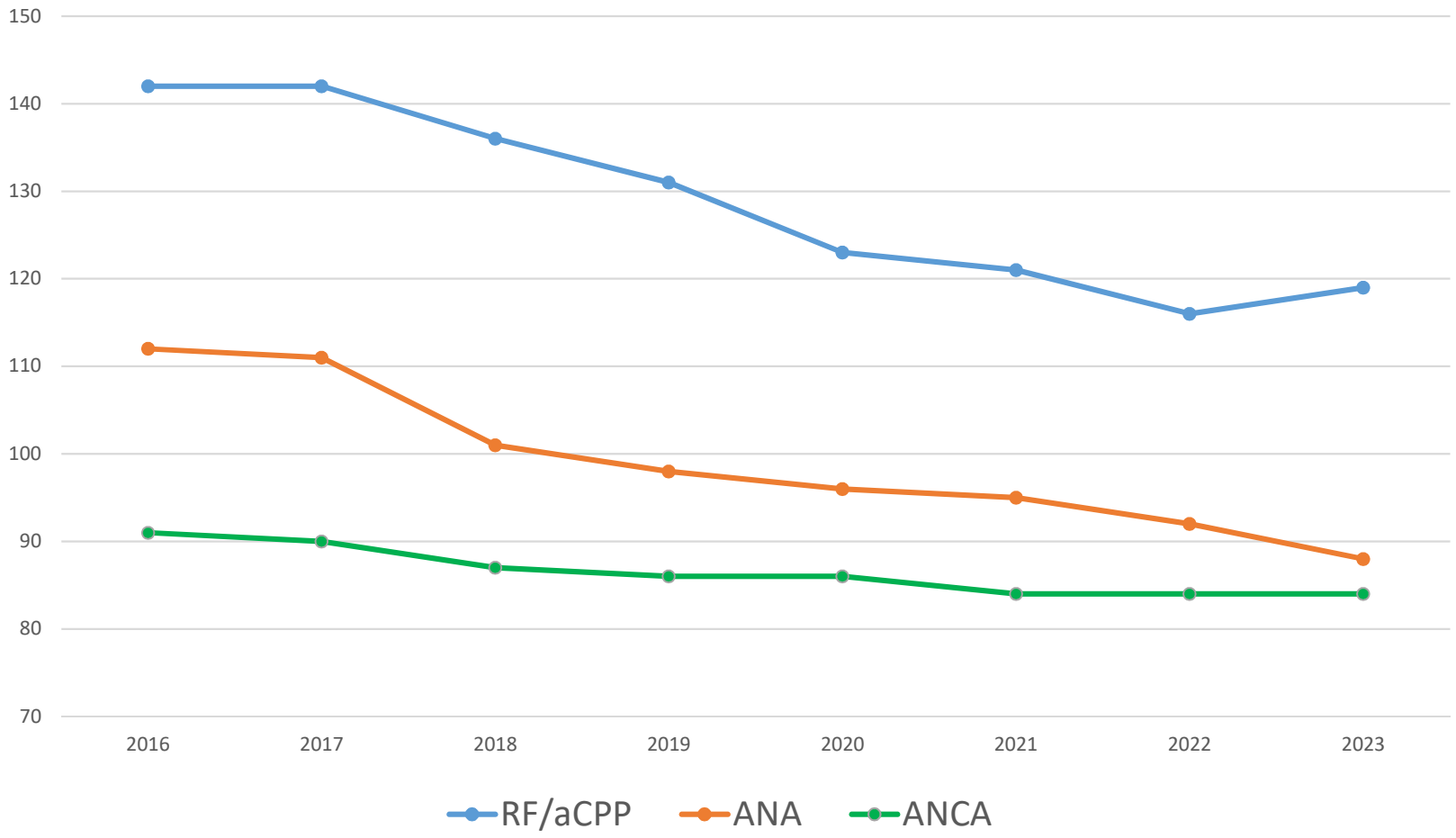
EQA: Participants



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EQA: RF/anti-CCP

EQA	Sample nr	RF	Anti-CCP
2016-2	SN/14322	+	-
2017-1	SN/14479	Strong +	Strong +
2018-1	SN/15559	Weak +	Strong +
2019-1	SN/15969	Strong +	Strong +
2020-1	SN/16984	+	Strong +
2021-1	SN/17805	-	Strong +
2022-1	SN/18837	+	Strong +
2023-1	SN/19347	+	-

1 sample for 2 EQA: RF/anti-CCP + ANA

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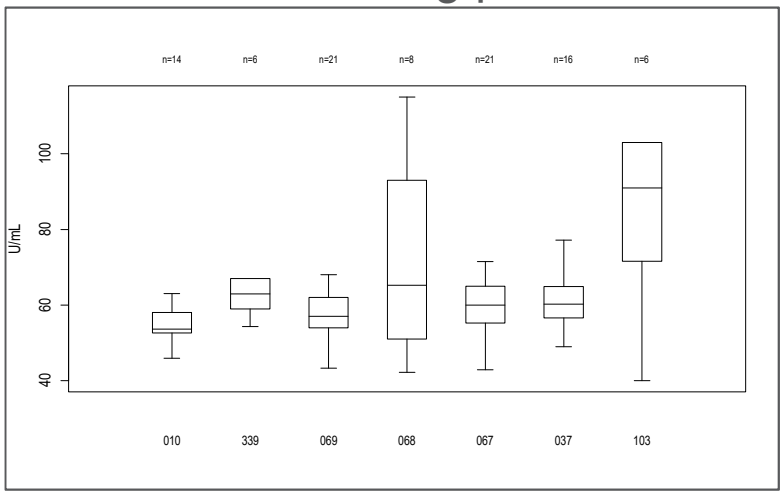
RF/anti-CCP: Take-home messages

Method variation

SN/19347

RF: usually overlap/concordance between methods
BUT weak >< strong pos



Anti-CCP: higher variation – Roche!

	2021	2022	2023
Abbott	107.8	143	0.6
Roche	496	>500	**38**
Siemens	72	74	<1.5
Inova		178	<1.2
ThermoSc	155	373	2.0
	St+	St+	-

Cut-off 17
=> +

!! Interference of anti-streptavidin antibodies: known but rare (<0.7%)

Results > range: dilute or not?

- Do like in routine
- Follow the insert of the kit

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ACR/EULAR 2010 criteria !!

2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative

Daniel Aletaha,[1] Tuhina Neogi,[2] Alan J Silman,[3] Julia Funovits,[1] David T Felson,[2] Clifton O Bingham III,[4] Neal S Birnbaum,[5] Gerd R Burmester,[6] Vivian P Bykerk,[7] Marc D Cohen,[8] Bernard Combe,[9] Karen H Costenbader,[10] Maxime Dougados,[11] Paul Emery,[12] Gianfranco Ferraccioli,[13] Johanna MW Hazes,[14] Kathryn Hobbs,[15] Tom WJ Huizinga,[16] Arthur Kavanaugh,[17] Jonathan Kay,[18] Tore K Kvien,[19] Timothy Laing,[20] Philip Mease,[21] Henri A Ménard,[22] Larry W Moreland,[23] Raymond L Naden,[24] Theodore Pincus,[25] Josef S Smolen,[1] Ewa Stanislawska-Biernat,[26] Deborah Symmons,[27] Paul P Tak,[28] Katherine S Upchurch,[18] Jiří Vencovský,[29] Frederick Wolfe,[30] Gillian Hawker,[31]

Menarini Diagnostics
- Zenit RA CCP (CLIA) -> AU/ml
- Zenit CCP ELISA -> U/ml, ratio

- Use a quantitative method
  - Correct toolkit + units!
- Analyse RF **and** anti-CCP
- Check cut-off

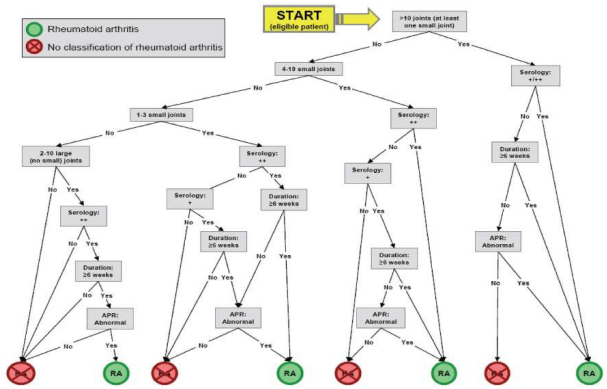

Table 3 The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)*	
2) with the synovitis not better explained by another disease†	
Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement§	
1 large joint¶	0
2–10 large joints	1
1–3 small joints (with or without involvement of large joints)**	2
4–10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)††	5
B. Serology (at least 1 test result is needed for classification)‡‡	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)§§	
Normal CRP *and* normal ESR 0	0
Abnormal CRP *or* normal ESR 1	1
D. Duration of symptoms¶¶	
<6 weeks	0
≥6 weeks	1

a score of 6 or greater being indicative of the presence of definite RA.

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EQA: ANCA

EQA	Sample nr	IIF	IA
2016-1	SN/14072	pANCA	MPO
2017-3	SN/14739	pANCA	MPO
2018-2	SN/15785	cANCA	PR3
	SN/15624	-	PR3
2019-2	SN/16467	pANCA	MPO
2020-2	SN/14741	pANCA	MPO
2021-2	SN/18126	cANCA	PR3
2022-2	SN/19094	pANCA	MPO
2023-3	SN/19999	cANCA	PR3

Publication

Nomenclature

Didactic sample

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ANCA: Take-home messages

Variability/Sensitivity IIF vs IA

Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis

Xavier Bossuyt[1], Jan-Willem Cohen Tervaert[2], Yoshihiro Arimura[3], Daniel Blockmans[4], Luis Felipe Flores-Suárez[5], Loïc Guillevin[6], Bernhard Hellmich[7], David Jayne[8], J. Charles Jennette[9], Cees G. M. Kallenberg[10], Sergey Moiseev[11], Pavel Novikov[11], Antonella Radice[12], Judith Anne Savige[13], Renato Alberto Sinico[14], Ulrich Specks[15], Pieter van Paassen[16], Ming-hui Zhao[17], Niels Rasmussen[18], Jan Damoiseaux[19] and Elena Csernok[7]

⇒ high-quality immunoassays can be used as the primary screening method for patients suspected of having the ANCA-associated vaculitides GPA and MPA without the categorical need for IIF

EQA SN/14739

Revised 2017 international consensus on ANCA testing in small vessel vasculitis: support from an external quality assessment

Sylvia Broeders [1], Sylvie Goletti [2], Jean-Paul Tomasi [2], Carolien Bonroy [3], René-Louis Humbel [4], Laurence Lutteri [5], Sofie Schouwers [6], Lieve Van Hoovels [7], Martine Vercammen [8], Xavier Bossuyt [9]

SN/15624: didactic sample

- Patient GPA under treatment: IIF neg, PR3
- High variability seen in both IIF and IA

  -> Lower titers & epitope spreading

>< RIZIV/INAMI nomenclature

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ANCA: nomenclature 2023

SN/19999

- Follow-up sample
- GPA patient
- cANCA-PR3
- 59/84 IIF -> OK
- 81/84 IA -> OK

Questions

- Have you adjusted your testing strategy for ANCA vasculitides in response to the new nomenclature?
- If not, do you plan to adjust your test strategy?

⇒ 80/84 answers

Adapted	To do	#	%	
Y	N	43	53.8	CHANGE 68/80! 85%
Y(part)	Y	2	2.5	
N	Y	23	28.8	
N	N	12	15.0	??

EQA: not possible to give the whole history of the follow-up sample -> MPO/PR3
routine: IIF + IA -> also in EQA: make correlation!

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EQA: ANA

EQA	Sample nr	IIF	Anti-dsDNA	Anti-ENA
2016-3	SN/14565	centromere	-	CENP-A/B
2017-2	SN/15085	(fine) speckled	-	SSA/SSB
	SN/15086	-	-	-
2018-3	SN/15843	AC-1	+	+
2019-3	SN/16557	AC-4	-	SSA/SSB
2020-3	SN/531	AC-3	-	CENP-A/B
2021-3	SN/18676	AC-5	-	RNP
	SN/18677	AC-5	/	/
	SN/18647	AC-2	/	/
2022-3	SN/19448	AC-3	-	CENP-B
	SN/19350	AC-22	/	/
2023-2	SN/19347	AC-4	-	SSA

Ringtest

UKNEQAS

ICAP

Didactic sample

1 sample for 2 EQA: RF/anti-CCP + ANA

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ANA: Take-home messages

SN/19448

- Centromere AC-3 -> CENP-A/B
- Additional borderline: SSB, Ro52, gp210, Th/To, AMA-M2

-> sensitivity/specificity of methods!

Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases

Xavier Bossuyt[1] ✉, *Ellen De Langhe*[2,3], *Maria Orietta Borghi*[4,5] *and Pier Luigi Meroni*[4]

Abstract | Antinuclear antibodies (ANAs) are valuable laboratory markers to screen for and support the diagnosis of various rheumatic diseases (known as ANA-associated rheumatic diseases). The importance of ANA testing has been reinforced by the inclusion of ANA positivity as an entry criterion in the 2019 systemic lupus erythematosus 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. A number of techniques are available for detecting antibodies to a selection of clinically relevant antigens (such as indirect immunofluorescence and solid phase assays). In this Review, we discuss the advantages and limitations of these techniques, as well as the clinical relevance of the differences between the techniques, to provide guidance in understanding and interpreting ANA test results. Such understanding not only necessitates insight into the sensitivity and specificity of each assay, but also into the importance of the disease context and antibody level. We also highlight the value of titre-specific information (such as likelihood ratios).

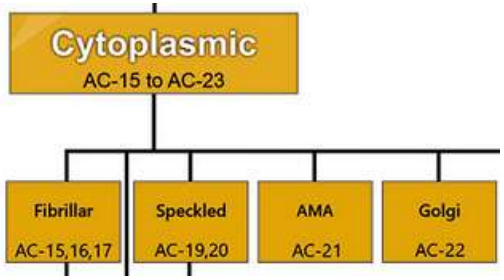
- The performance characteristics of IIF assays and SPAs are disease-dependent; IIF assays are more sensitive than SPA for screening for systemic sclerosis (and systemic lupus erythematosus) but not Sjögren syndrome.

Choose well!!

ANA: Take-home messages

SN/19350

- Didactic sample
- Golgi-like pattern AC-22
  - 93.3 % IIF +
  - 83.3 % correct pattern
- ICAP competent level



- No clinical association

Anti-Golgi autoantibodies are not clinically associated with systemic autoimmune diseases

Pieter Vermeersch,[1] Karolien Van den Bergh,[1] Daniel Blockmans,[2] Rene Westhovens,[3] Xavier Bossuyt[1]

ANA: Take-home messages

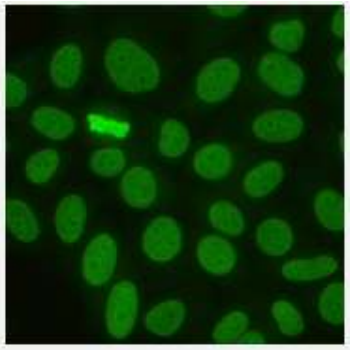
SN/19347 – SN/16557 – SN/18647

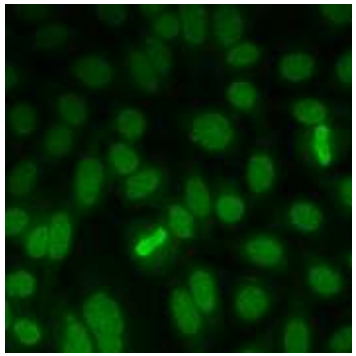
IIF:

- SSA on HEp-2000

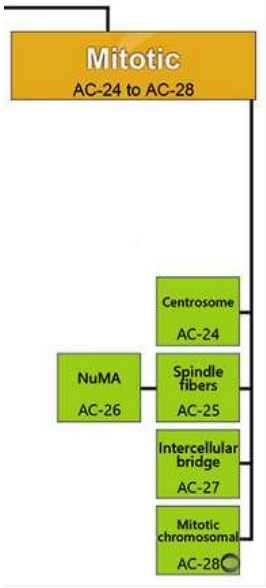
Note #1. Anti-SS-A/Ro60 staining on HEp-2000 slides (IMMUNO CONCEPTS) is different from other HEp-2 slides.

- Homogeneous (AC-1) and DFS70 (AC-2): Nuclear - positive mitosis





≠ mitotic patterns (AC-24->28)



https://www.anapatterns.org/trees-2021.php

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ANA: Take-home messages

SN/19347

Anti-dsDNA:

Different methodologies (buffer, Ag source, conjugate,…)

-> difference performance

-> difference in detection of Ab

-> choice of cut-off

- 97.4 %: dsDNA neg
- Diesse (cut-off: 20)

Result	Cut-off	Conclusion
18.8 IU/ml	<10	+

Belgian recommendations on ANA, anti-dsDNA and anti-ENA antibody testing

M. Van Blerk[1,2], X. Bossuyt[2,3], R. Humbel[2,4], A. Mewis[2,5], G. Servais[2,6], J. P. Tomasi[2,7], C. Van Campenhout[1,2], L. Van Hoovels[2,8], M. Vercammen[2,9], J. Damoiseaux[10], W. Coucke[1], P. Van de Walle[1]

=> It is not recommended to use a dot technique for anti-dsDNA antibody detection.

=> The method used for determining anti-dsDNA antibodies should be specified when reporting the result.

ANA: Take-home messages

SN/19347

Anti-ENA:

Fine speckled AC-4 – SSA/Ro60

- 57/58 dot -> 1/58 Ro52 only tested
- 22/22 ELISA/CLIA/FEIA
- 4/4 microarray

-> Need to test basic Ag

-> Adapt method cf IIF

-> Dot/line: specificity

=> Correlation IIF/anti-ENA

Belgian recommendations on ANA, anti-dsDNA and anti-ENA antibody testing

M. Van Blerk[1,2], X. Bossuyt[2,3], R. Humbel[2,4], A. Mewis[2,5], G. Servais[2,6], J. P. Tomasi[2,7], C. Van Campenhout[1,2], L. Van Hoovels[2,8], M. Vercammen[2,9], J. Damoiseaux[10], W. Coucke[1], P. Van de Walle[1]

It is recommended to type the anti-ENA antibodies for at least :
SSA/Ro60, SSB, Sm, RNP, CENP-B, Scl-70, Jo-1

Carolien Bonroy, Martine Vercammen, Walter Fierz, Luis E.C. Andrade, Lieve Van Hoovels, Maria Infantino, Marvin J. Fritzler, Dimitrios Bogdanos, Ana Kozmar, Benoit Nespola, Sylvia Broeders, Dina Patel, Manfred Herold, Bing Zheng, Eric Y.T. Chan, Raivo Uibo, Anna-Maija Haapala, Lucile Musset, Ulrich Sack, Gabor Nagy, Tatjana Sundic, Katarzyna Fischer, Maria-José Rego de Sousa, Maria Luisa Vargas, Catharina Eriksson, Ingmar Heijnen, Ignacio García-De La Torre, Orlando Gabriel Carballo, Minoru Satoh, Kyeong-Hee Kim, Edward K.L. Chan, Jan Damoiseaux, Marcos Lopez-Hoyos and Xavier Bossuyt* for the European Federation of Laboratory Medicine (EFLM) Working Group "Autoimmunity Testing," the European Autoimmune Standardization Initiative (EASI) and International Consensus on Antinuclear Antibody Patterns (ICAP)

Detection of antinuclear antibodies: recommendations from EFLM, EASI and ICAP

09:30 – 09:55 Detection of antinuclear antibodies: recommendations from EFLM, EASI and ICAP: clinical and methodological aspects including automated microscopy

Carolien Bonroy (Department of Diagnostic Sciences, Ghent University, Ghent, Belgium; Department of Laboratory Medicine, University Hospital Ghent, Ghent, Belgium)

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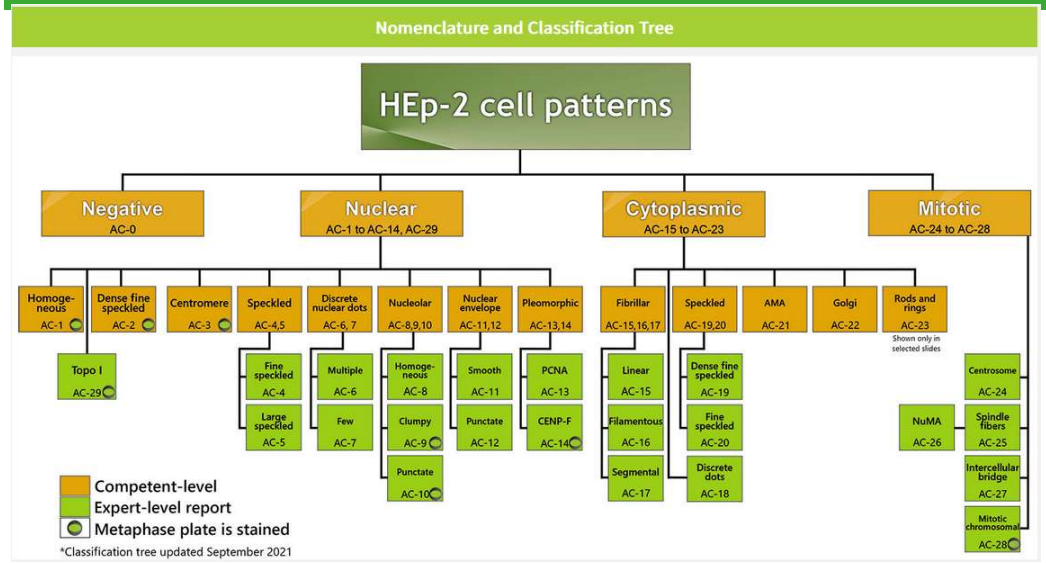
ANA: ICAP nomenclature 2018 -> 2021



https://www.anapatterns.org/trees-2021.php

ICAP Smartphone App – connecting to our new generation of users

International Consensus on ANA Patterns (ICAP): inbedding in het Nederlandse taalgebied

Jan Damoiseaux, Xavier Bossuyt, Sylvia Broeders, Dörte Hamann, Karina de Leeuw, Maarten Limper, Henny Otten, Caroline Roozendaal, Sofie Schouwers, Marco Schreurs, Rogier Thurlings, Paul van Daele, Renate van der Molen, Lieve van Hoovels, Martine Vercammen

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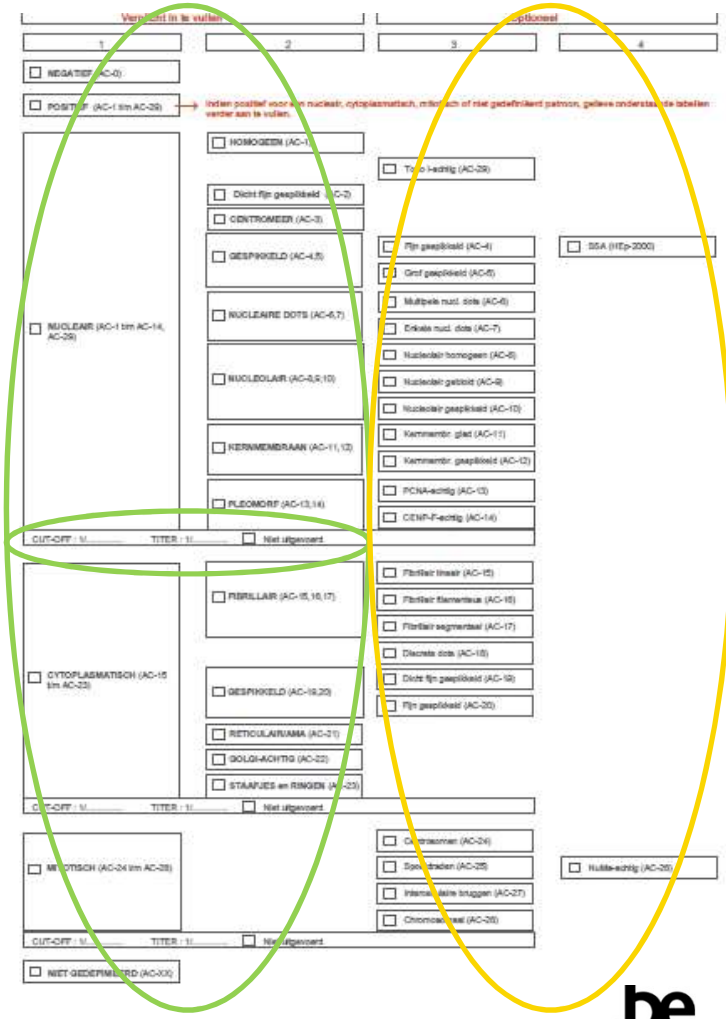
SN/15843: EQA



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Survey: ICAP

ANA IIF pattern survey

Study Objective

Current laboratory and clinical practices in reporting and interpreting ANA patterns in Belgium.

& beyond

Materials and methods

- **International Consensus on Antinuclear Antibody Patterns (ICAP)**
  - Consensus ANA pattern definition
  - Level of competency (competent/expert) to identify them
- **Questionnaires**
  - Content: reporting, familiarity, clinical significance of patterns
  - Scope: Belgian laboratories licensed to perform ANA IIF testing
    Belgian rheumatologists

EASI European Autoimmunity Standardisation Initiative

ICAP

UK NEQAS International Quality Expertise

Van Hoovels *et al. Autoimmun Highlights* (2020) 11:17
https://doi.org/10.1186/s13317-020-00139-9

Autoimmunity Highlights

ORIGINAL RESEARCH Open Access

Current laboratory and clinical practices in reporting and interpreting anti-nuclear antibody indirect immunofluorescence (ANA IIF) patterns: results of an international survey

Lieve Van Hoovels[1,2*], Sylvia Broeders[3], Edward K. L. Chan[4], Luis Andrade[5], Wilson de Melo Cruvinel[6], Jan Damoiseaux[7], Markku Viander[8], Manfred Herold[9], Wim Coucke[3], Ingmar Heijnen[10], Dimitrios Bogdanos[11], Jaime Calvo-Alén[12], Catharina Eriksson[13], Ana Kozmar[14], Liisa Kuhi[15], Carolien Bonroy[16,17], Bernard Lauwerys[18,19], Sofie Schouwers[20], Laurence Lutteri[21], Martine Vercammen[22,23], Miroslav Mayer[24], Dina Patel[25], William Egner[25], Kari Puolakka[26], Andrea Tesija-Kuna[14], Yehuda Shoenfeld[27,28], Maria José Rego de Sousa[29], Marcos Lopez Hoyos[30], Antonella Radice[31] and Xavier Bossuyt[2,32]

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Survey: ANA methodology

A SURVEY ON METHODOLOGICAL ASPECTS OF ANTI-NUCLEAR ANTIBODY TESTING

The use of Indirect Immunofluorescence for ANA testing in Belgium

Vercammen M, Broeders S, Bonroy C, Coucke W, Lutteri L, Schouwers S, Van Hoovels L, Bossuyt X. Belgian EASI team - Sciensano

14th Dresden Symposium on Autoantibodies

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DE GRUYTER

Clin Chem Lab Med 2023; 61(7): 1199–1208

EFLM Paper

Martine Vercammen, Carolien Bonroy, Sylvia Broeders, Edward K.L. Chan, Nicola Bizzaro, Dimitrios P. Bogdanos, Luis Andrade, Wim Coucke, Wilson de Melo Cruvinel, Ana Kozmar, Liisa Kuhi, Laurence Lutteri, Maria Jose Rego de Sousa, Sofie Schouwers, Lieve Van Hoovels and Xavier Bossuyt*, on behalf of the EFLM Working Group on Autoimmunity Testing

Analytical aspects of the antinuclear antibody test by HEp-2 indirect immunofluorescence: EFLM report on an international survey

09:55 – 10:20 Detection of antinuclear antibodies: recommendations from EFLM, EASI and ICAP: QA & verification, a practical approach

Martine Vercammen (Department of Laboratory Medicine, AZ Sint-Jan, Brugge, Belgium)

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Standardisation in NIS

Autoimmune diagnostics: complex!!!

- Different kits/providers
- Different methodologies
- Different specificities (Ag souce, avidity,…)
- ….

-> Difference in results!!

=> Standardisation is needed!!

Standardisation of PR3-ANCA and MPO-ANCA: evaluation of certified reference materials

Xavier Bossuyt ,[1] Doreen Dillaerts,[1] Michael Mahler ,[2] Dirk Roggenbuch,[3,4] Ulrich Leinfelder,[5] Friederike Hammar,[5] Wolfgang Schlumberger,[6] Nina Olschowka,[7] Jan Damoiseaux [8]

=> Taken together, using the certified reference material for PR3-ANCA and MPO-ANCA squeezes test results within the same order of magnitude. However, depending on the assays compared, differences and poor correlation between individual results remain.

UKNEQAS collaboration:

- SN/18676: AC-5, dsDNA neg, RNP
- SN/18677: AC-5
- SN/18647: Anti-DFS70/LEDGF-p75 (anti-DFS70)

-> Can we standardize?

Harmonisation in NIS

Is this more feasible?

ICAP nomenclature -> IIF aspect reporting

Ring test:

- SN/15085: (fine) speckled, SSA/SSB
- SN/15086: negative

-> Use of automated microscope helps in harmonisation ?

Usermeeting automated IIF microscopes
"Results of the Ringtest 2017"
Thursday November 30th 2017

→

EQA
Autom >< Manual

General: test result interpretation < universal way of test reporting

-> Based on cut-off: establishment?

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Harmonisation in NIS

Use of Likelihood ratio for test result reporting?!

11:00 - 11:30 **LR in autoimmune diagnostics: basic principles**
Xavier Bossuyt (Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium; Department of Laboratory Medicine, University Hospital Leuven, Leuven, Belgium)

-> Survey BE + EASI 2023

Current application and interest of likelihood ratio reporting in autoimmune serology: results of a Belgian and EASI forum survey

Lieve Van Hoovels[1,2], Sylvia Broeders[3], Jan Damoiseaux[4], Wim Coucke[3], Carolien Bonroy[5,6], Sylvie Goletti[7], Laurence Lutteri[8], Sofie Schouwers[9], Martine Vercammen[10,11], Xavier Bossuyt[2,12]
on behalf of the Belgian expert committee on non-infectious serology of Sciensano and the Belgian EASI working group

[1]OLV Hospital Aalst, Department of Laboratory Medicine, Aalst, Belgium; [2]Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium; [3]Sciensano, Quality of laboratories, Brussels, Belgium; [4]Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, Netherlands; [5]Department of Laboratory Medicine, Ghent University, Ghent, Belgium; [6]Department of Diagnostic Sciences, Ghent University, Ghent, Belgium; [7]Department of Laboratory Medicine, ULB Bruxelles, Brussels, Belgium; [8]Department of Laboratory Medicine, CHU Liège, Liège, Belgium; [9]GZA Hospital, Department of Laboratory Medicine, Antwerp, Belgium; [10]AZ St-Jan Hospital, Bruges, Department of Laboratory Medicine, Bruges, Belgium; [11]Research group REIM, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [12]Department of Laboratory Medicine, UZ Leuven, Leuven, Belgium

16th Dresden Symposium on autoimmune antibodies

11:30 - 11:50 **LR: Results of the 2023 survey and practical implementation in the lab**
Sofie Schouwers (Department of Laboratory Medicine, GZA Hospitals/ZAS, Antwerp, Belgium)

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Pipeline

- EQA RF/anti-CCP, ANCA, ANA
- EQA Coeliac Disease
- Survey on APS (BE + NL) -> EQA?

11/03/2024
EQA RF/anti-CCP

13:00 – 13:25 APS new classification criteria and daily practice in laboratory diagnosis of APS Katrien Devreese (Coagulation Laboratory, Department of Diagnostic Sciences, Ghent University Hospital, Ghent, Belgium)

13:25 – 13:50 Method comparison in Antiphospholipid antibody measurement
Lieve Van Hoovels (Department of Laboratory Medicine, OLV Ziekenhuis, Aalst, Belgium; Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium)

- Publications
  - LR application on EQA results
  - …
- Alternative ‘tool’ for the NIS EQA on paper

ACKNOWLEDGEMENTS

Expert committee NIS

RBSLM

QUESTIONS?

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Contact

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Presentations, which are allowed, will be published on the Sciensano website

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