Value-added reporting

X. Bossuyt

COMMUNICATING DIAGNOSTIC ACCURACY

Communicating diagnostic accuracy

Question 1

- Sensitivity: 95%

- Specificity: 90%

- Pre-test probability: 2.5%

- Post-test probability ???

- 10%

- 20%

- 50%

- 80%

- 90%

Vermeersch P, Bossuyt X.
Arch Intern Med. 2010;170:734-5.

Communicating diagnostic accuracy

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- Post-test probability ???

- 10%

- **20% (9% of respondents)**

- 50%

- 80%

- 90%

Communicating diagnostic accuracy
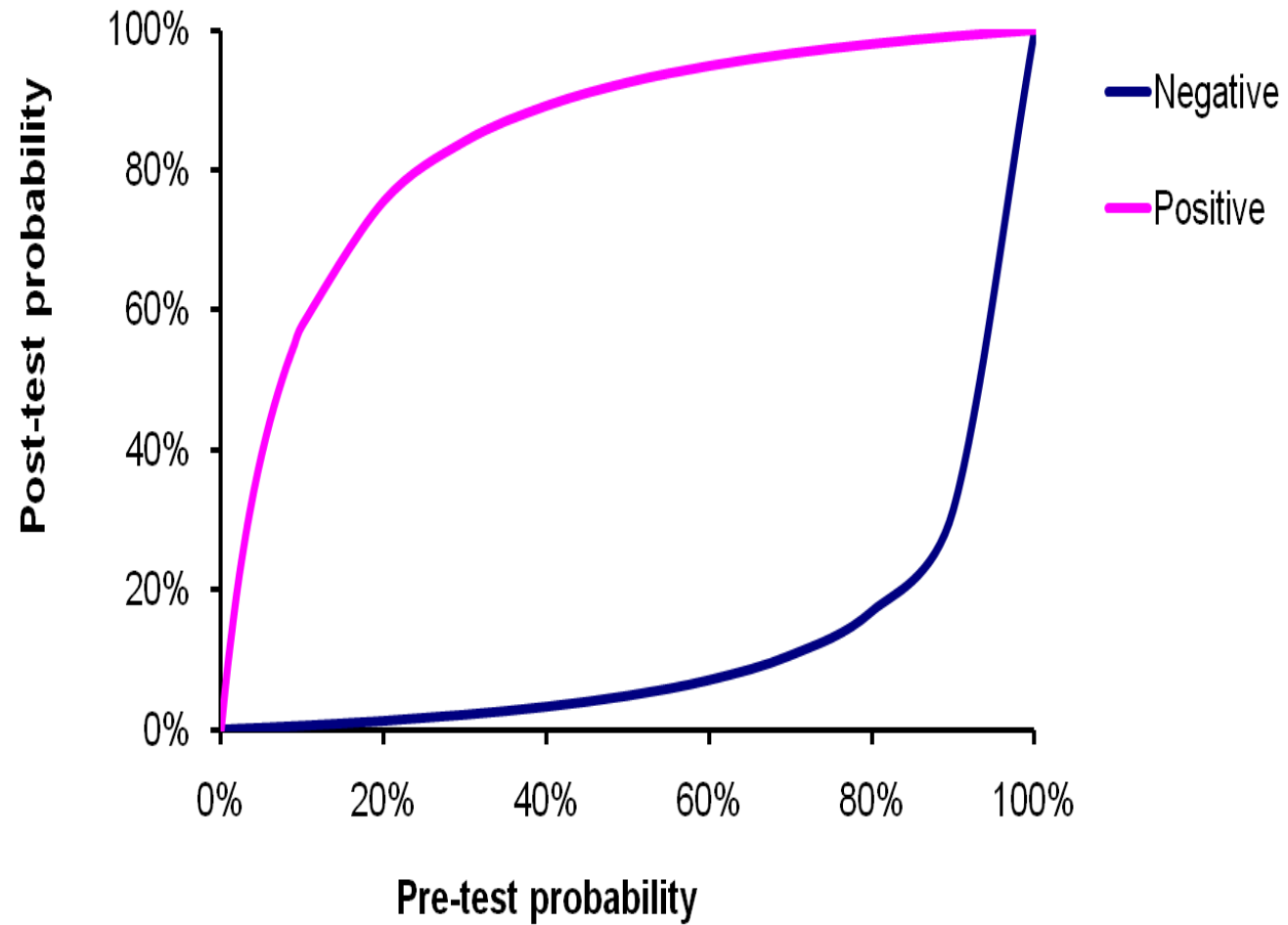
Question 2

- LR 10

    A pos test result occurs 10 times more frequently in patients with the disease compared to patients without the disease

- Pre-test probability: 2.5%

- 10%

- 20%

- 50%

- 80%

- 90%

Vermeersch P, Bossuyt X.
Arch Intern Med. 2010;170:734-5.

Communicating diagnostic accuracy

Question 2

- LR 10

    A pos test result occurs 10 times more frequently in patients with the disease compared to patients without the disease

- Pre-test probability: 2.5%

- 10%
- **20% (35% of respondents)**
- 50%
- 80%
- 90%

Vermeersch P, Bossuyt X.
Arch Intern Med. 2010;170:734-5.

Communicating diagnostic accuracy

Question 3

Vermeersch P, Bossuyt X.
Arch Intern Med. 2010;170:734-5.

Diagnostic task	Base rate	Sensitivity	False-positive rate	Positive predictive value
Breast cancer	1%	80%	10%	

Diagnostic task	Base rate	Sensitivity	False-positive rate	Positive predictive value
Breast cancer	1% 100 of 10,000	80% 80 of 100	10% 990 of 9900	

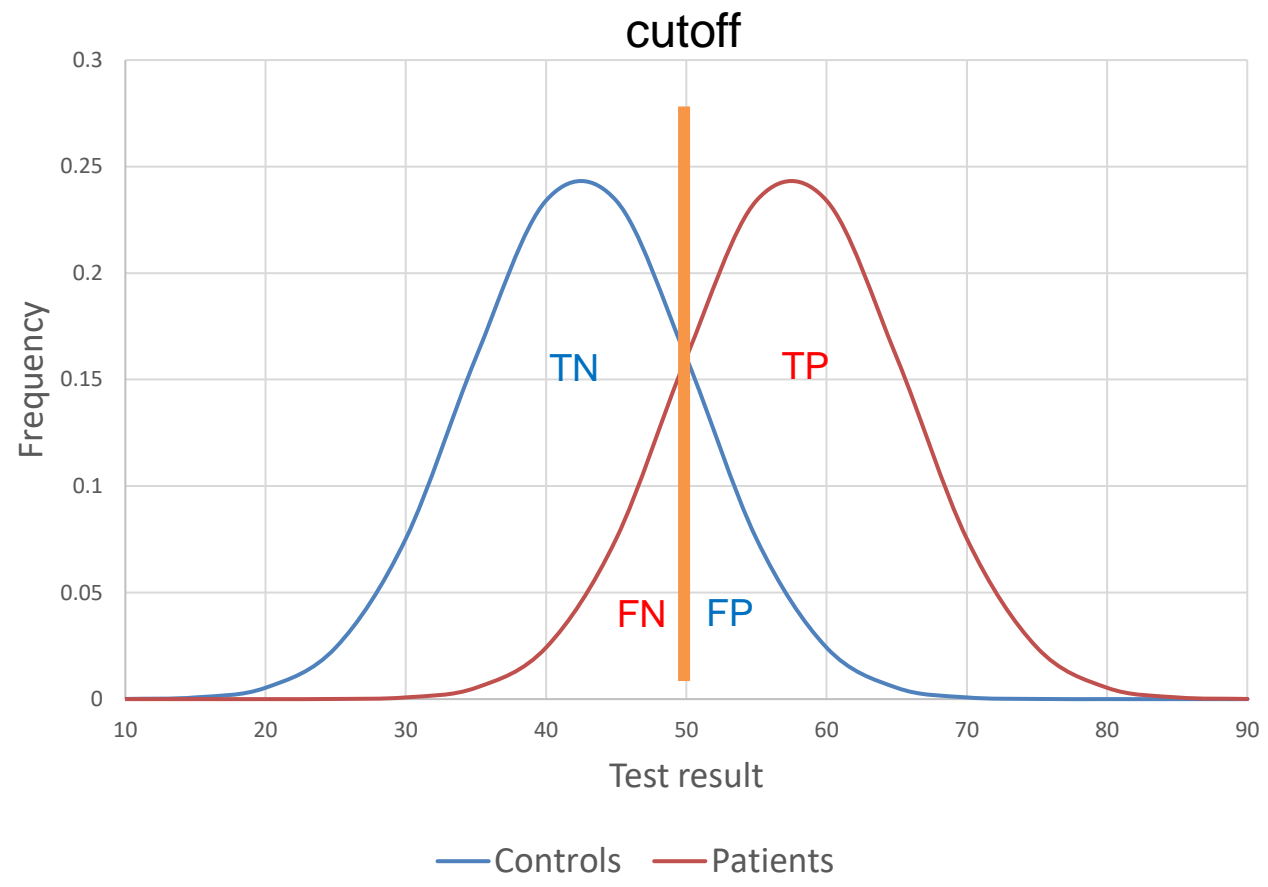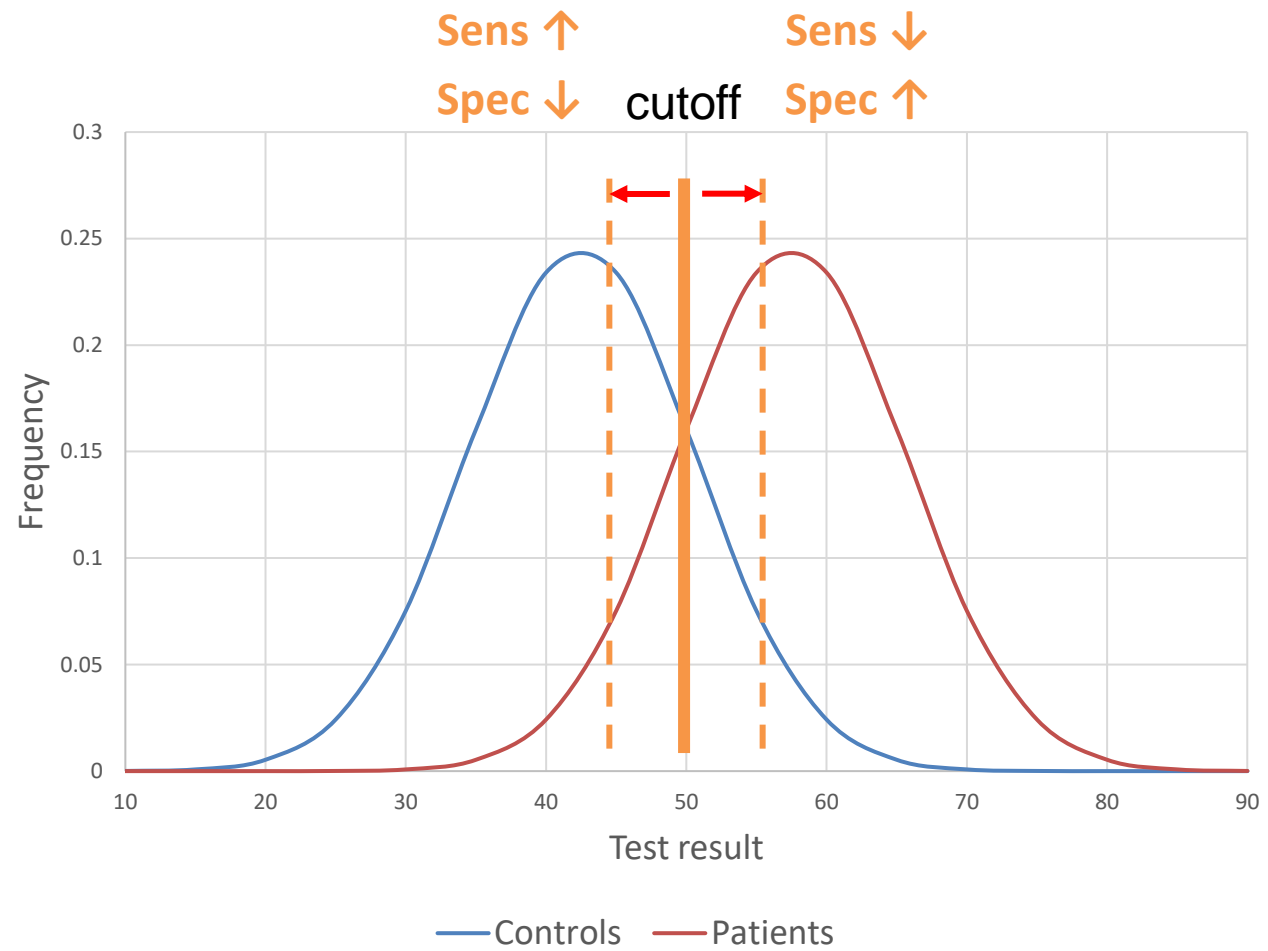| Diagnostic task	Base rate	Sensitivity	False-positive rate	Positive predictive value
Breast cancer	1%	80%	10%	7%
	100 of 10,000	80 of 100	990 of 9900	80 of 1070
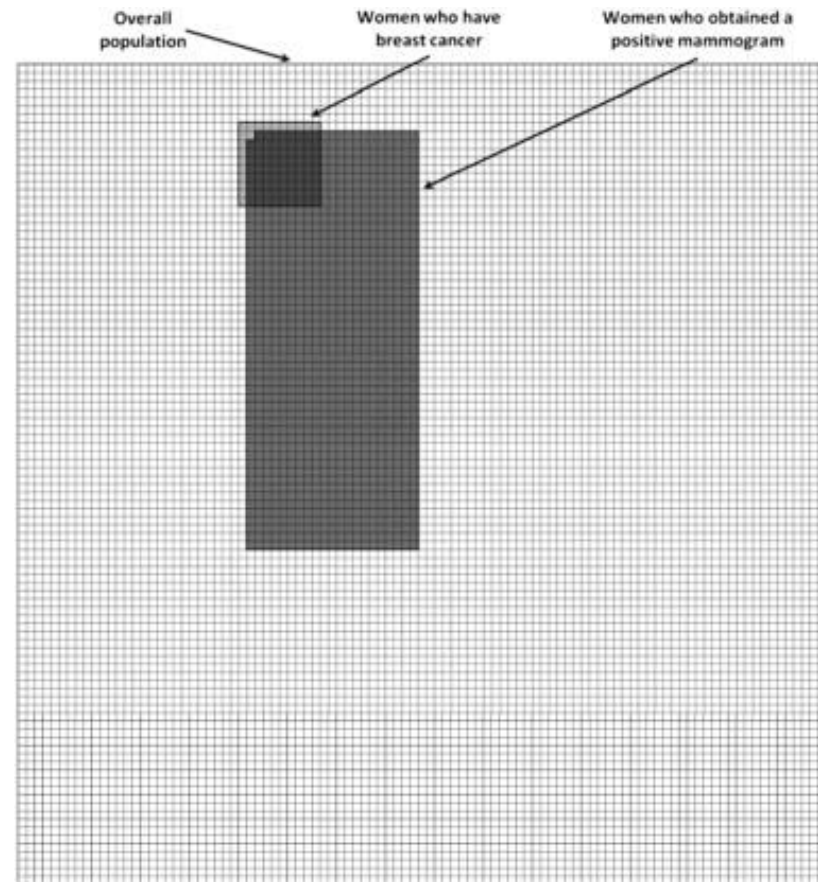
Fig. 1 Visual aid representing the overall number of women at risk, the number of women who have breast cancer, and the number of women who obtained a positive mammogram.
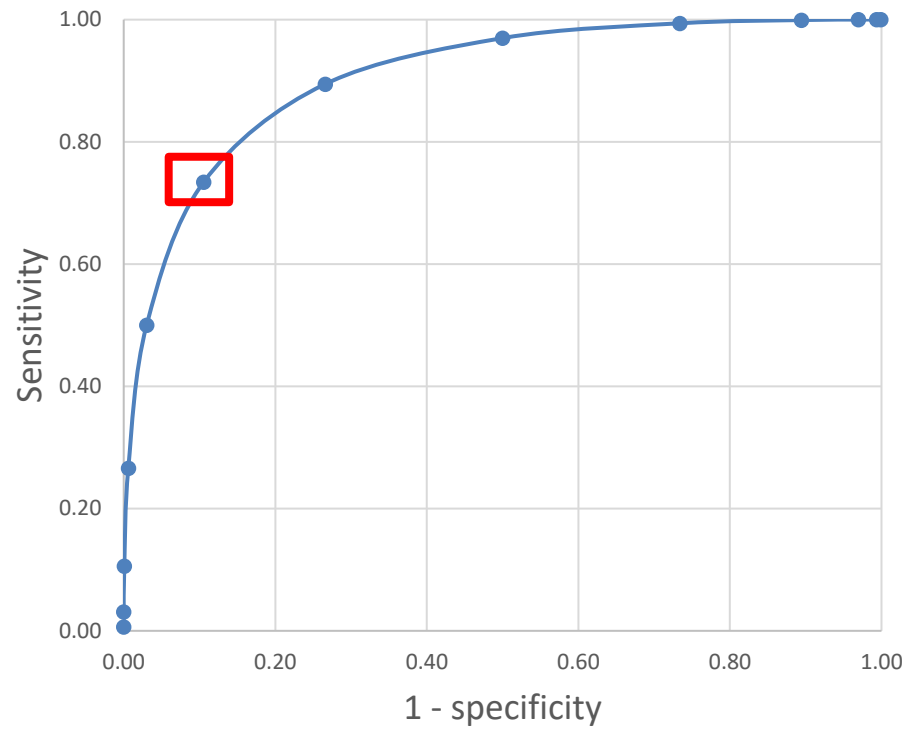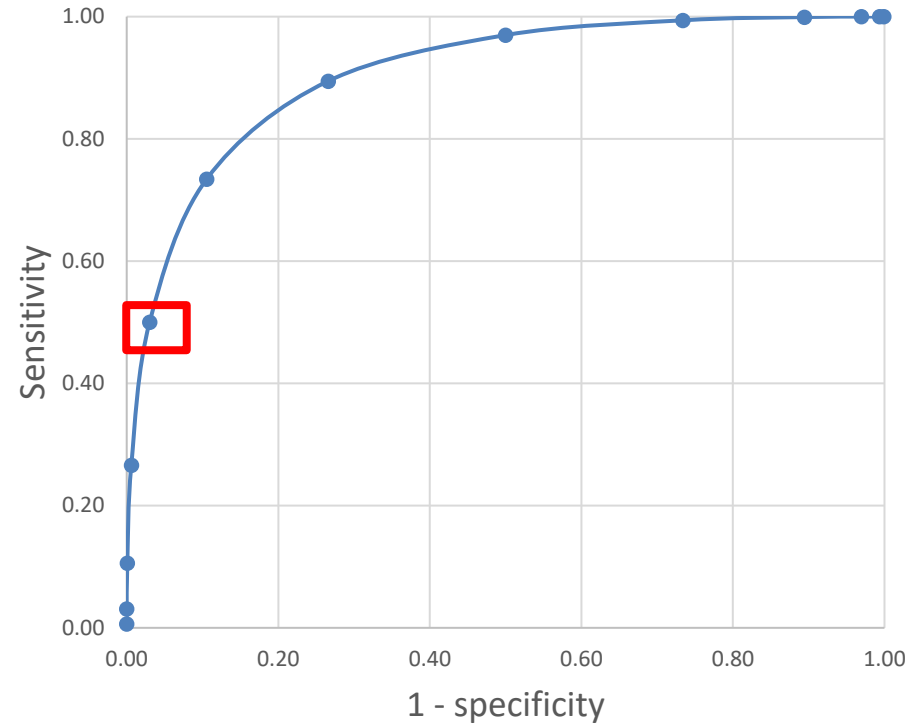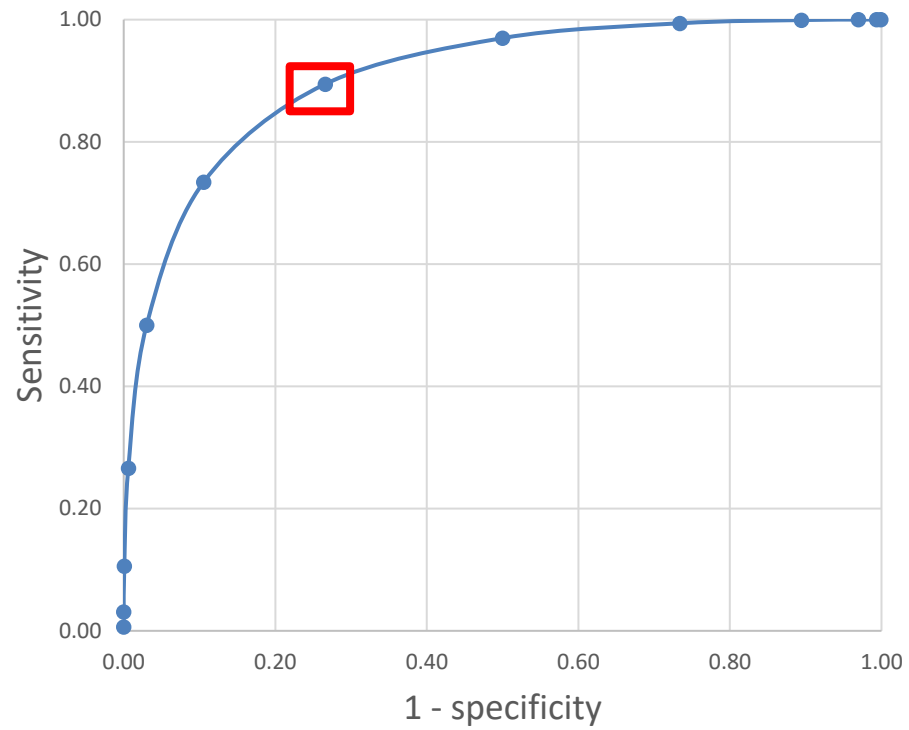
DIAGNOSTIC ACCURACY: Basics
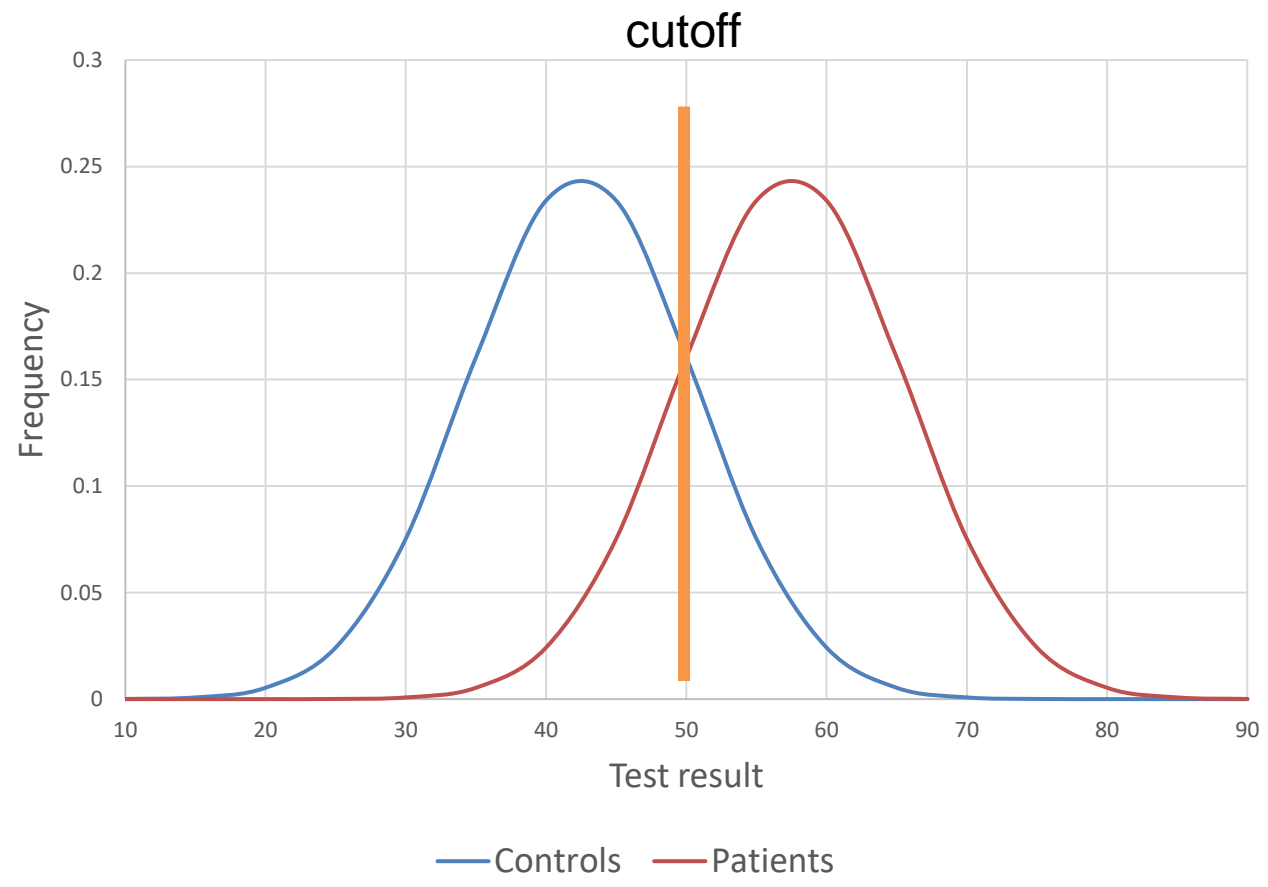
Cutoff	Sensitivity	Specificity
15	1,00	0,00
20	1,00	0,01
25	1,00	0,03
30	1,00	0,11
35	0,99	0,27
40	0,97	0,50
45	0,89	0,73
50	0,73	0,89
55	0,50	0,97
60	0,27	0,99
65	0,11	1,00
70	0,03	1,00
75	0,01	1,00

Cutoff	Sensitivity	Specificity
15	1,00	0,00
20	1,00	0,01
25	1,00	0,03
30	1,00	0,11
35	0,99	0,27
40	0,97	0,50
45	0,89	0,73
50	0,73	0,89
55	0,50	0,97
60	0,27	0,99
65	0,11	1,00
70	0,03	1,00
75	0,01	1,00
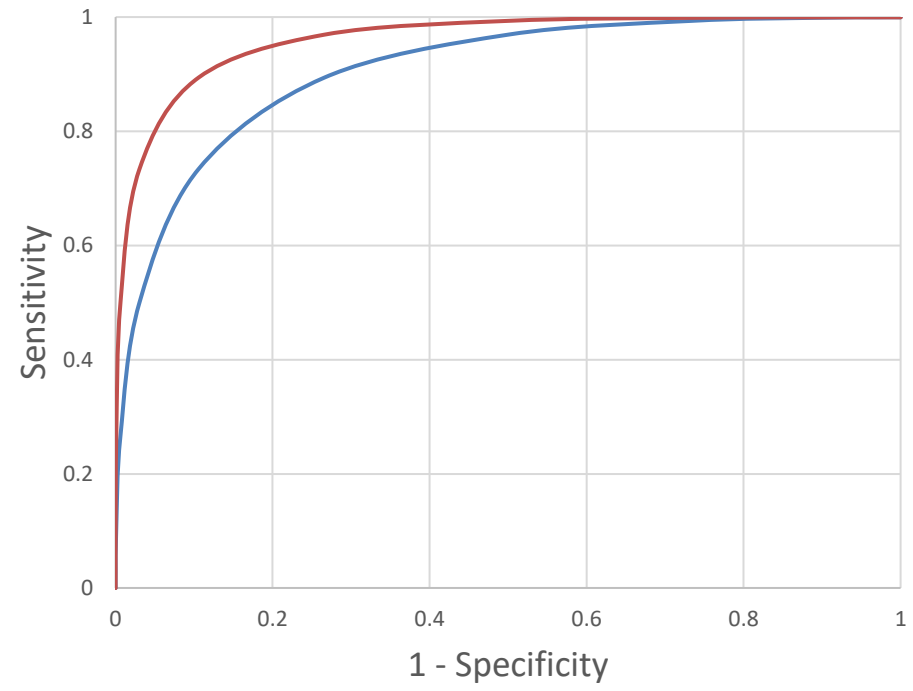
Anti-CCP: sensitivity 67%

                   specificity 95% for RA.

What is the probability that an individual with a positive result has RA

when the pre-test probability is:

- 1%

Prevalence in population

- 10%

- Female, 50 years old
- Recent onset undifferentiated arthritis
- Intermittent asymmetric tender and swollen small joints of the hands (n=5)
- CRP: 10 mg/L

- 80%

- Female, 50 years old
- Symmetric arthritis of upper and lower extremities
- Tender and swollen small joints (n=12)
- CRP: 70 mg/L

Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Likelihoods	RA	non RA
anti-CCP+	0,67	0,05
anti-CCP-	0,33	0,95

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Likelihoods	RA	non RA				
anti-CCP+	0,67	0,05				
anti-CCP-	0,33	0,95				
pre-test prob (1%)	RA	non RA				
	0,01	0,99	1			

Likelihoods	RA	non RA				
anti-CCP+	0,67	0,05				
anti-CCP-	0,33	0,95				
pre-test prob (1%)	RA	non RA				
	0,01	0,99	1			
joint probability	RA	non RA				
anti-CCP+	0,0067	0,0495	0,056			
anti-CCP-	0,0033	0,9405	0,944			
	0,01	0,99	1			

Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Likelihoods	RA	non RA			
anti-CCP+	0,67	0,05			
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anti-CCP+	0,0067	0,0495	0,056		
anti-CCP-	0,0033	0,9405	0,944		
	0,01	0,99	1		
	RA	non RA			
anti-CCP+	6,7	49,5	0,12	PPV	6,7/(6,7+49,5)
anti-CCP-	3,3	940,5	0,997	NPV	940,5/(940,5+3,3)
	10	990			

Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Likelihoods	RA	non RA			
anti-CCP+	0,67	0,05			
anti-CCP-	0,33	0,95			
pre-test prob (10%)	RA	non RA			
	0,1	0,9			
Joint probability	RA	non RA			
anti-CCP+	0,067	0,045	0,11		
anti-CCP-	0,033	0,855	0,89		
	0,1	0,9	1		
	RA	non RA			
anti-CCP+	67	45	0,60 PPV	67/(67+45)	
anti-CCP-	33	855	0,96 NPV	855/(855+33)	
	100	900			

Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Likelihoods	RA	non RA			
anti-CCP+	0,67	0,05			
anti-CCP-	0,33	0,95			
pre-test prob (80%)	RA	non RA			
	0,8	0,2			
joint probability	RA	non RA			
anti-CCP+	0,536	0,01			
anti-CCP-	0,264	0,19			
	0,8	0,2	1		
	RA	non RA			
anti-CCP+	536	10	0,98	PPV	
anti-CCP-	264	190	0,42	NPV	
	800	200			
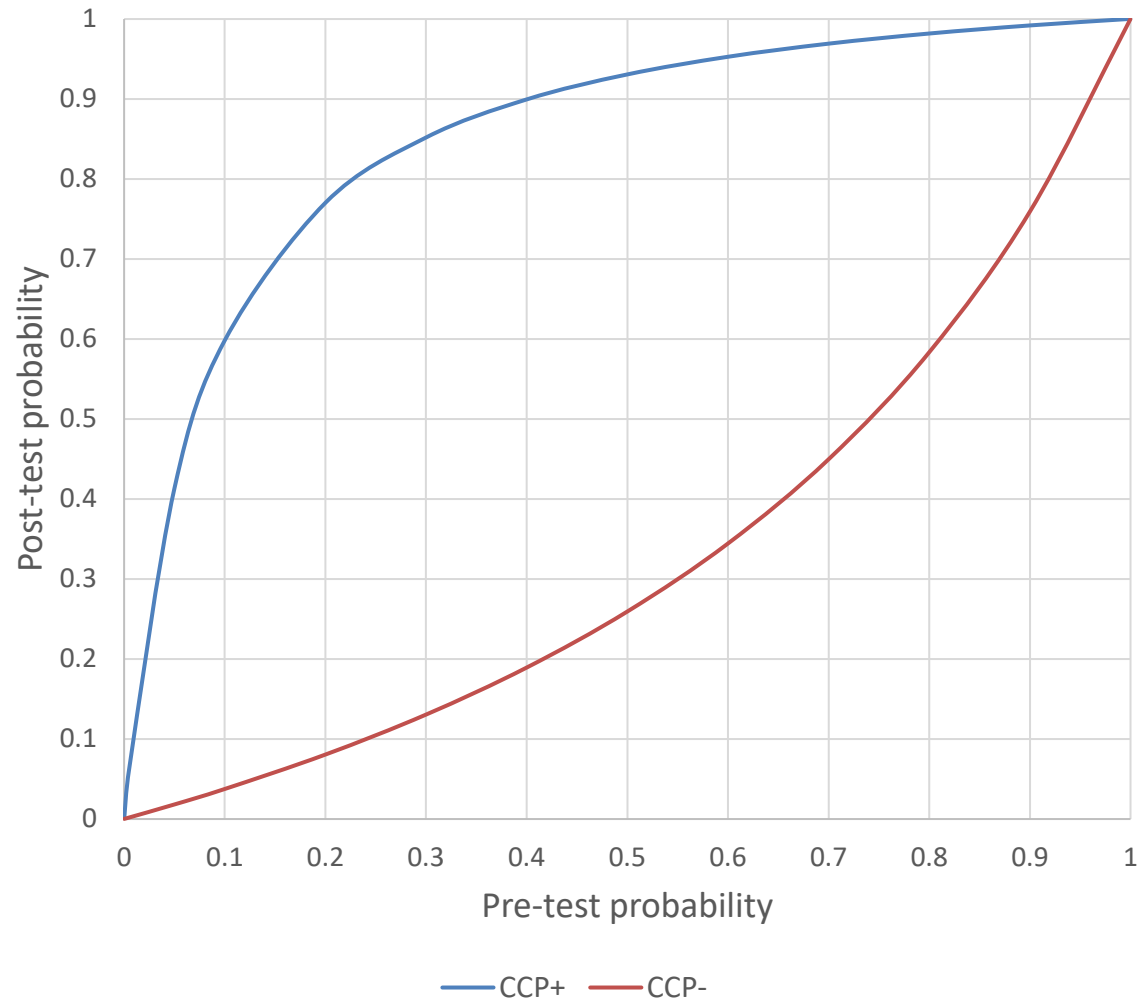
Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

pre-test prob			0,01	0,10	0,80
post-test prob voor positief resultaat			0,12	0,60	0,98
post-test prob voor negatief resultaat			0,003	0,04	0,58

Likelihood en likelihood ratio

Likelihoods	RA	non RA		LR(+)	LR(-)			
anti-CCP+	0,67	0,05		13,4			*0,67/0,05*	*Sens/(1 – Spec)*
anti-CCP-	0,33	0,95			0,35		*0,33/0,95*	

LR	Interpretation
1	No clinical difference
2-5 or 0,2-0,5	Small difference, may be relevant in certain clinical settings
5-10 or 0,1-0,2	Modest, but substantial difference in pretest-posttest probability
>10 or <0,1	Clinically important differences in pretest-post-test difference

Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Post-test odds

=

Pre-test odds x LR

probability → odds

odds = prob/(1-prob)

odds → probability

probability = odds/(1+odds)

Likelihoods	RA	non RA	LR(+)	LR(-)		
anti-CCP+	0,67	0,05	13,4			*0,67/0,05*
anti-CCP-	0,33	0,95		0,35		*0,33/0,95*
pre-test probability			0,10	0,01	0,80	
pre-test odds			0,11	0,01	4,00	Odds = Prob/(1-prob)
			0,1/0,9	0,01/0,99	0,8/0,2	
post-test odds voor positief resultaat			1,49	0,14	53,60	**Post-test odds = Pre-test odds x LR**
			0,11x13,4		4x13,4	Bayesian Theorem
post-test odds voor negatief resultaat			0,04	0,00	1,39	
			0,11x0,35		4x0,35	
post-test probability voor positief resultaat			0,60	0,12	0,98	Probability = Odds/(1+odds)
			1,49/(1+1,49)			
post-test probability voor negatief resultaat			0,037	0,003	0,58	
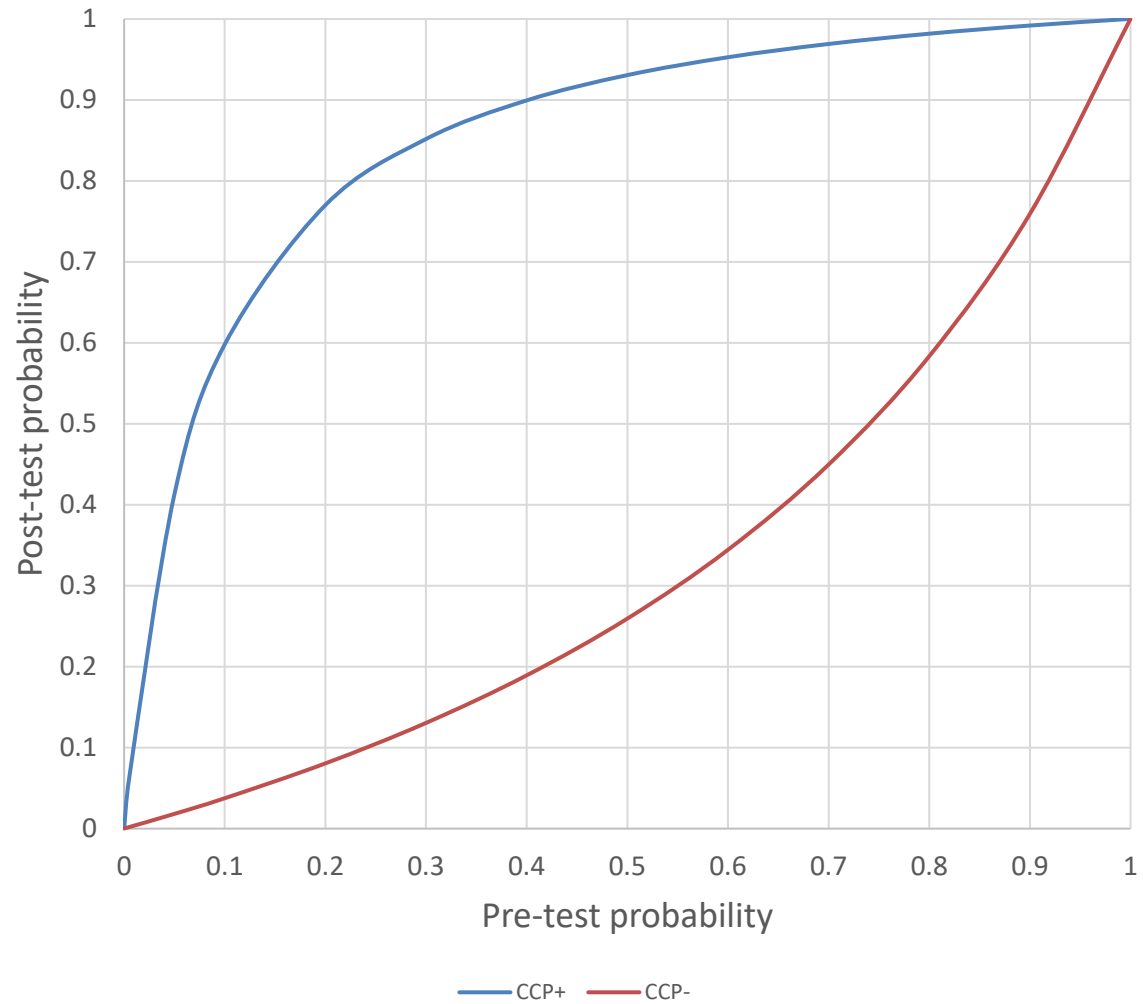
Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

pre-test prob			0,01	0,10	0,80
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Bossuyt X. Autoimmunity Reviews 2009;8:543-548.

Test result interval-specific likelihood ratios

Bossuyt X. et al.
Ann Rheum Dis. 2009;68:287-9.
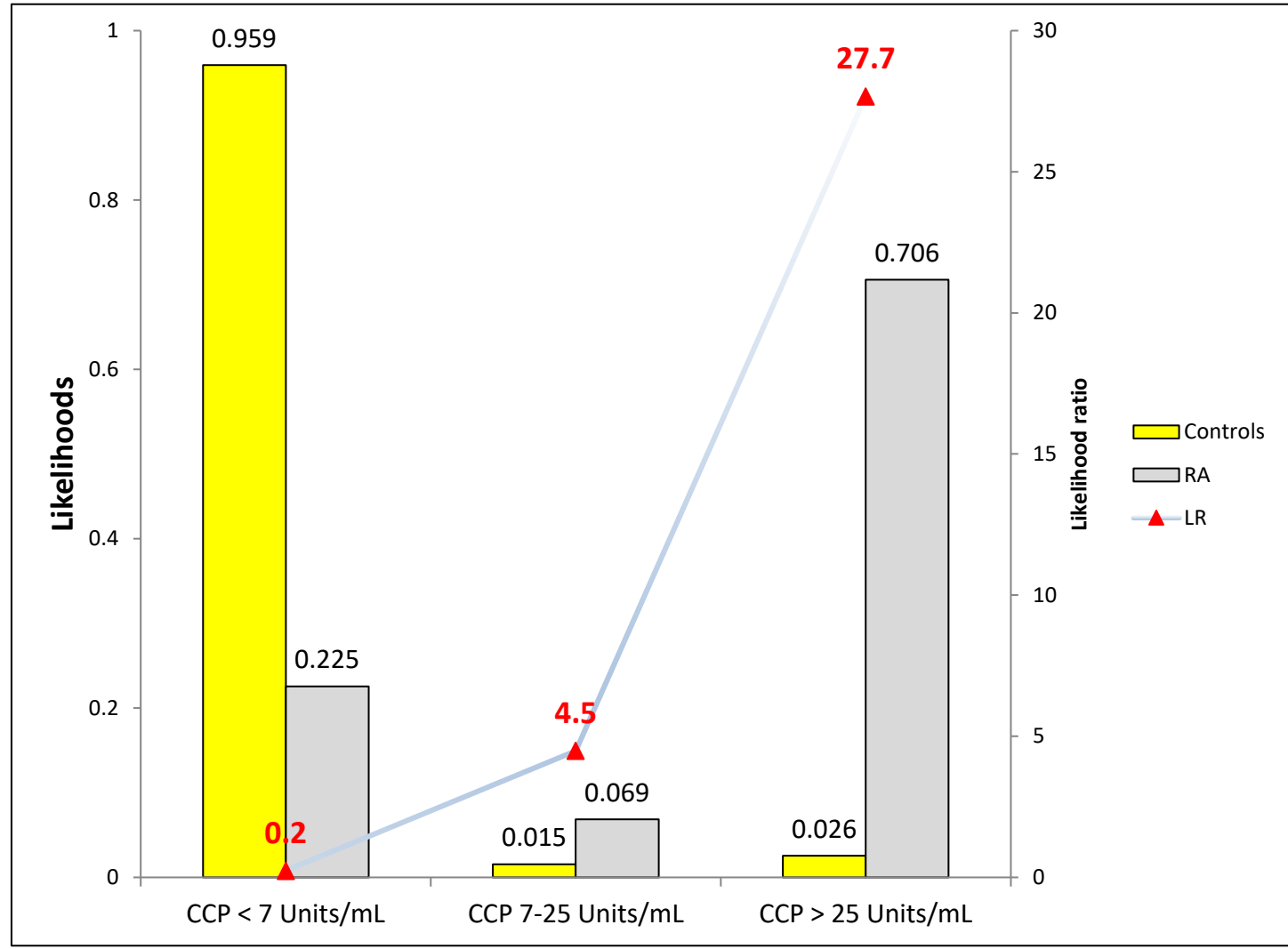
Post-test probability in function of pre-test probability

Bossuyt X. et al.
Ann Rheum Dis. 2009;68:287-9.
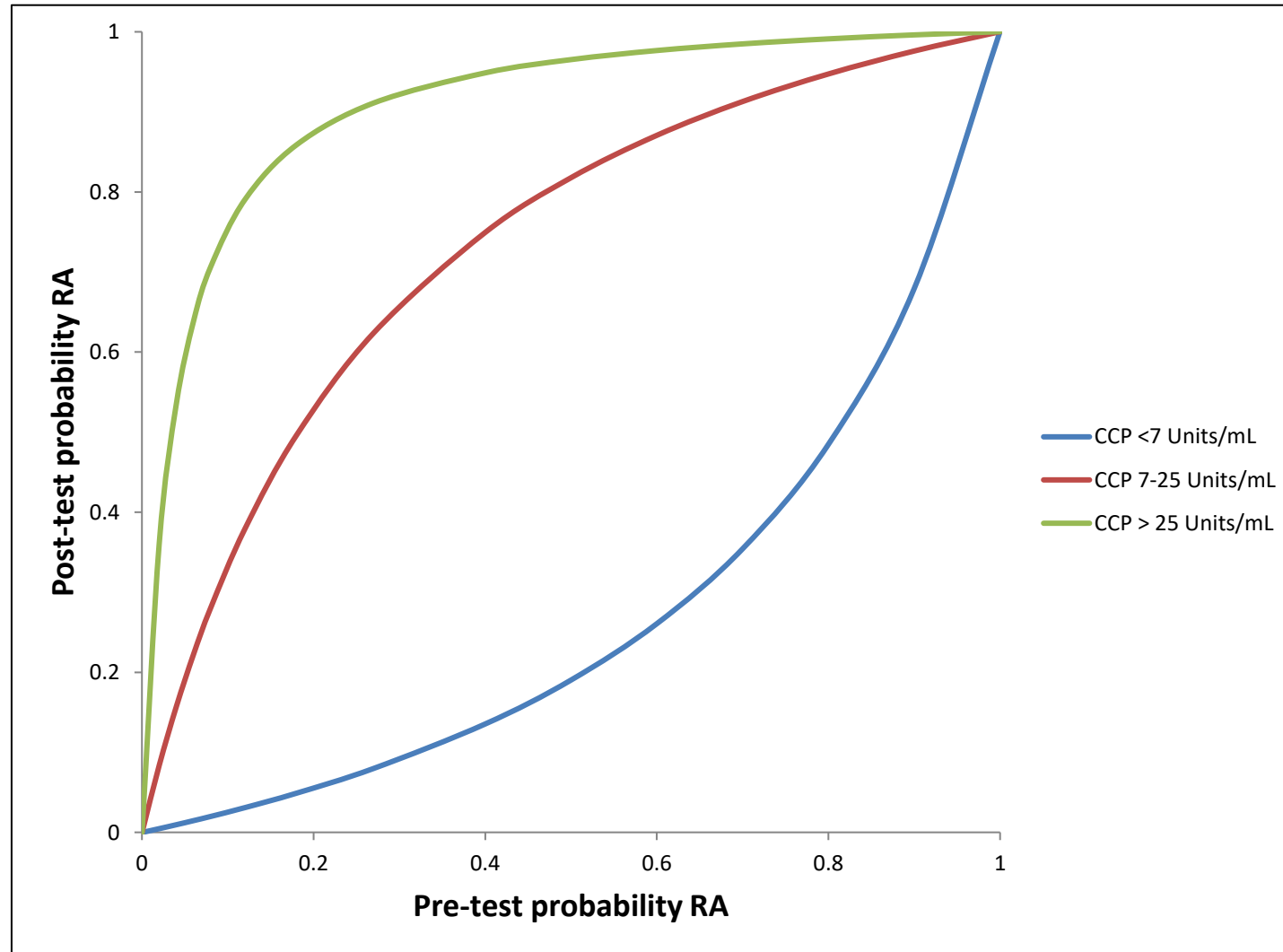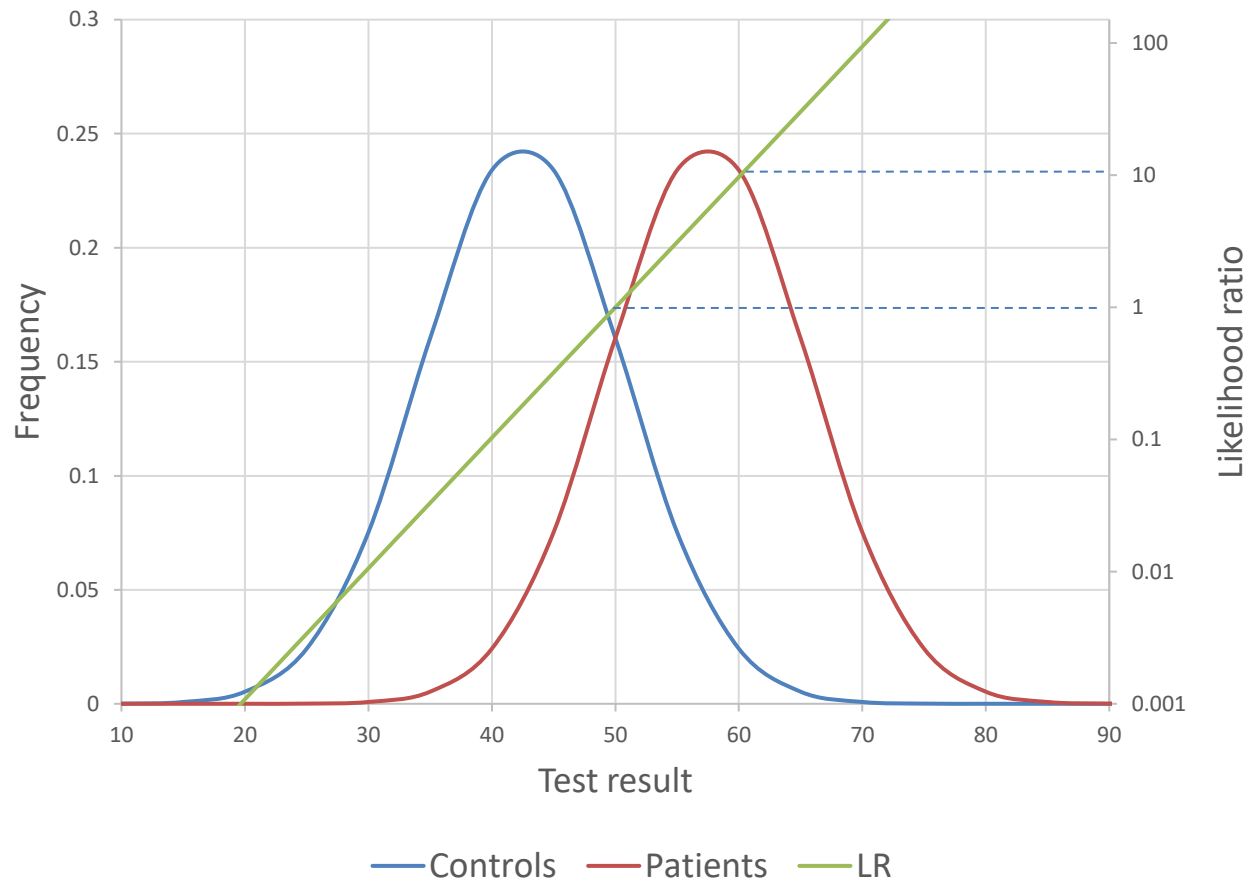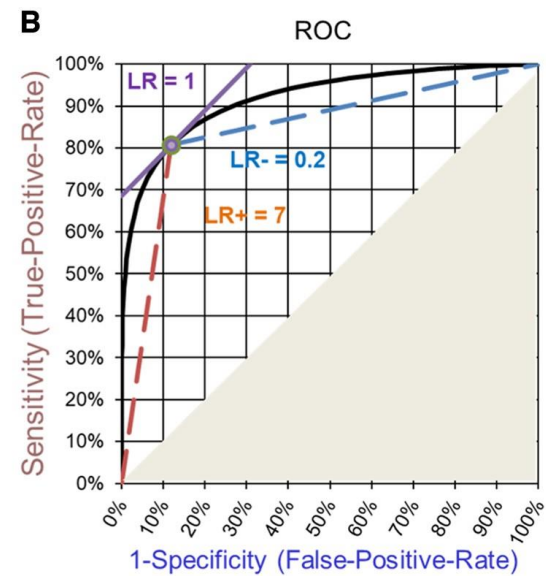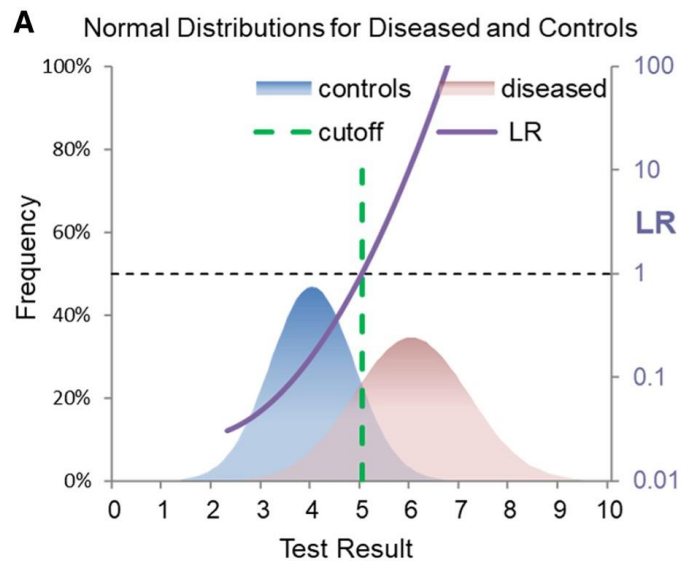
Fierz W, Bossuyt X.

Likelihood Ratios as Value Proposition for Diagnostic Laboratory Tests.

J Appl Lab Med. 2020;5:1061-1069.

ROC
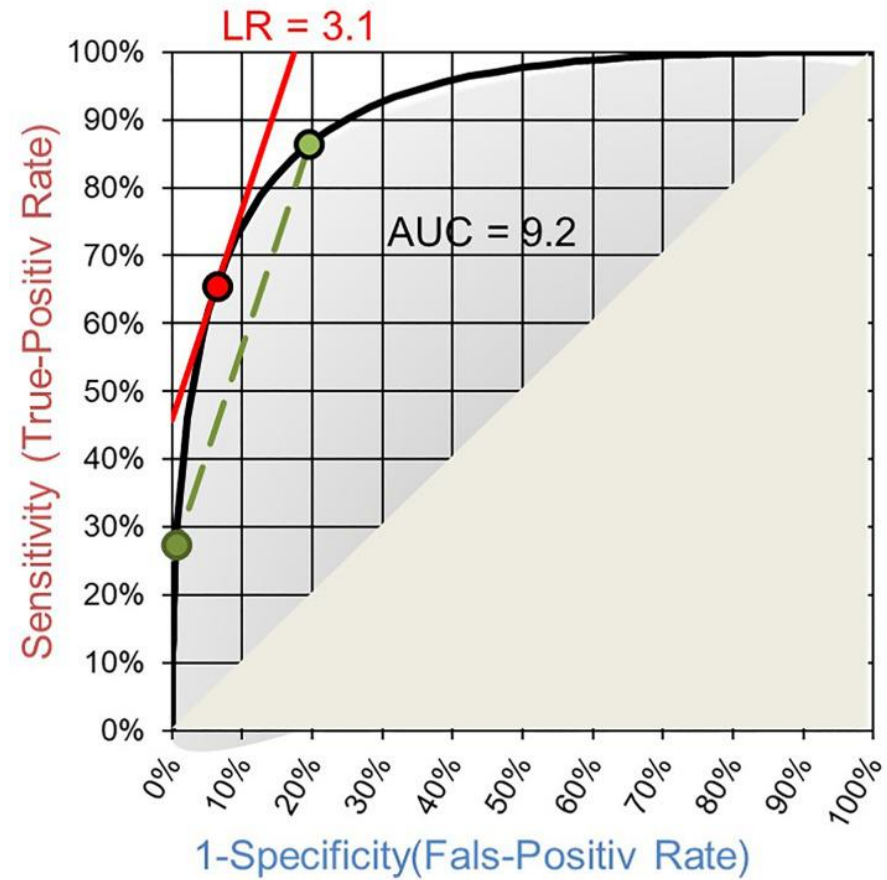
Fierz W, Bossuyt X.

Likelihood Ratio Approach and Clinical Interpretation of Laboratory Tests.

Front Immunol. 2021 Apr 16;12:655262.

Bossuyt, X. *et al.* (2017) *Nat. Rev. Rheumatol.*
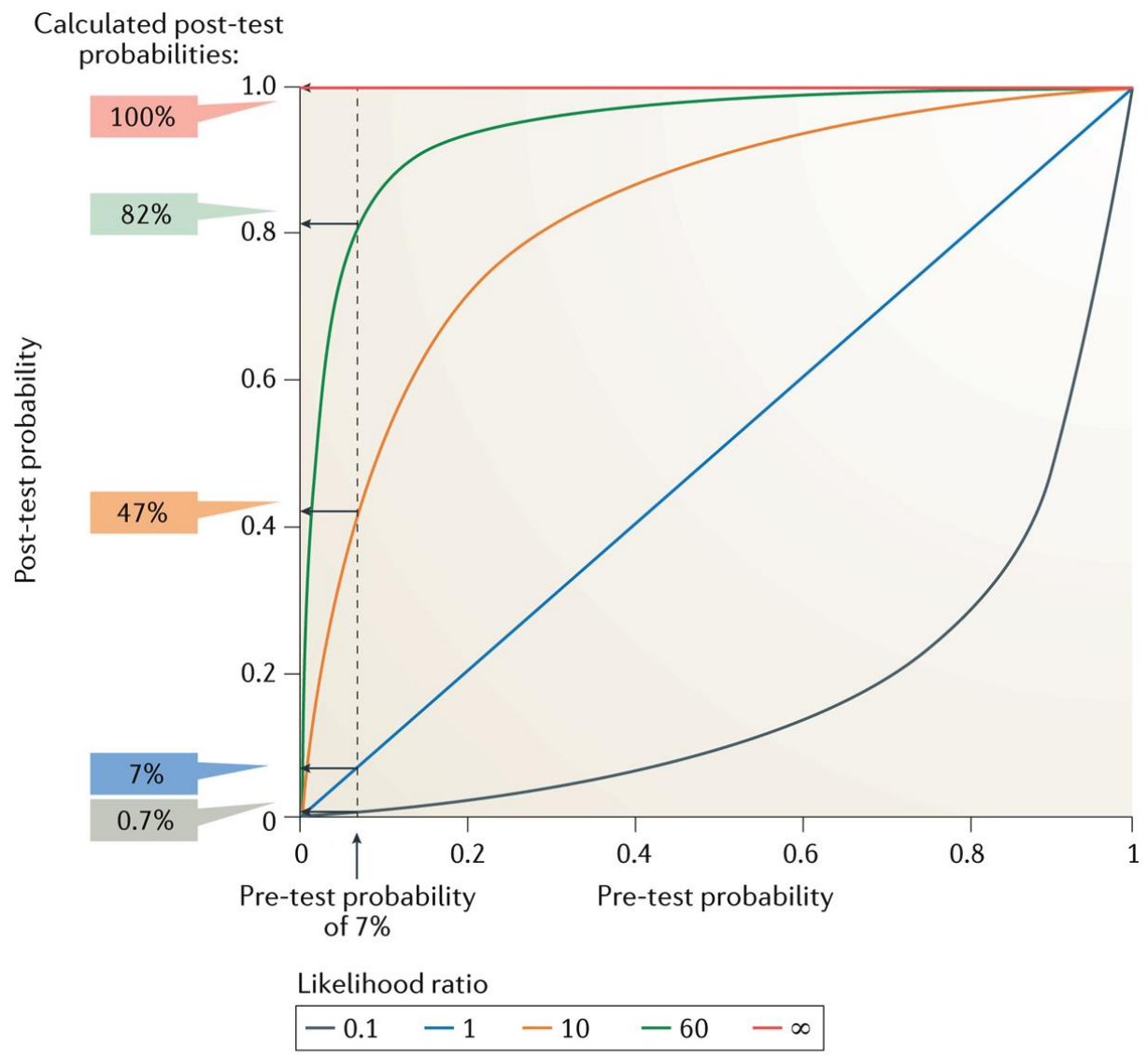
ANCA-ASSOCIATED VASCULITIS

Visual representation of the 1999 recommendations and revised 2017 recommendations
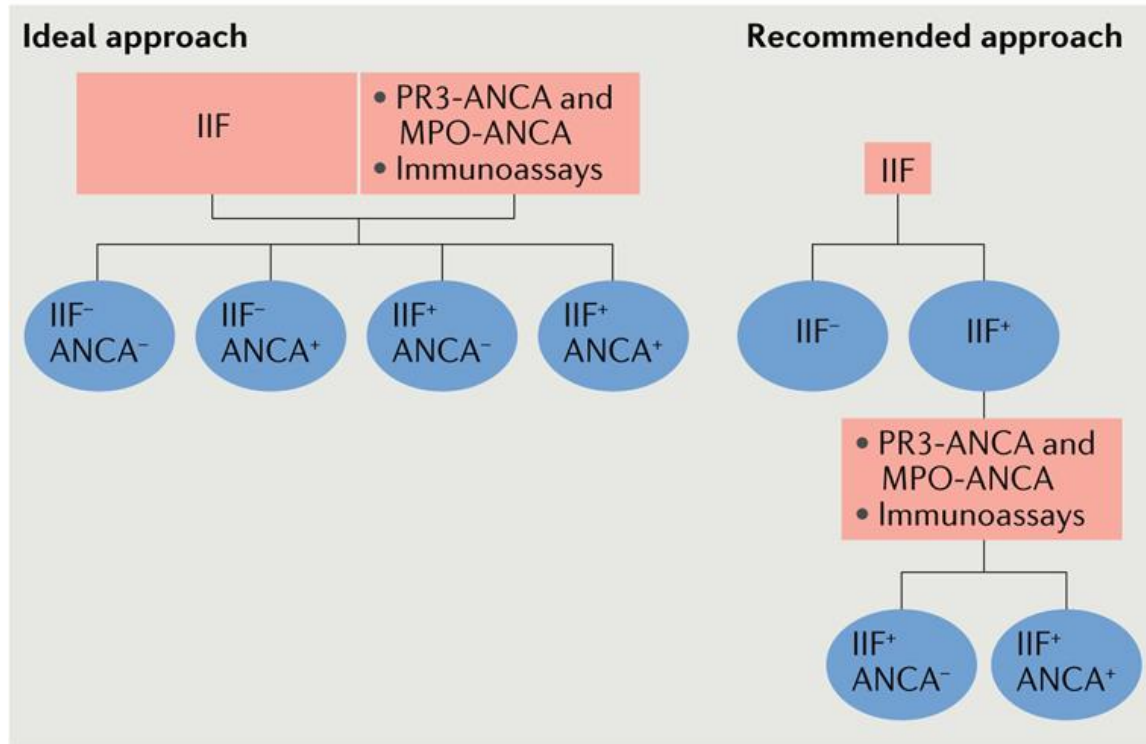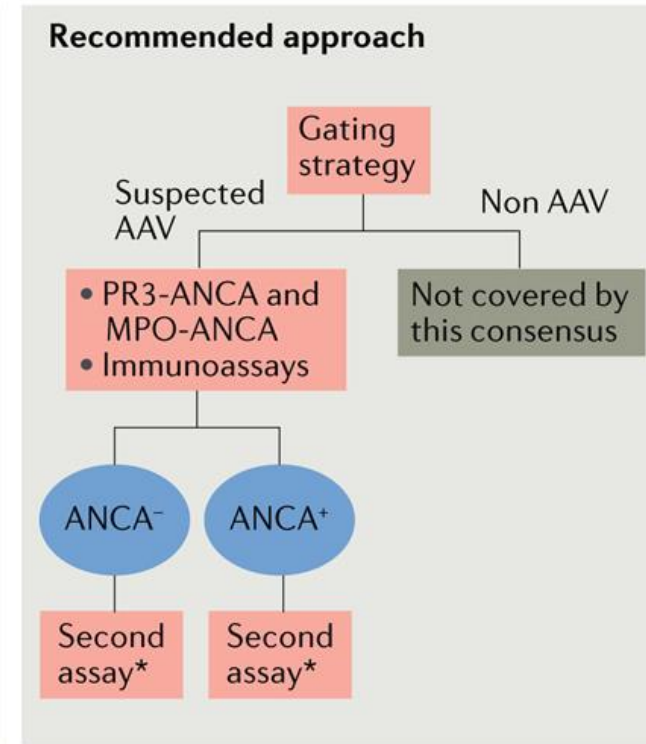
Bossuyt, X. *et al.* (2017) *Nat. Rev. Rheumatol.*

Manufacturer	Threshold	Sensitivity	Specificity	LR(+)	LR(-)
ELISA Quantalite - Inova	10,0	0,90	0,95	18,2	0,10
Cutoff: 20 Units	19.0	0,87	0,98	35,0	0,13
	37,0	0,77	0,99	79,4	0,23
	159,1	0,17	1,00	+∞	0,83

Bossuyt et al. Rheumatology (Oxford). 2017 ;56:1533-1541.

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	37,0	0,77	0,99	79,4	0,23
	159,1	0,17	1,00	+∞	0,83

Manufacturer	Interval	Fraction of patients	Fraction of controls	LR	95% CI
QuantaLite - Inova	0,0 - 10,0	0,096	0,949	0,10	0,07 to 0,15
20 Units	10.1 - 19,0	0,040	0,026	1,50	0,74 to 3,16
	19,0 - 37,0	0,092	0,014	6,51	3,34 to 12,67
	37,0 - 159,1	0,602	0,011	55,59	29,76 to 103,81
	159,1 - 250,0	0,171	0,000	∞	19,55 to ∞

Bossuyt et al. Rheumatology (Oxford). 2017 ;56:1533-1541.

Manufacturer Cutoff	Interval	Fraction of patients	Fraction of controls	LR	95% CI
QuantaLite - Inova	0,0 - 10,0	0,096	0,949	0,10	0,07 to 0,15
20 Units	10.1 - 19,0	0,040	0,026	1,50	0,74 to 3,16
	19,0 - 37,0	0,092	0,014	6,51	3,34 to 12,67
	37,0 - 159,1	0,602	0,011	55,59	29,76 to 103,81
	159,1 - 250,0	0,171	0,000	∞	19,55 to ∞
QuantaFlash - Inova	0,0 - 12,5	0,096	0,949	0,10	0,07 to 0,15
20 CU	12,5 - 23,8	0,032	0,026	1,23	0,56 to 2,70
	23,8 - 78,2	0,155	0,015	10,25	5,66 to 18,58
	78,2 - 1049,8	0,629	0,010	64,63	33,50 to 124,67
	1049,8 - 3500,0	0,088	0,000	∞	9,85 to ∞
FEIA EliA - Thermo Fisher	0,0 - 2,1	0,100	0,950	0,10	0,07 to 0,15
PR3: equivocal 2-3 IU/L	2,1 - 5,0	0,084	0,025	3,36	1,89 to 5,97
MPO: equivocal 3,5-5 IU/L	5,0 - 16,0	0,179	0,015	11,83	6,60 to 21,20
	16,0 - 142,0	0,570	0,010	58,49	30,26 to 113,05
	142,0 - 180,0	0,068	0,000	∞	7,55 to ∞
BioPlex 2200 BioRad	0,0 - 0,5	0,092	0,950	0,10	0,06 to 0,14
1AI	0,5 - 1,3	0,024	0,024	1,00	0,41 to 2,45
	1,3 - 6,2	0,235	0,016	14,48	8,36 to 25,08
	6,2 - 8,0	0,649	0,010	66,67	34,58 to 128,54
ELISA Euro-diagnostica	0,0 - 2,8	0,120	0,951	0,13	0,09 to 0,18
equivocal 5-7 IU/mL	2,8 - 4,5	0,028	0,023	1,23	0,53 to 2,85
	4,5 - 11,9	0,052	0,015	3,41	1,63 to 7,18
	11,9 – 247.0	0,713	0,011	65.89	35,40 to 122,67
	247.0 - 3000,0	0,088	0,000	∞	10,28 to ∞
ELISA Orgentec	0,0 - 4,4	0,120	0,950	0,13	0,09 to 0,18
PR3: 10 U/mL	4,4 - 7,9	0,016	0,025	0,64	0,22 to 1,83
MPO: 5 U/mL	7,9 - 22,4	0,104	0,015	6,84	3,62 to 12,89
	22,4 - 230,0	0,745	0,010	76,49	39,76 to 147,13
	230,0 - 450,0	0,016	0,000	∞	1,56 to ∞
ELISA Euroimmun	0,0 - 7,4	0,084	0,949	0,09	0,06 to 0,13
20 U/mL	7,4 - 26,7	0,044	0,026	1,69	0.84 to 3,40
	26,7 - 115,7	0,231	0,015	15,25	8,65 to 26,88
	115,7 - 200,0	0,641	0,010	66,85	34,15 to 126,99
CytoBead	0,0 - 4,6	0,159	0,948	0,17	0,13 to 0,23
equivoval 4,5-5 IU/mL	4,6 - 5,6	0,036	0,027	1,32	0,63 to 2,79
	5,6 - 14,3	0,151	0,015	9,99	5,49 to 18,09
	14,3 - 68,2	0,363	0,010	37.22	18,04 to 72,78
	68,2 - 180,0	0,291	0,000	∞	33,42 to ∞

Bossuyt et al. Rheumatology (Oxford). 2017 ;56:1533-1541.
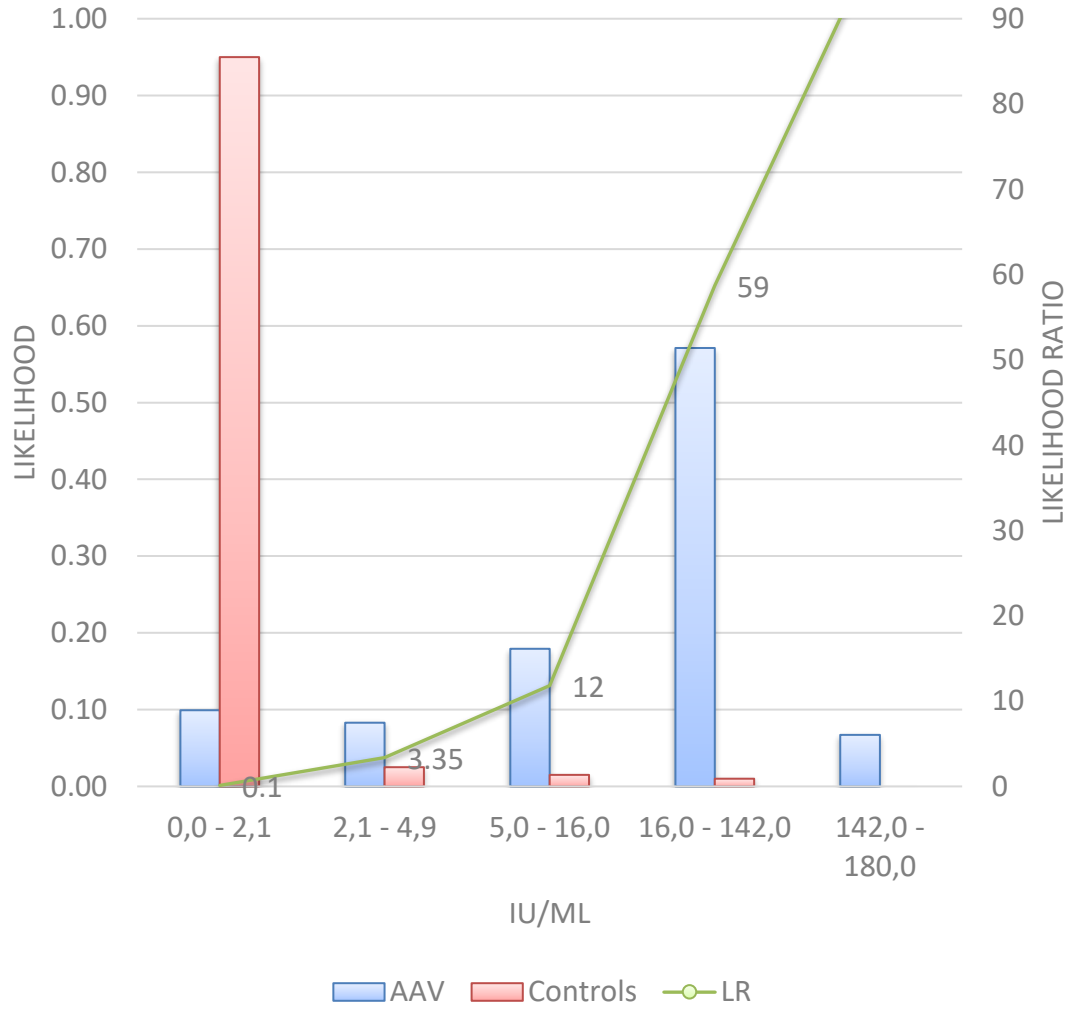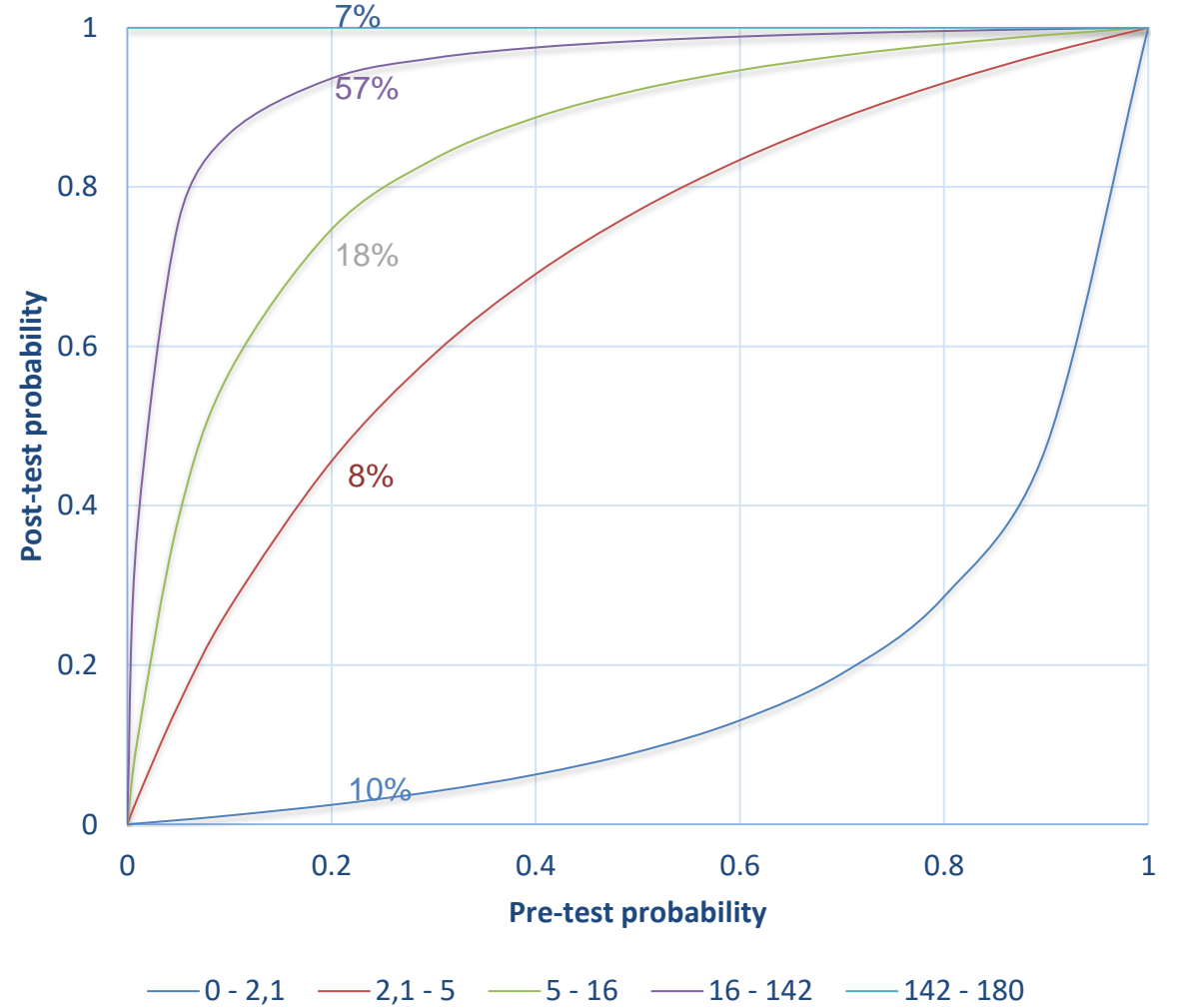
Clinical condition	Pre-test probability
none*	0.00003
sinus*	0.01
sinus and pulmonary*	0.10
sinus and glomerulonephritis*	0.30
sinus, pulmonary and glomerulonephritis*	0.85
rapidly progressive glomerulonephritis°	0.47
hematuria, proteinuria, creatinine >3 mg/dl°	0.21
Hematuria, proteinuria, creatinine 1.5-3 mg/dl°	0.07
Hematuria, proteinuria, creatinine <1.5 mg/dl°	0.02

*: pre-test probabilities from Langford [*Cleve Clin J Med. 1998;65:135-40*]
o   sinus: radiographic evidence of mucosal thickening involving one or more sinuses
o   pulmonary: radiographic presence of pulmonary infiltrates or nodules, or both
o   glomerulonephritis: urinalysis demonstrating hematuria and red blood cell casts

°: pre-test probabilities from Jennette et al. [Kidney Int. 1998;53:796-8]. The conditions are for an adult patient (>18 years).

EliA

7%

57%

18%

8%

10%

Post-test probability

Pre-test probability

0 - 2,1   2,1 - 5   5 - 16   16 - 142   142 - 180
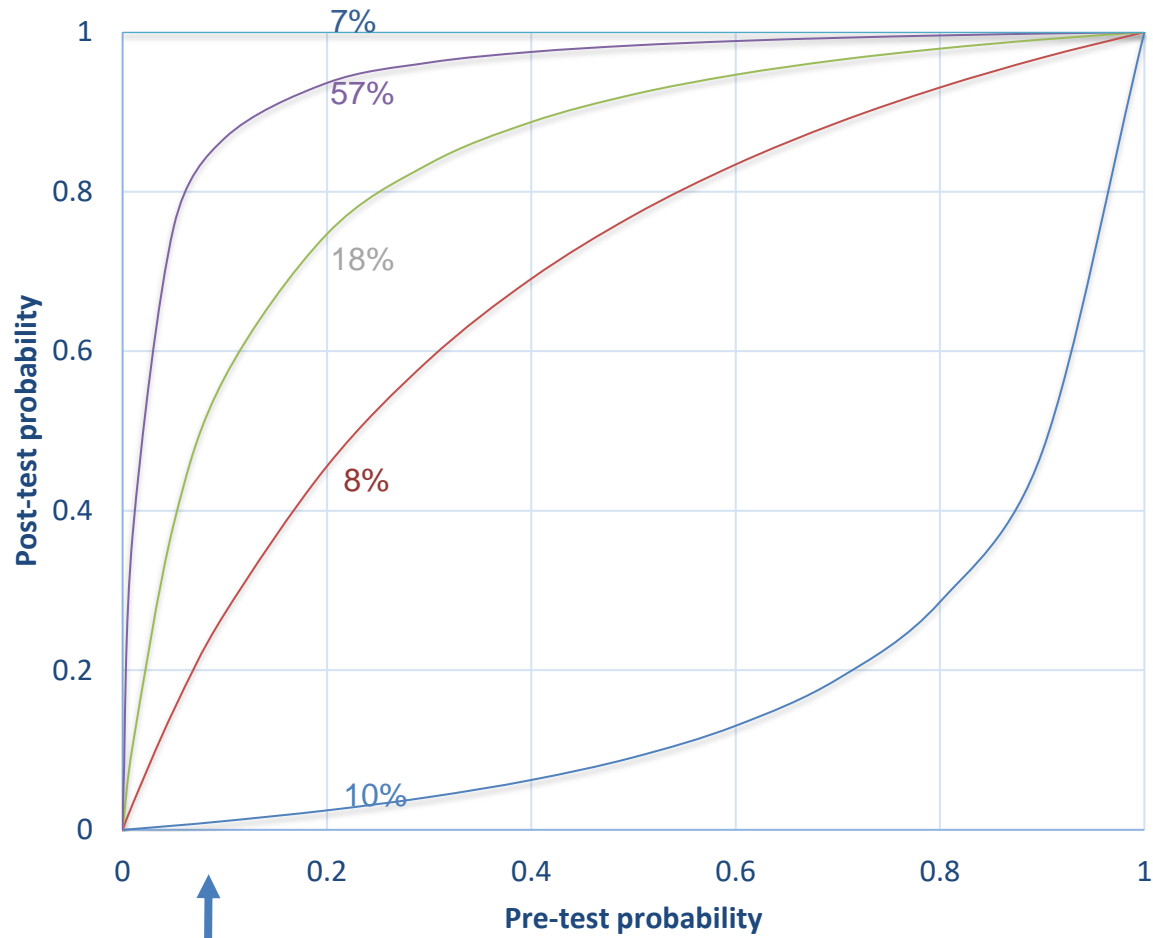
10 % pre-test probability
radiographic evidence of mucosal thickening involving one or more sinuses
radiographic presence of pulmonary infiltrates or nodules, of both

EliA
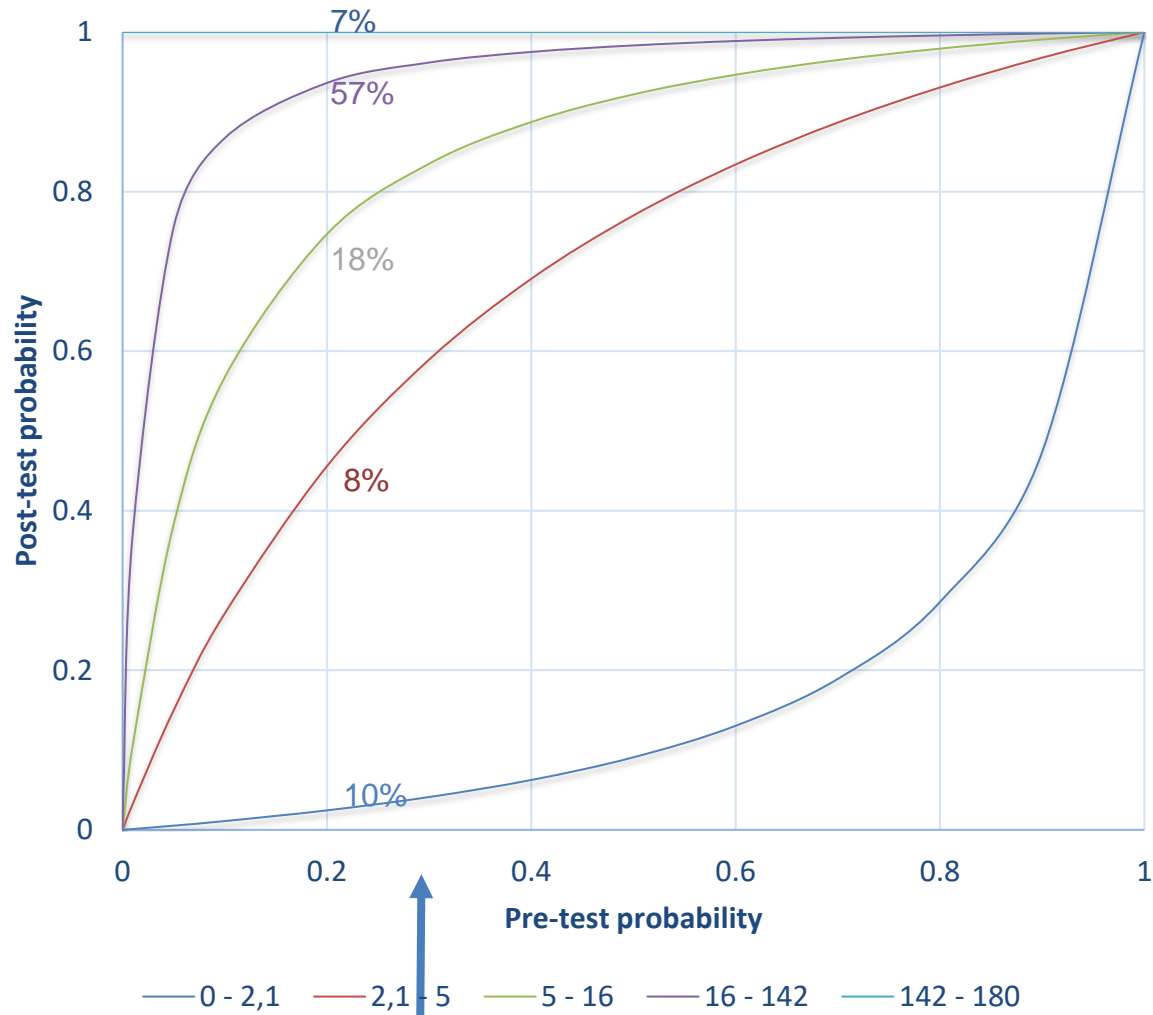
EliA

85 % pre-test probability
radiographic evidence of mucosal thickening involving one or more sinuses
radiographic presence of pulmonary infiltrates or nodules, or borh
urinalysis demonstrating hematuria and red blood cell casts

Letter to the Editor

Xavier Bossuyt*, Jan Damoiseaux, Niels Rasmussen, Pieter van Paassen, Bernard Hellmich, Bo Baslund, Daniel Blockmans, Pieter Vermeersch, Marcos Lopez-Hoyos, Martine Vercammen, Elisa Barret, Friederike Hammar, Ulrich Leinfelder, Michael Mahler, Nina Olschowka, Dirk Roggenbuck, Wolfgang Schlumberger, Roger Walker, Johan Rönnelid, Jan-Willem Cohen Tervaert, Elena Csernok and Walter Fierz
for (i) the European Federation of Laboratory Medicine (EFLM) Task and Finish Group "Autoimmunity Testing," (ii) the European Autoimmune Standardization Initiative (EASI) and the (iii) European Consensus Finding Study Group on autoantibodies (ECFSG)

Harmonization of antineutrophil cytoplasmic antibodies (ANCA) testing by reporting test result-specific likelihood ratios: position paper

Bossuyt X. et al. CCLM 2021

Bossuyt X. et al. CCLM 2021

A

Likelihood Ratio (x-axis), Assay-specific Unit (y-axis)
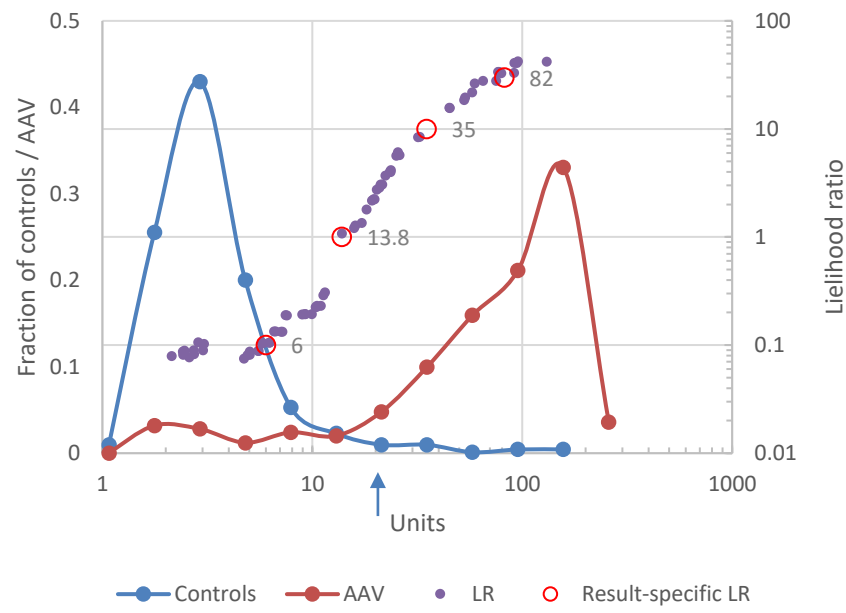
Legend:
- Inova (ELISA - QUANTA Lite)
- Inova (CLIA - QUANTA Flash)
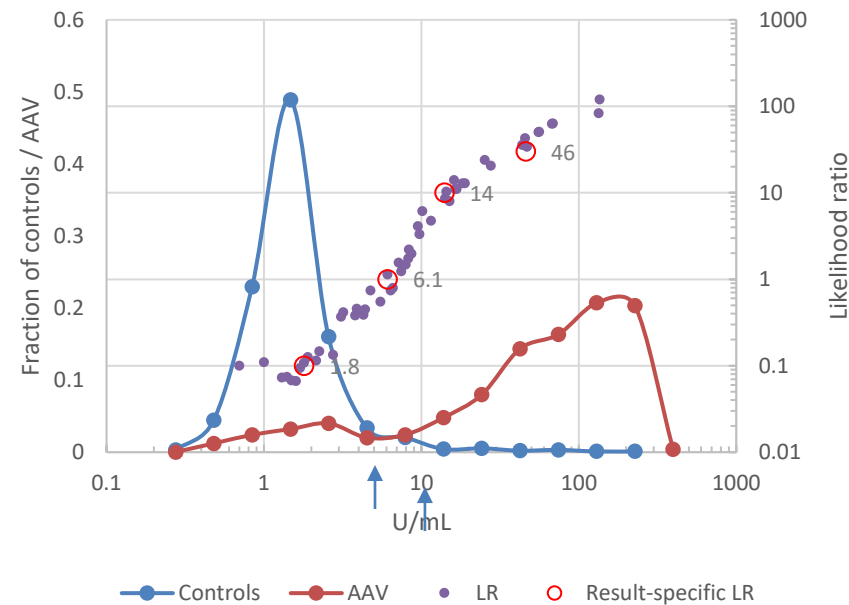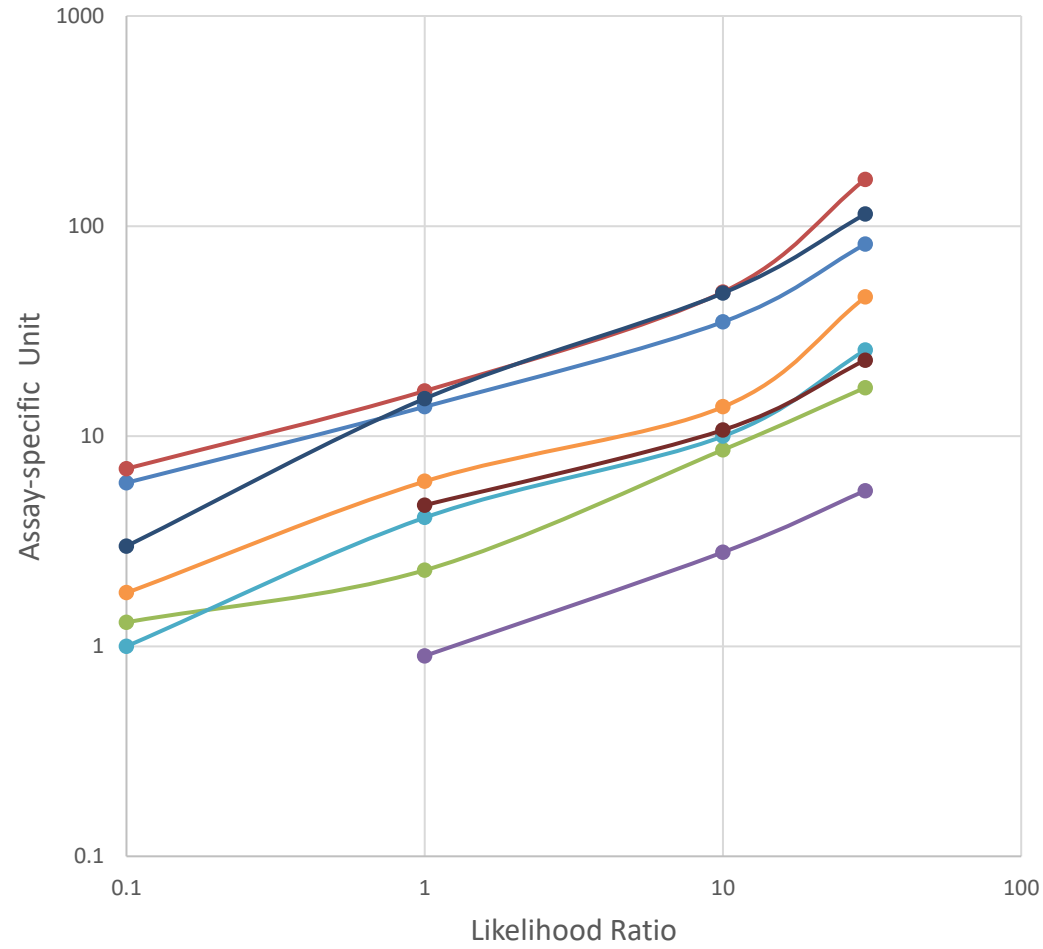- Thermo Fisher (FEIA - EliA)
- BioRad (MIA - Bioplex 2200)
- Svar Life Science (Capture ELISA)
- Orgentec (ELISA)
- Euroimmune (ELISA)
- Medipan (CytoBead)
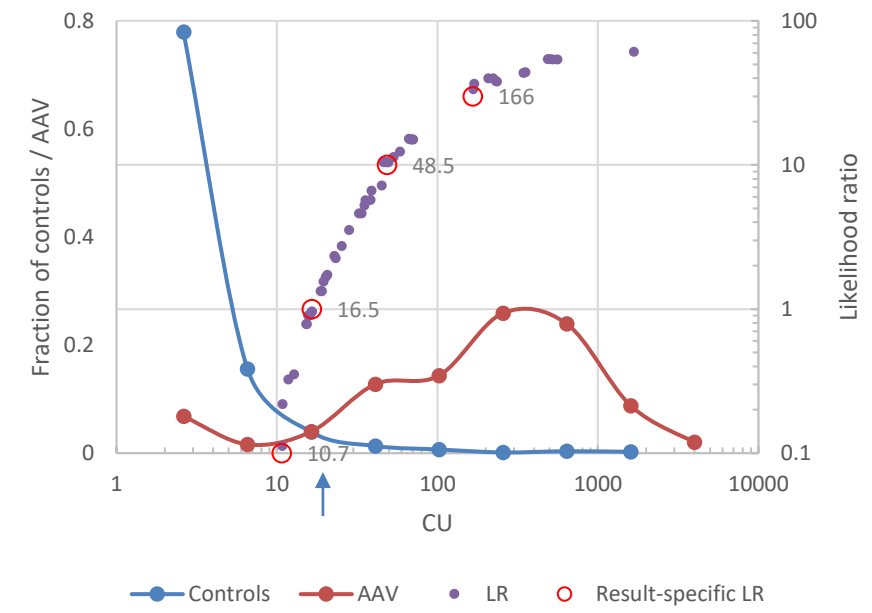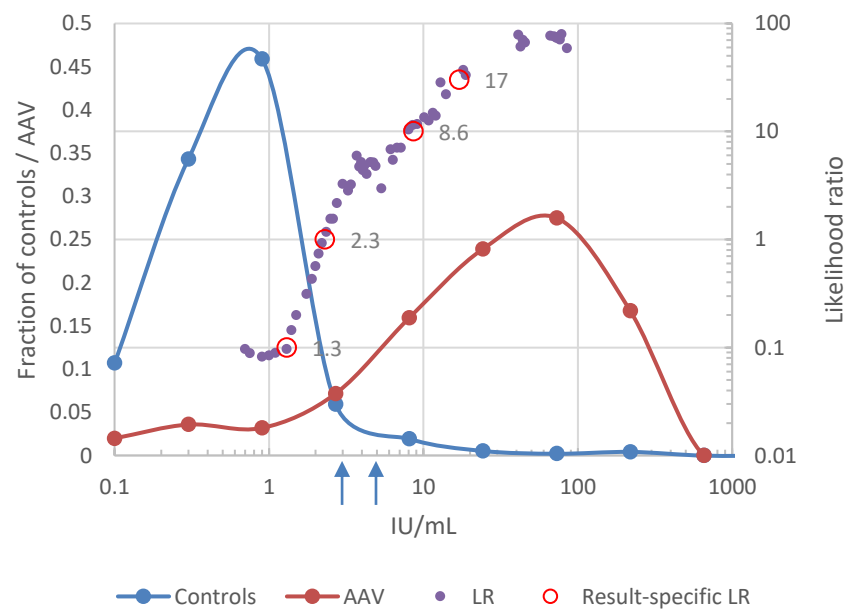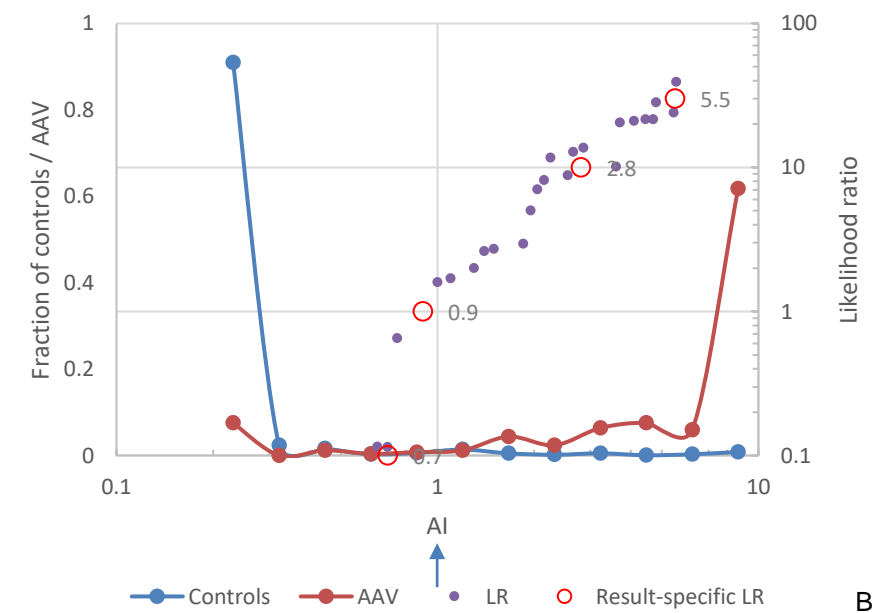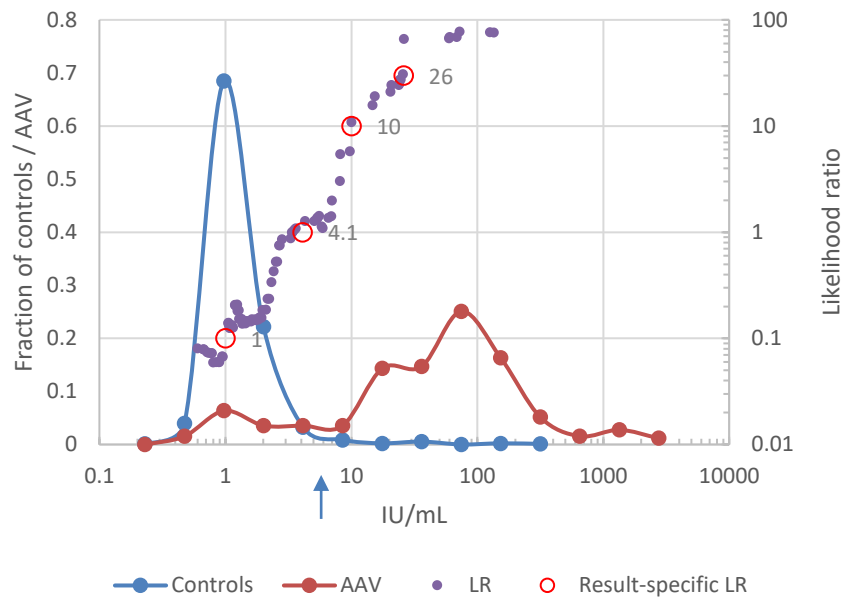
	Threshold associated with LR				cutoff
	0.1	1	10	30	
Units	6	13.8	35	82	20
CU	7	16.4	48.5	167	20
IU/mL	1.3	2.3	8.6	17	3/5*
AI		0.9	2.8	5.5	1
IU/mL	1	4.1	10	25.7	7
U/mL	1.8	6.1	13.8	46	10/5*
U/mL	3	15.1	48	114	20
IU/mL		4.7	10.7	23	5

* first value for PR3-ANCA, second value for MPO-ANCA

Bossuyt X. et al. CCLM 2021

		Fraction of AAV patients with results associated with LR				
		<0.1	0.1 -1	1-10	10-30	>30
Inova (ELISA - QUANTA Lite)	Units	0.072	0.040	0.096	0.267	0.526
Inova (CLIA - QUANTA Flash)	CU	0.088	0.012	0.124	0.194	0.578
Thermo Fisher (FEIA - EliA)	IU/mL	0.088	0.028	0.139	0.124	0.622
BioRad (MIA - Bioplex 2200)	AI			0.088	0.151	0.661
Svar Life Science (Capture ELISA)	IU/mL	0.036	0.108	0.024	0.183	0.649
Orgentec (ELISA)	U/mL	0.068	0.060	0.052	0.203	0.618
Euroimmune (ELISA)	U/mL	0.072	0.032	0.100	0.151	0.645
Medipan (CytoBead)	IU/mL			0.120	0.143	0.570

2019 pauci-immune vasculitis with acute renal insufficiency							
		PR3-ANCA			MPO-ANCA		
Euroimmun	n=5	LR >30	5		LR <0.1	5	
Inova Diagnostics - QuantaFlash	n=7	LR >30	7		LR <0.1	6	1 not reported
Inova Diagnostics - QuantaLite	n=3	LR >30	3		LR <0.1	3	
Svar Life Science	n=1	LR >30	1		LR <0.1		1 not reported
Thermo Fisher - EliA	n=43	LR >30	43		LR <0.1	42	1 not reported

Source: Sylvia Broeders – Sciensano, Belgium / M. Vercammen

Current immunoassays for ANCA classically apply a single cut-off point with a dichotomous interpretation (positive/negative).

Here we propose to employ test result-specific LRs to align test result interpretation across assays and manufacturers and to convey clinical information intrinsic to the antibody level.
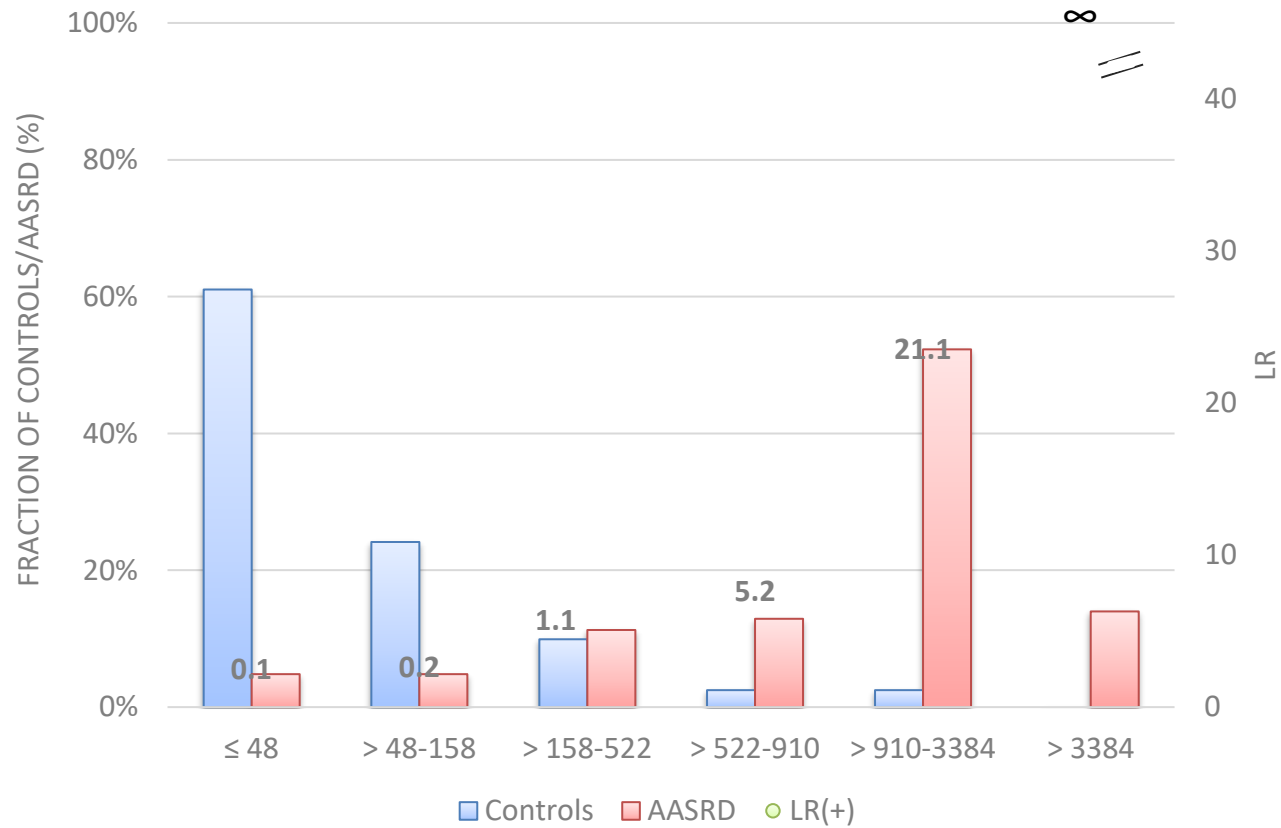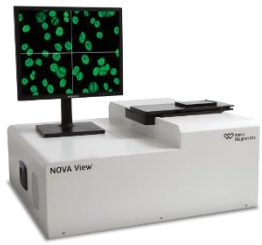
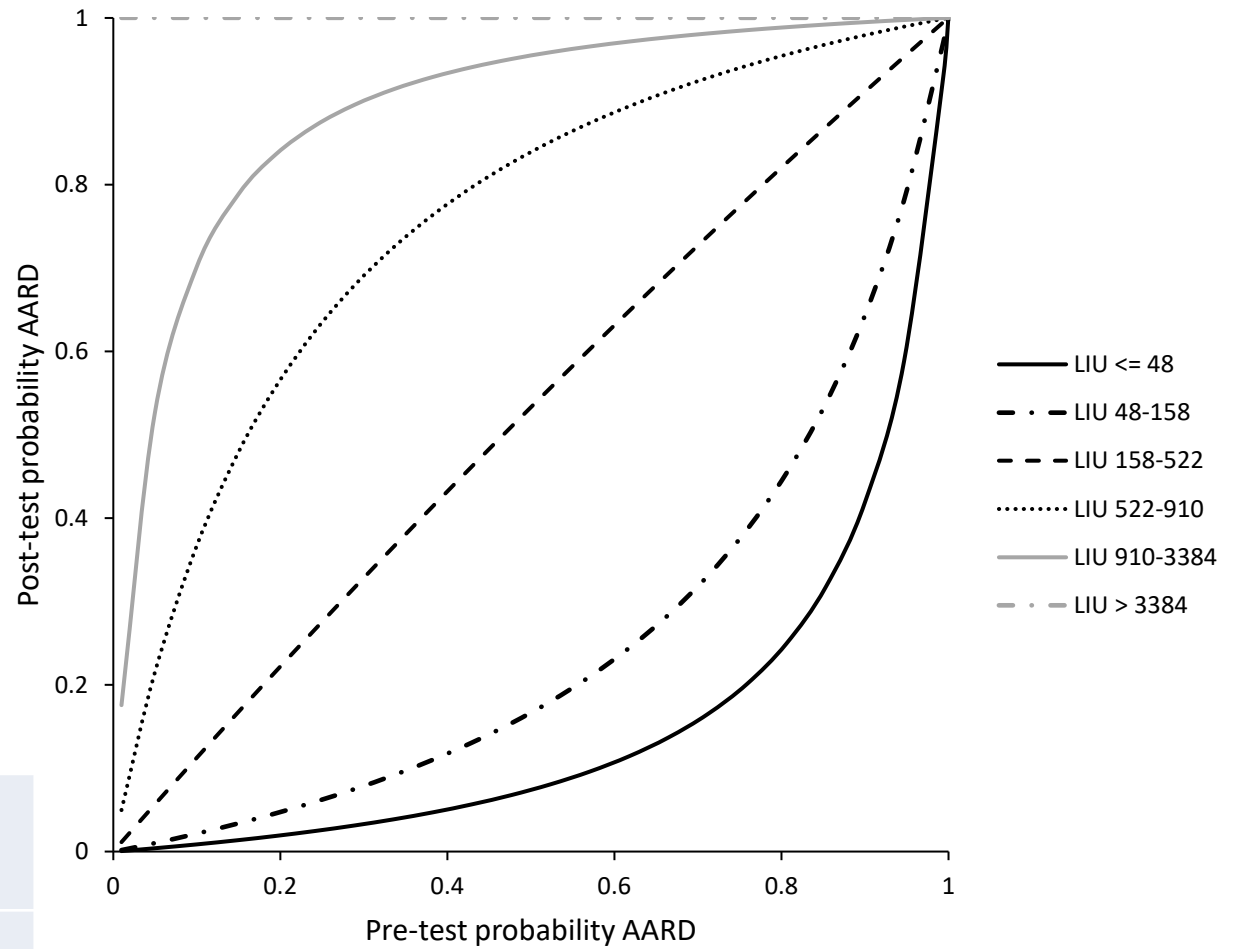# ANTINUCLEAR ANTIBODIES

ANA

ANA by IIF and solid phase assay

480 diagnostic samples from AARD patients

[SLE (n=119), primary SjS (n=65), SSc (n=220), IIM (n=50), MCTD (n=56)]

767 controls

[diseased controls (n=314), chronic fatigue syndrome (n=150) and blood donors (n=279)].

Claessens et al. Autoimmun Rev. 2018;17:533-540.
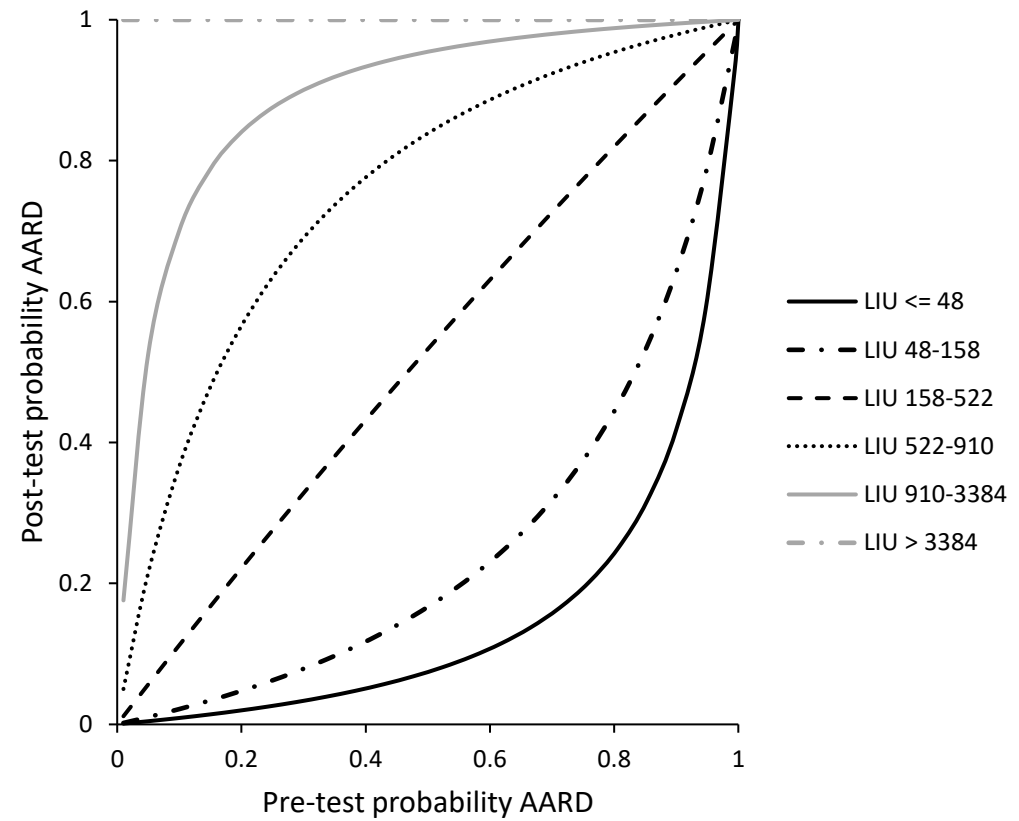
Pre-test probability

1%: a young women with hair loss and polyarthralgia

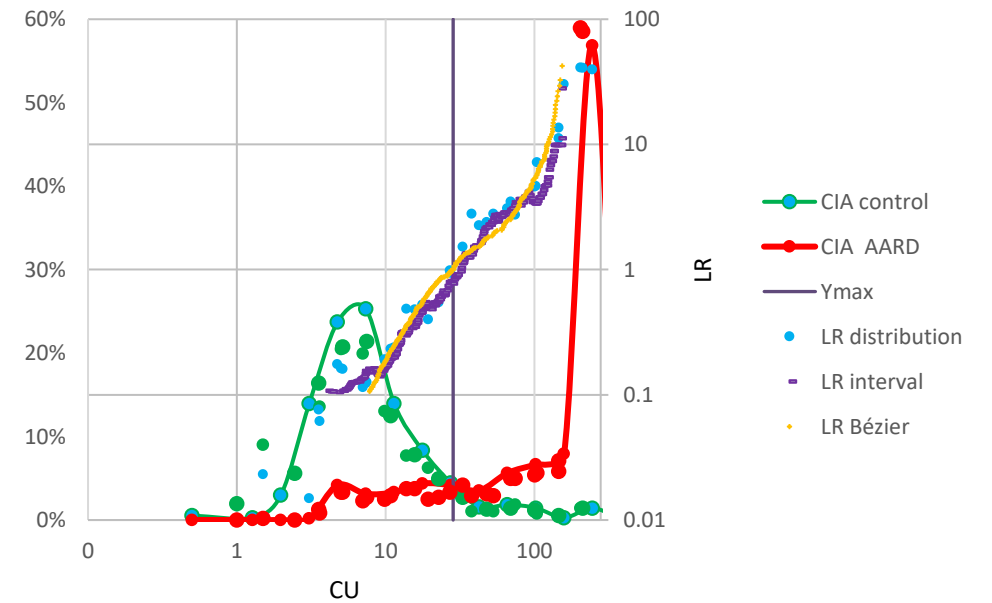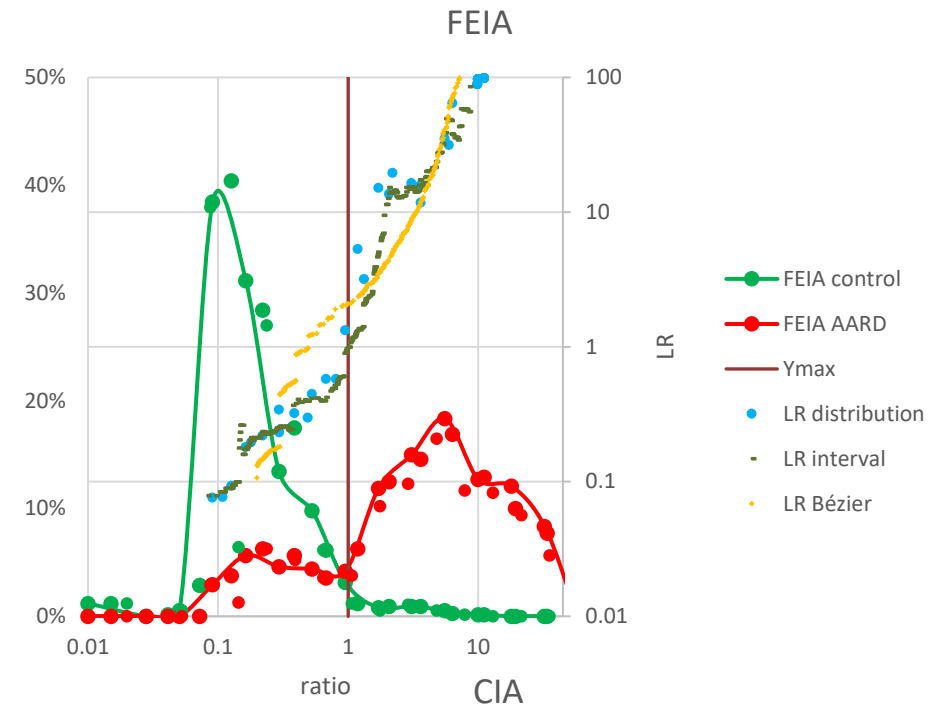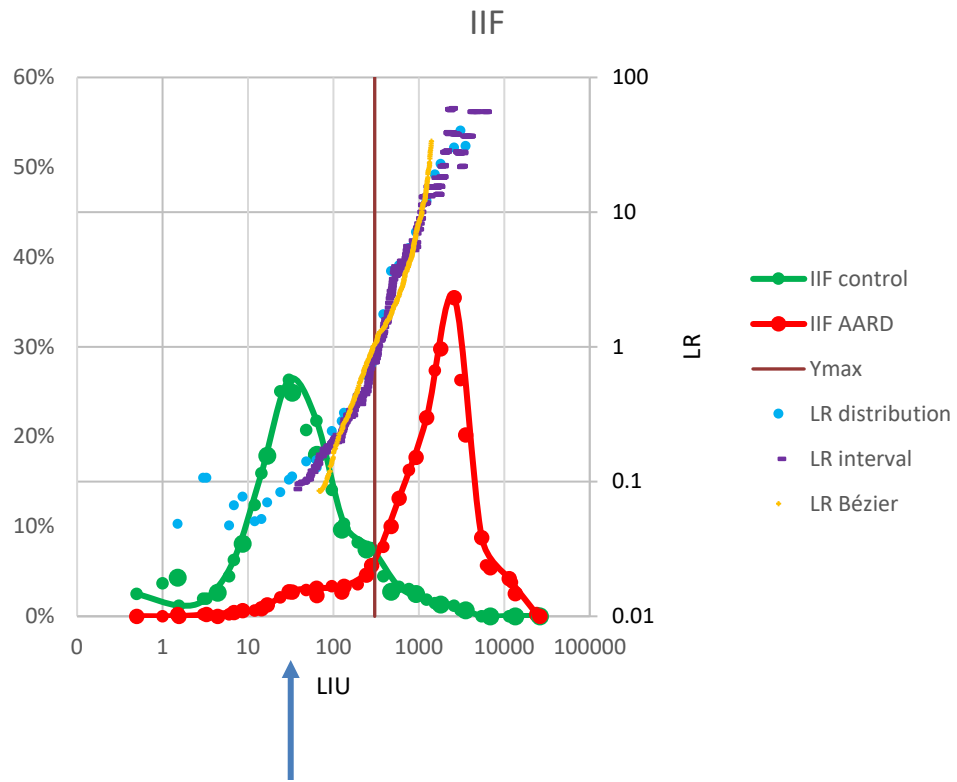**10%**: a young women wit photosensitivity and mild leucopenia (3000-3500/mm^3)

50%: a young women with photosensitivity , malar rash and symmetrical polyarthritis

Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. J Immunol Res. 2014;2014:315179.

IIF NovaView	FEIA - EliA	CLIA QUANTAFLASH
	dsDNA	dsDNA
	SSA/Ro 60 kDa	Ro60
	SSA/Ro 52 kDa	Ro52
	SSB/La	SSB
	U1-RNP (70, A, C)	Sm/RNP
	Sm	
	Scl-70	Scl-70
	Centromere B	Centromere B
	Fibrillarin	
		Ku
		Th/To
	RNA-Pol III	RNA Pol III
	PM-Scl	PM-Scl
	Jo-1	Jo-1
	Mi-2	Mi-2
	Rib-P	Rib-P
	PCNA	PCNA

B

0.8 (0.2-2.6)
0,8% of AARD/1% of controls

7.3 (3.6-15)
8.6% of AARD/1,2% of controls

181 (59-563)
71% or AARD/0.39% of controls

0.06 (0.04-0.1)
3.9% of AARD/59,9% of controls

0.23 (0.16-0.32)
7.5% of AARD/32,7% of controls

1.8 (1.1-2.7)
8.1% of AARD/4.7% of controls

FEIA CTD screen (Ratio)

IIF NOVA View (LIU)

Legend: PM/DM, SSc, SjS, SLE, MCTD, BD, CFS, DC, cutoff NV, NV 95% spec., cutoff FEIA

Bossuyt et al. Ann Rheum Dis 2019

IIF

FEIA

CIA

Bossuyt X et al. Autoimmun Rev.
2019;18:102386

	IIF NOVAView (LIU)	CIA CTD Screen Plus (CU)	FEIA EliA CTD Screen (ratio)
LR 0.1	30	7	0.1
LR 0.33	133	13	0.3
LR 1	307	27	0.9
LR 3	445	66	1
LR 10	1000	145	3
Company cutoff	48	20	1

Bossuyt X et al. Autoimmun Rev. 2019;18:102386

Leuchten et al. Performance of Antinuclear Antibodies for Classifying Systemic Lupus Erythematosus: A Systematic Literature Review and Meta-Regression of Diagnostic Data. Arthritis Care Res (Hoboken). 2018;70:428-438.

13.080 SLE patients and 7.539 controls: ANA 1:80 has a high sensitivity for SLE

Aringer M et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis*. 2019;78:1151-1159.

ANA (1:80) is entry criterion

Aringer M et al. European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) SLE classification criteria item performance. *Ann Rheum Dis*. 2021 Feb

ANA are a useful screening test, but not specific. The classification criteria should not be used as diagnostic criteria
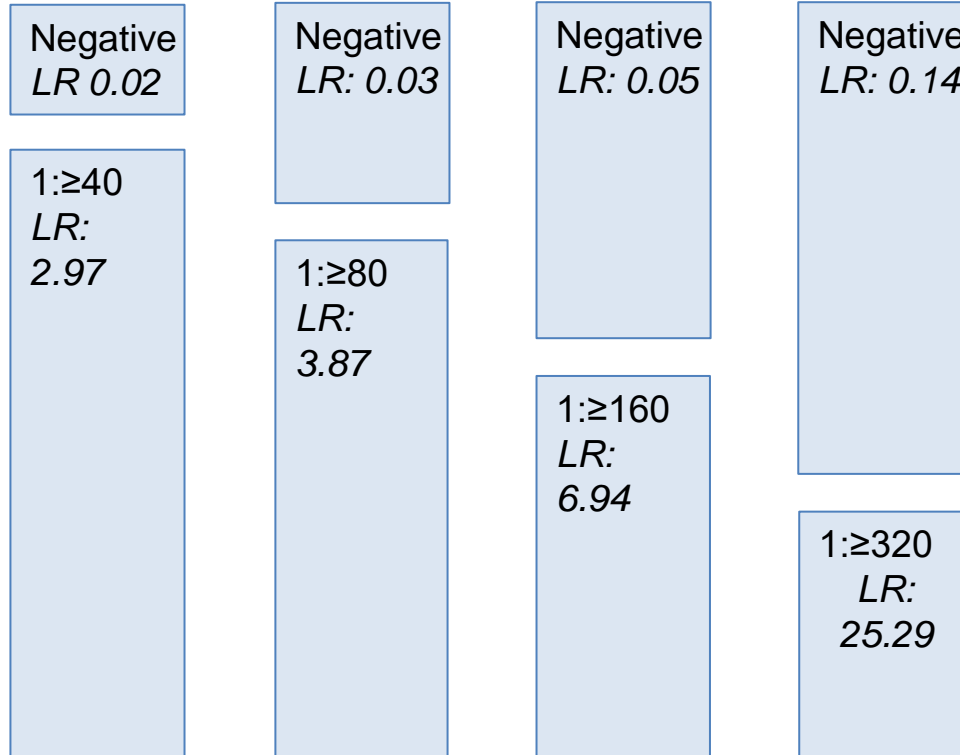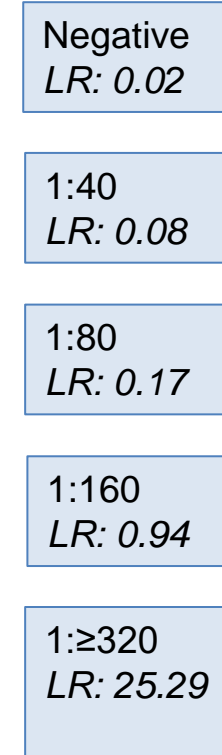
Conclusion

By defining test result-specific LRs, clinical interpretation of ANA test results can be improved and harmonized across assays and suppliers.

Rheumatoid arthritis
RF - ACPA

11 European hospitals – 7 countries:

Universitätsklinik für Innere Medizin III (Austria)

University Hospital Leuven (Belgium)
University Hospital Ghent (Belgium)
OLV Hospital Aalst (Belgium)

National Institute of Rheumatology and Physiotherapy Budapest (Hungary)

Centre Hospitalier de Luxembourg (Luxembourg)

University Medical Centre Ljubljana (Slovenia)

Sahlgrenska Academy at University of Göthenburg (Sweden)
University Hospital Linköping (Sweden)

University Hospital Basel (Switzerland)
Kantonspital Aarau (Switzerland).

NIBSC (National Institute for Biological Standards and Control), Hertfordshire, UK

Collaborators

Van Hoovels L.
Vander Cruyssen B.
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Eszter N.
Pullerits R.
Čučnik S.
Dahle C.
Heijnen I.
Bernasconi L.
Benkhadra F.
Bogaert L.
Van Den Bremt S.
Van Liedekerke A.
Vanheule G.
Robbrecht J.
Studholme L.
Wirth C.
Müller R.B.
Kyburz D.
Sjowall C.
Kastbom A.
Ješe R.
Jovancevic B.
Emese K.
Jacques P.
Aletahah D.
Verschueren P.
Steiner G.
Bossuyt X.

Industrial collaborations

RF

Thermo Fisher Scientific
Roche Diagnostics
Diazym
Cambridge Life Science
Orgentec
Abbott
Ortho-Clinical diagnostics
Beckman Coulter
Siemens

ACPA

Abbott
Bio-Rad Laboratories
Euroimmun
ids, UK
Orgentec
Roche Diagnostics
Siemens
Svar Life Science
Thermo Fisher Scientific

Rheumatoid arthritis

ACR 1987

1. Morning stiffness
2. Arthritis of 3 or more joint areas

3. Arthritis of hand joints
4. Symmetric arthritis

5. Rheumatoid nodules

6. Serum rheumatoid factor

7. Radiographic changes

ACR/EULAR 2010

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

B. Serology (at least 1 test result is needed for classification)††

Negative RF *and* negative ACPA	≤ ULN	0
Low-positive RF *or* low-positive ACPA	> ULN but ≤ 3x ULN	2
High-positive RF *or* high-positive ACPA	> 3x ULN	3

C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡

Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1

D. Duration of symptoms§§

<6 weeks	0
≥6 weeks	1

≥ 6/10=RA

	RA patients *n=398*	Healthy controls *n=200*	Consecutive disease controls *n=656*	Cohorts of disease controls *n=217*
				OA (25)
				PsA (25)
				ReA (20)
				SPA (25)
				SLE (50)
				SS (48)
				GPA/MPA (24)

398 RA
1073 controls

	TF RF IgM	R RF	D RF	C RF IgM	O RF IgM	A RF	O RF	B RF	S RF
Unit	IU/mL	IU/mL	kIU/mL	U/mL	U/mL	IU/mL	IU/mL	IU/mL	IU/mL
Manufacturer's cut-off	5	14	20	15.3	20	30	12	14	14
Sensitivity (%)	62.3 (57.3-67.1)	64.3 (59.4-69.0)	54.5 (49.5-59.5)	53.3 (48.2-58.3)	**51.8 (46.7-56.8)**	**51.8 (46.7-56.8)**	**74.4 (69.8-78.6)**	65.8 (60.9-70.5)	68.2 (63.2-73.0)
Specificity (%)	89.8 (87.8-91.5)	89.8 (87.8-91.5)	**93.9 (92.3-95.3)**	91.7 (89.9-93.3)	91.7 (89.9-93.3)	**93.9 (92.2-95.2)**	**72.4 (69.6-75.1)**	88.4 (86.4-90.3)	86.5 (84.2-88.5)
LR +	6.1 (5.0-7.4)	6.3 (5.2-7.6)	9.0 (7.0-11.6)	6.4 (5.1-8.0)	6.2 (5.0-8.2)	**8.4 (6.5-10.8)**	**2.7 (3.4-3.0)**	5.7 (4.8-6.8)	5.0 (4.2-6.0)
LR -	0.4 (0.4-0.5)	0.4 (0.3-0.4)	0.5 (0.4-0.5)	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.4 (0.3-0.4)

|	TF ACPA	R ACPA	Sv ACPA	I ACPA	O ACPA	A ACPA	E ACPA	B ACPA	S ACPA
Unit	U/mL	U/mL	U/mL	AU/mL	U/mL	U/mL	RU/mL	U/ml	U/mL
Manufacturer's cut-off	10	17	25	5	20	5	5	3	5
Sensitivity	63.1 (58.1-67.8)	62.6 (57.6-67.3)	61.3 (56.3-66.1)	62.6 (57.6-67.3)	**57.8 (52.8-62.7)**	62.1 (57.1-66.8)	**64.6 (59.7-69.3)**	60.8 (55.5-65.8)	63.5 (58.3-68.5)
Specificity	**97.6 (96.5-98.4)**	96.7 (95.5-97.7)	97.4 (96.3-98.3)	96.4 (95.5-97.7)	**97.8 (96.7-98.6)**	97.7 (96.6-98.5)	**94.9 (93.4-96.1)**	96.8 (95.5-97.8)	97.3 (96.1-98.2)
LR +	**26.3 (17.7-38.3)**	19.2 (13.7-26.6)	23.5 (16.2-34.1)	19.2 (13.7-26.8)	25.8 (17.2-38.7)	26.6 (17.0-39.5)	**12.6 (9.6-16.5)**	18.9 (13.3-26.9)	23.4 (16.0-34.4)
LR -	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.4 (0.4-0.4)	0.4 (0.3-0.4)	0.4 (0.4-0.5)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.4 (0.4-0.5)	0.4 (0.4-0.5)
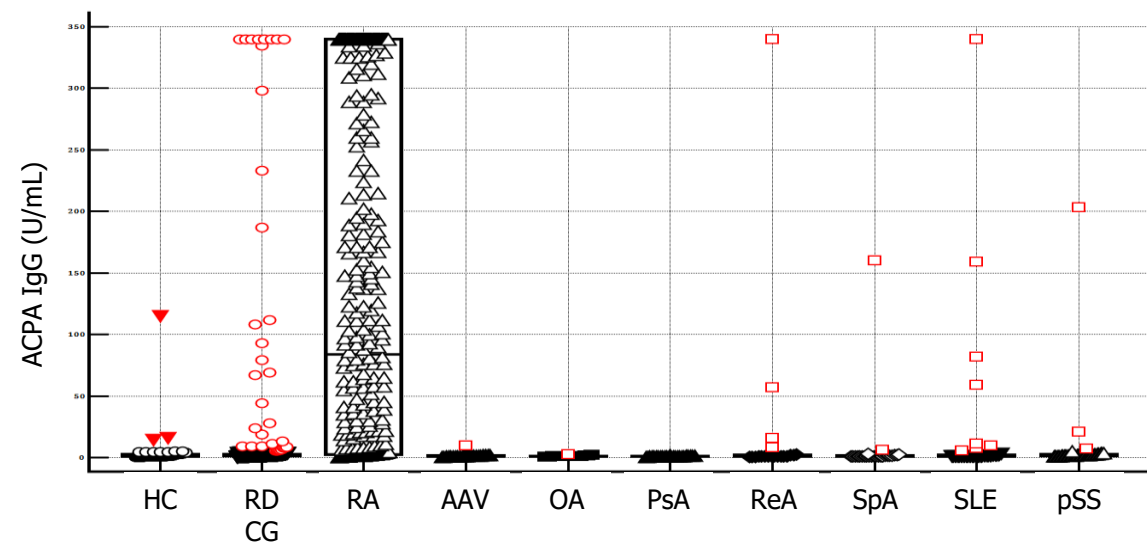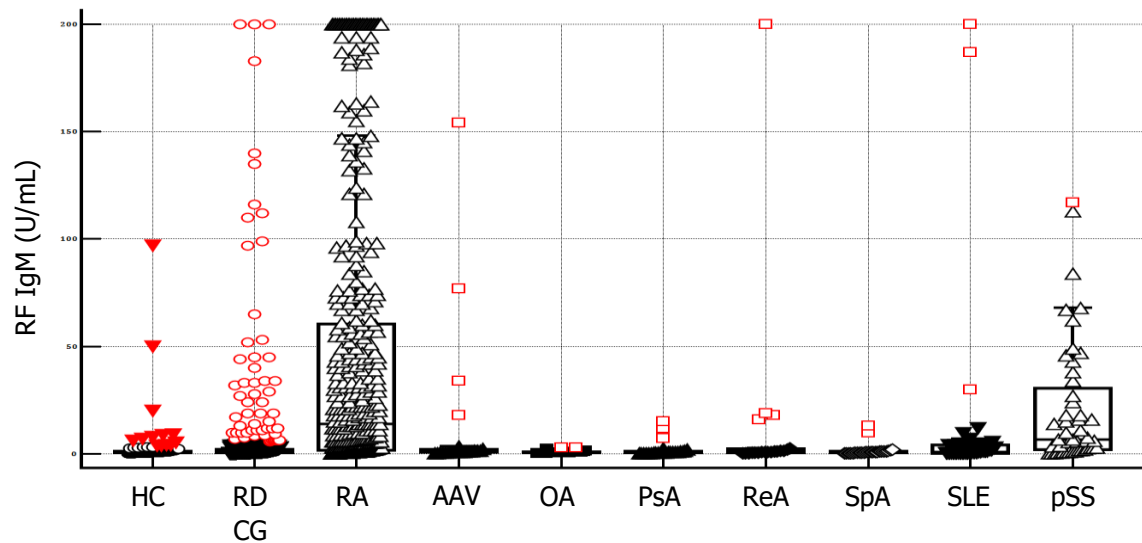
RF Beckman Coulter
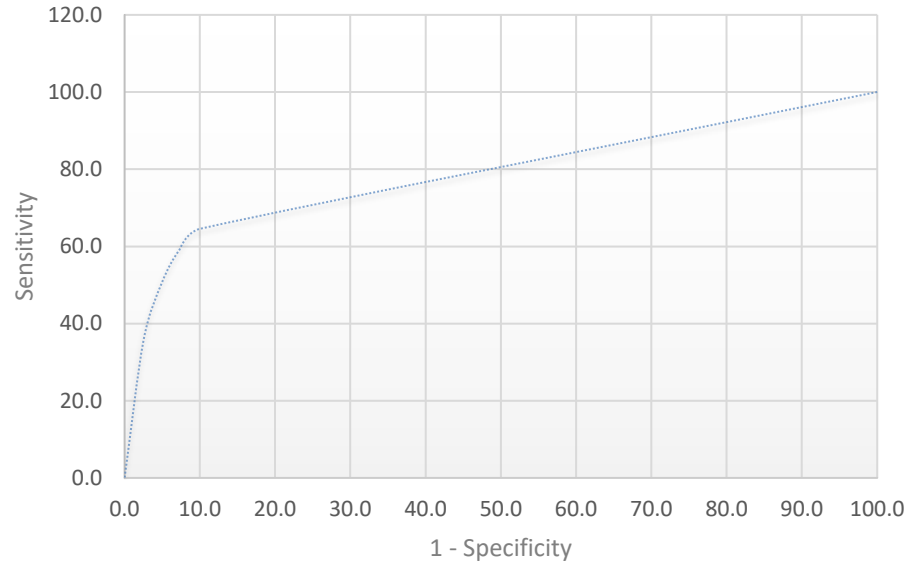
Cutoff company: 14 IU/mL

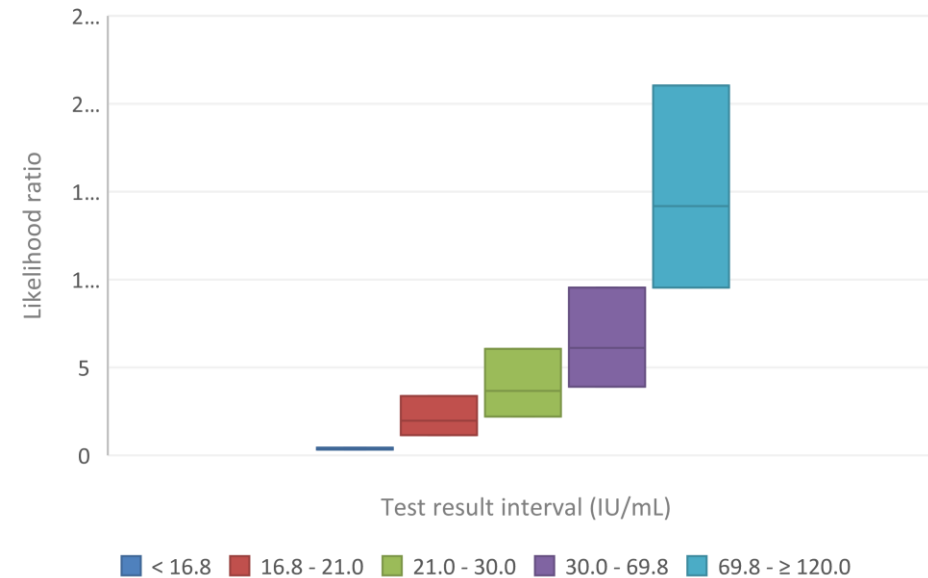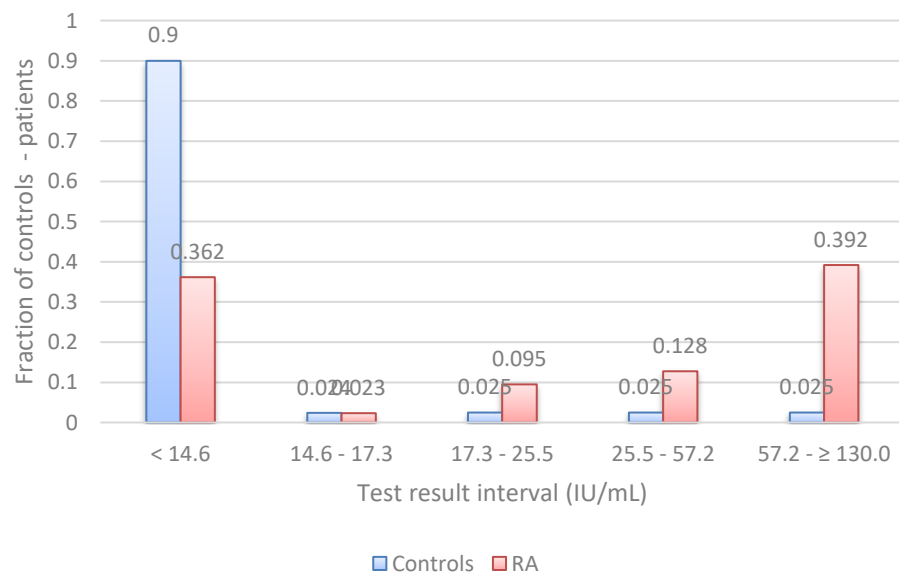| threshold	1-specificity	sensitivity	slope
	100,0	100,0	0,39
16,8	10,1	64,6	1,97
21	7,3	59,1	3,67
30	4,9	50,5	6,12
69,8	2,5	35,7	14,16
	0,0	0,0	

RF Beckman Coulter
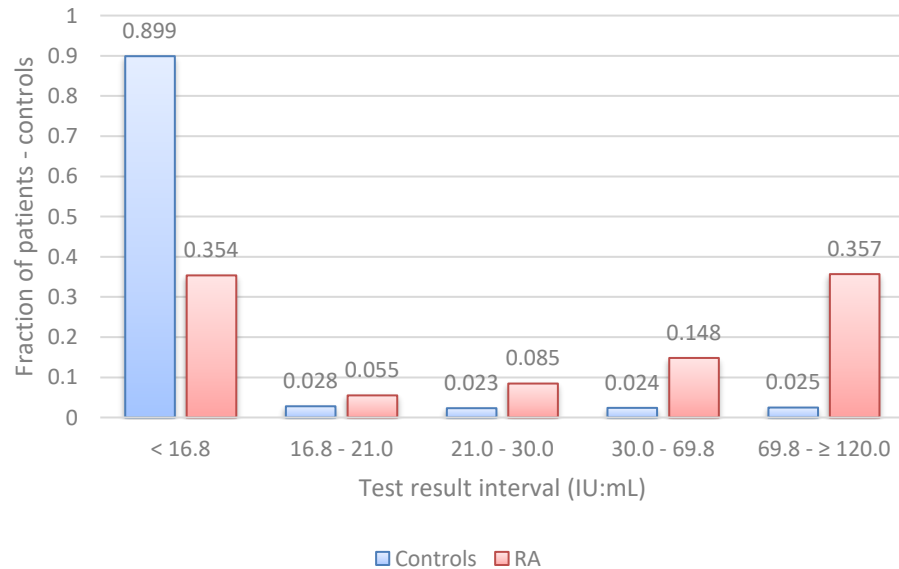
RF Beckman Coulter
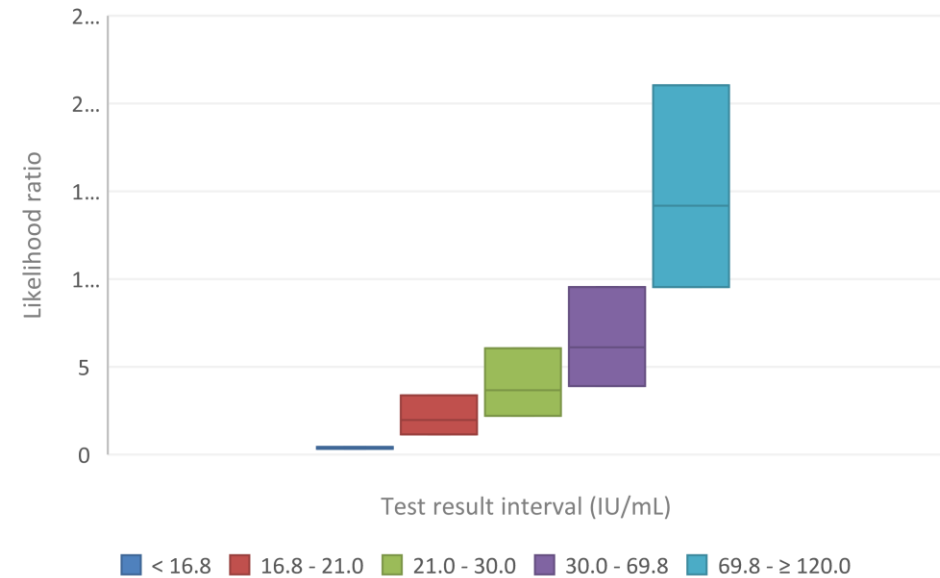
RF Roche
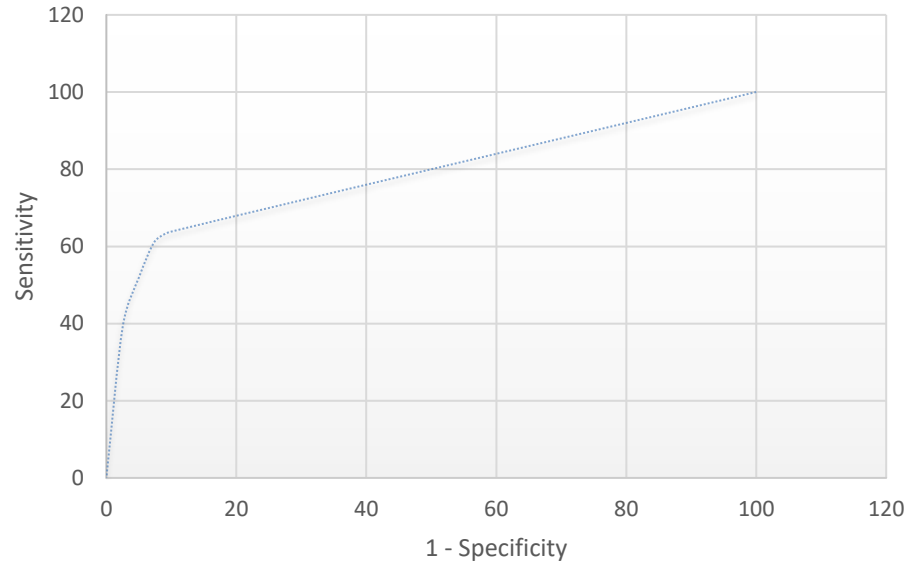
Cutoff company: 14 IU/mL

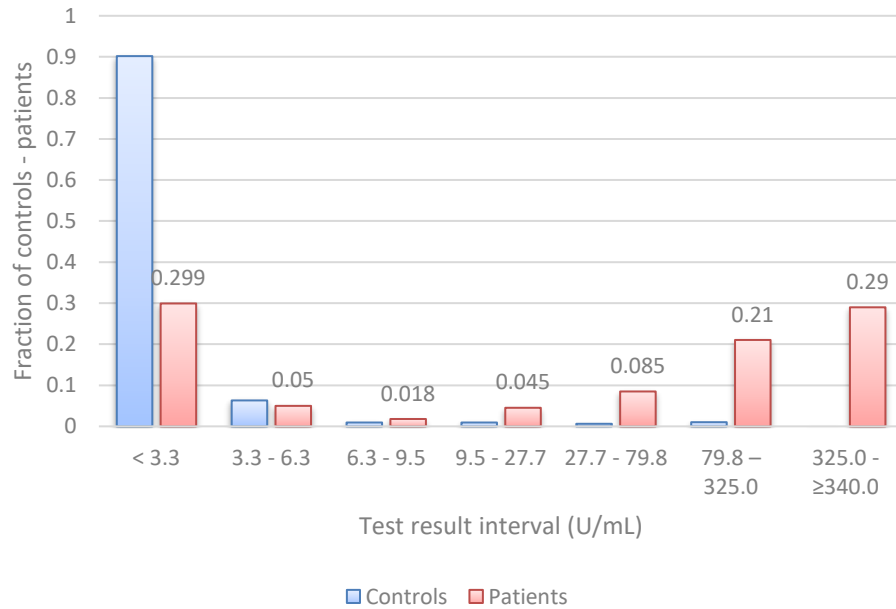| threshold	1 - Specificity	Sensitivity	slope
	100	100	0,40
14.6	10,0	63,8	0,93
17.3	7,6	61,6	3,79
25.5	5,0	52,0	5,10
57.2	2,5	39,2	15,56
	0	0	

RF Roche
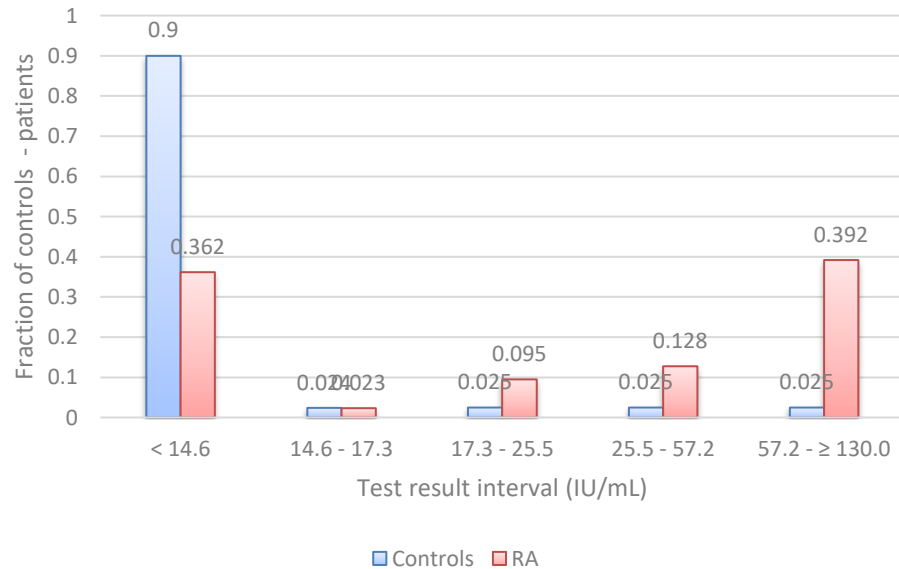
RF Roche

RF Beckman Coulter

Fraction of patients - controls vs Test result interval (IU:mL)

Test result interval	Controls	RA
< 16.8	0.899	0.354
16.8 - 21.0	0.028	0.055
21.0 - 30.0	0.023	0.085
30.0 - 69.8	0.024	0.148
69.8 - ≥ 120.0	0.025	0.357

RF Beckman Coulter

Likelihood ratio vs Test result interval (IU/mL)

Legend: < 16.8 | 16.8 - 21.0 | 21.0 - 30.0 | 30.0 - 69.8 | 69.8 - ≥ 120.0

RF Roche

Fraction of controls - patients vs Test result interval (IU/mL)

Test result interval	Controls	RA
< 14.6	0.9	0.362
14.6 - 17.3	0.024	0.023
17.3 - 25.5	0.025	0.095
25.5 - 57.2	0.025	0.128
57.2 - ≥ 130.0	0.025	0.392

RF Roche

Likelihood ratio vs Test result interval (IU/mL)

Legend: < 14.6 | 14.6 - 17.3 | 17.3 - 25.5 | 25.5 - 57.2 | 57.2 - ≥ 130.0

ACPA Thermo Fisher

Threshold	1 - Specificity	Sensitivity	slope
	100	100	
3.3	9,79	70,1	0,79
6.3	3,45	65,08	1,89
9.5	2,52	63,32	4,82
27.7	1,58	58,79	15,98
79.8	1,03	50	27,80
325.0	0,28	29,15	104,11
	0	0	

ACPA Thermo Fisher
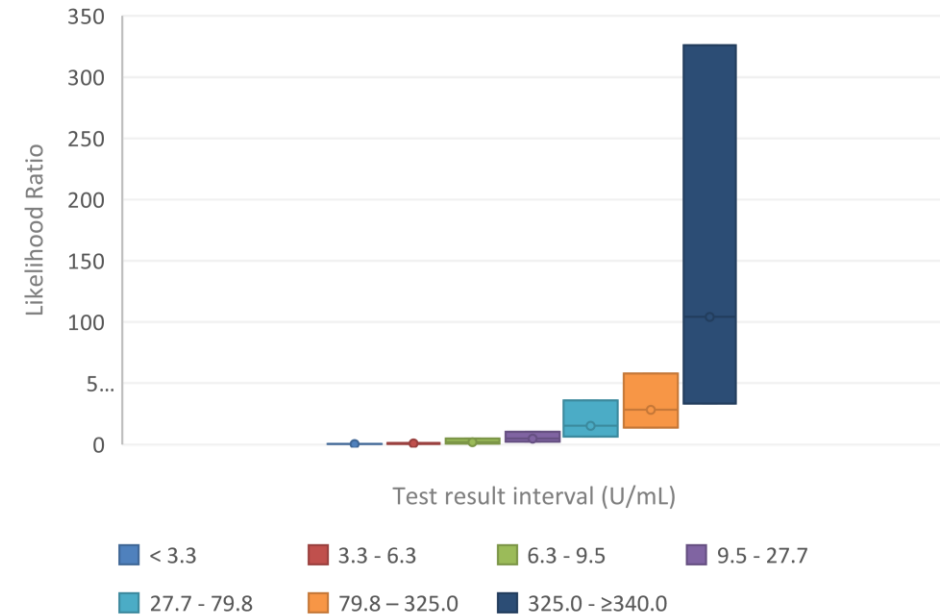
ACPA Thermo Fisher
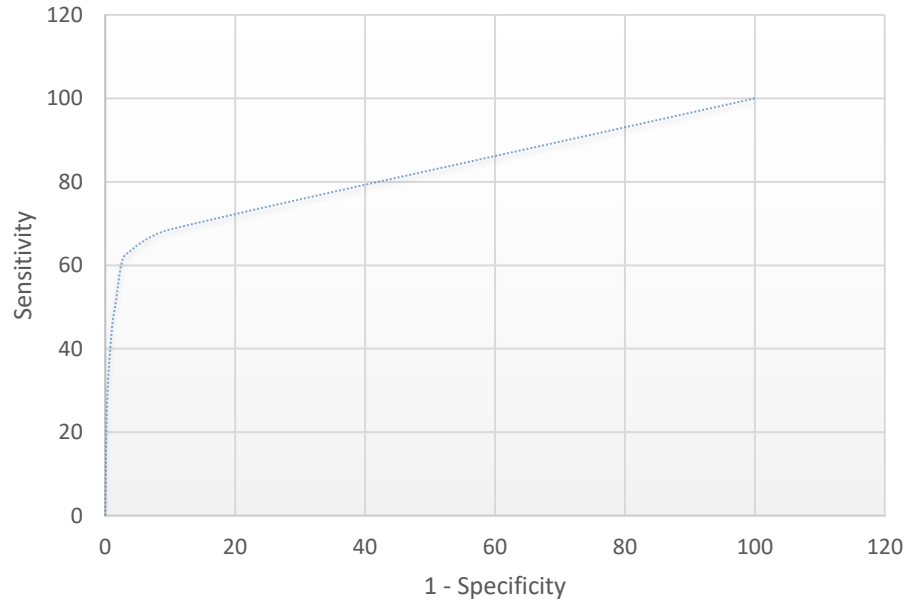
ACPA Orgentec

Threshold	1- Specificity	Sensitivity	Slope
	100	100	0,35
5.8	9,97	68,59	0,86
10.6	3,54	63,07	2,47
14.7	2,52	60,55	10,43
60.4	1,58	50,75	10,51
137.7	1,03	44,97	23,77
926.0	0,28	27,14	96,93
	0	0	

ACPA Orgentec
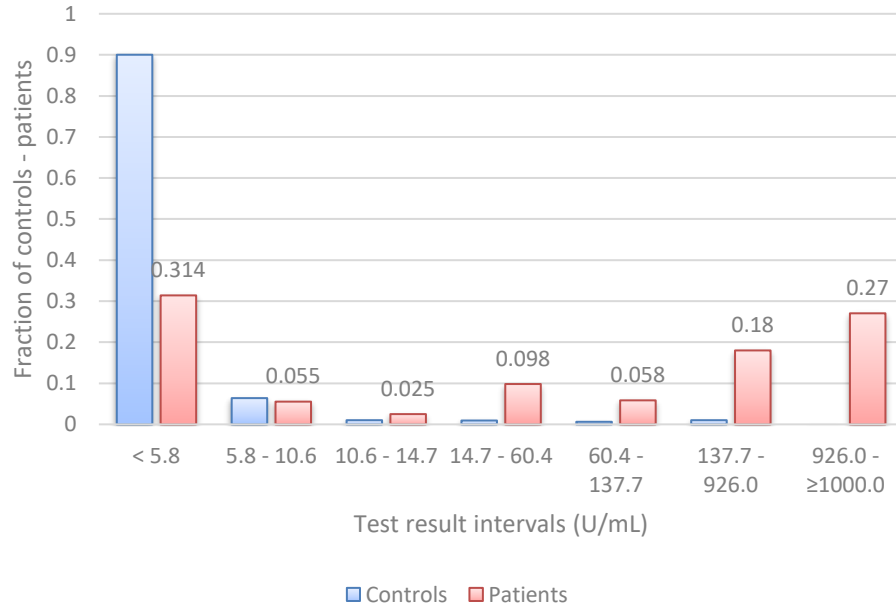
ACPA Orgentec
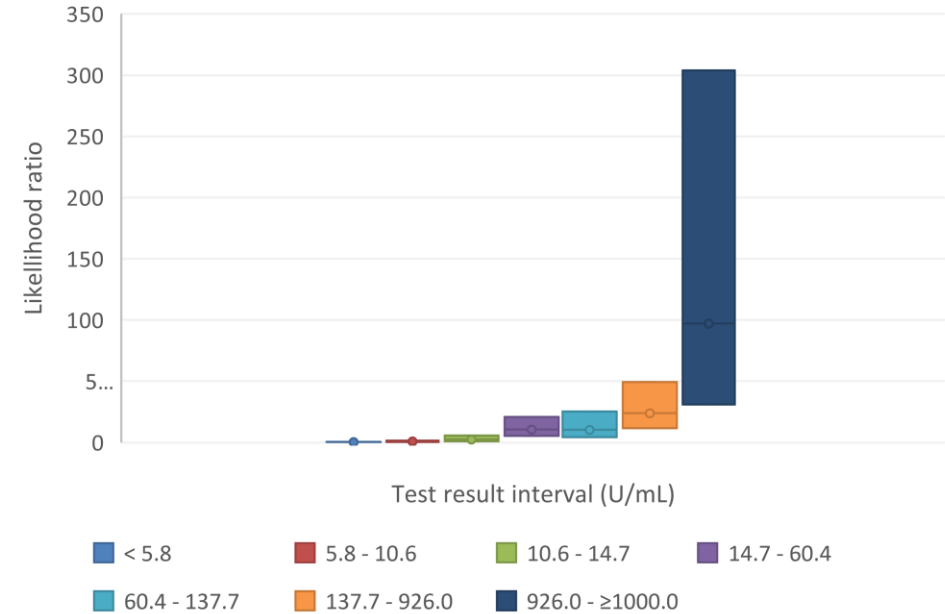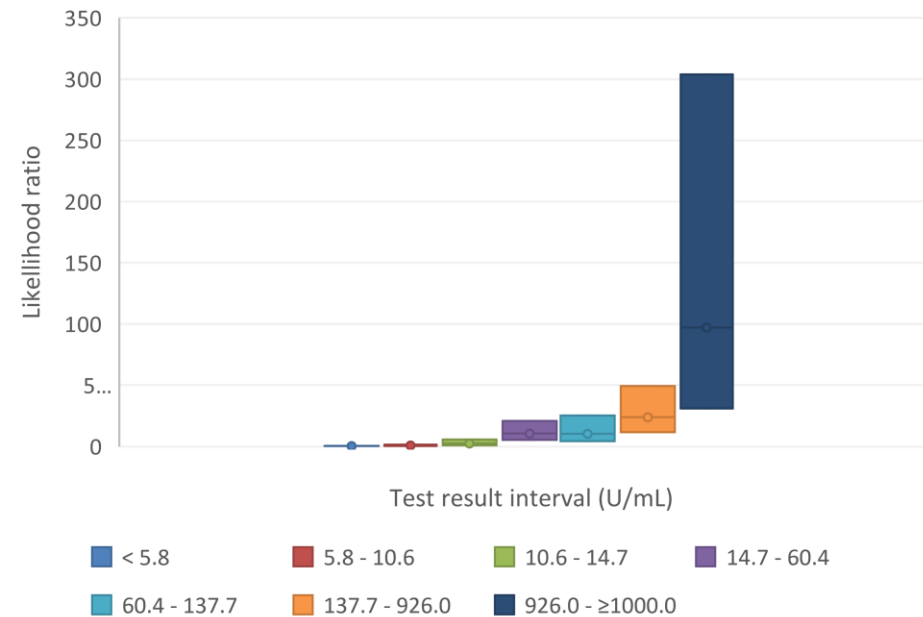
ACPA Thermo Fisher
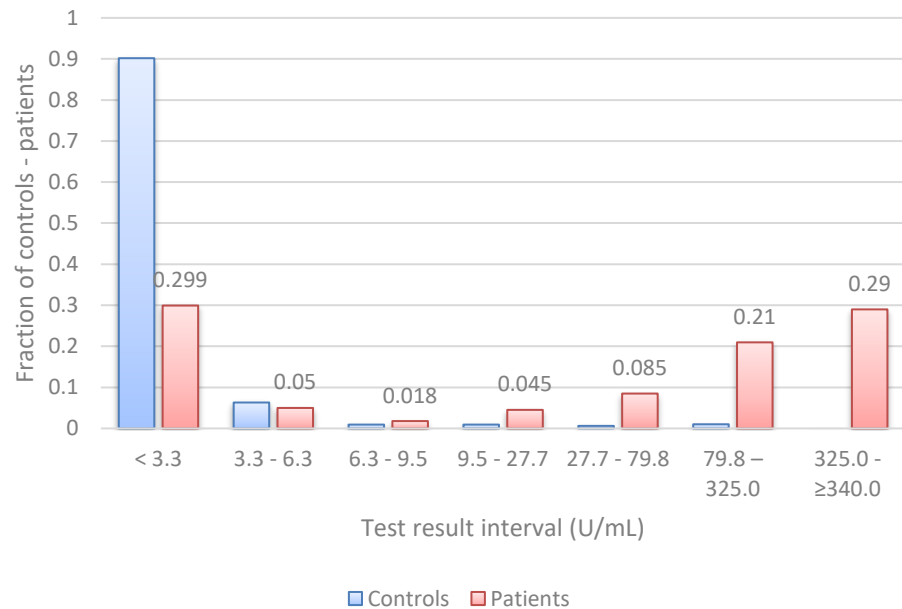
ACPA Thermo Fisher
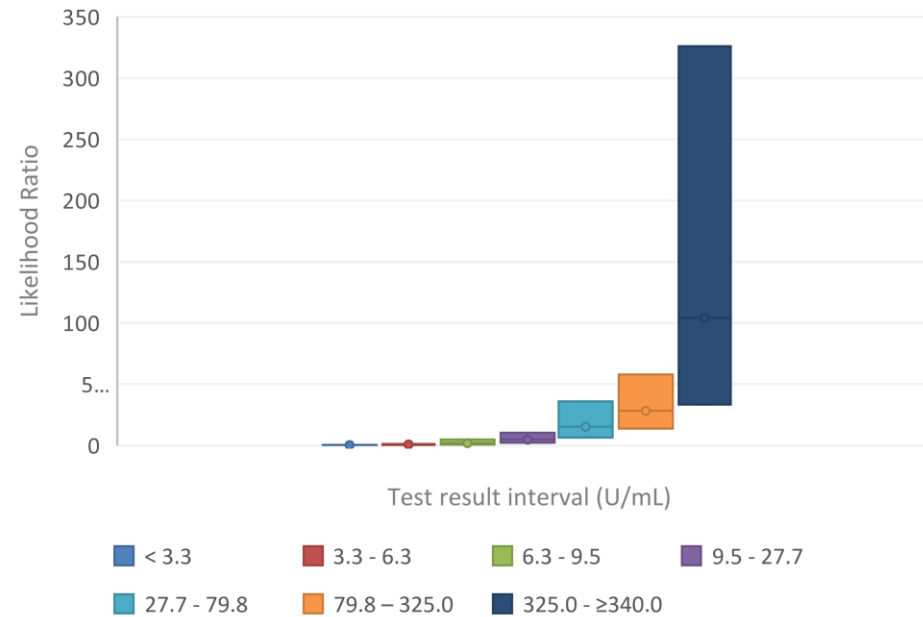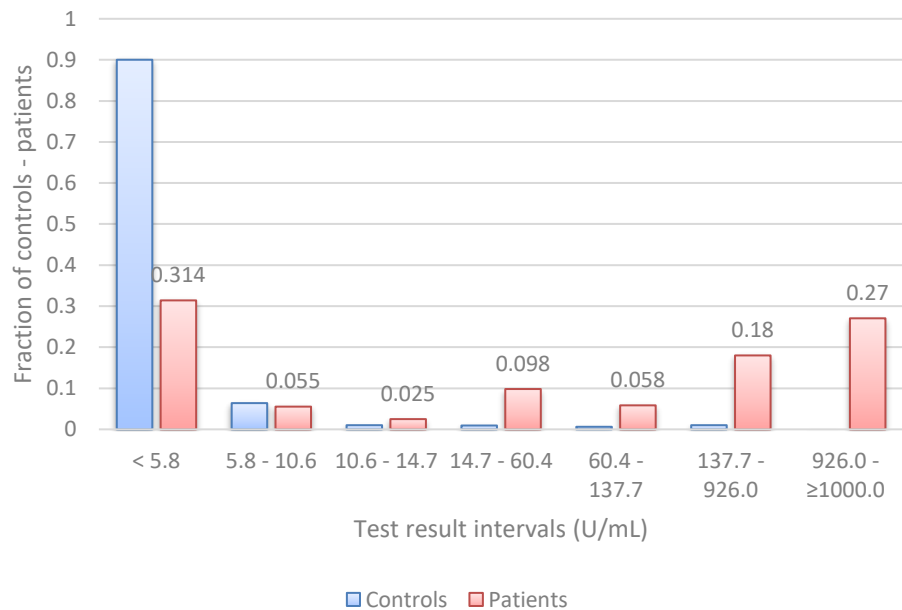
ACPA Orgentec

ACPA Orgentec

	Interval	Fraction of controls	Fraction of patients	LR	95% CI
TF IgM	< 5.0	0.897	0.377	0.42	0.37 - 0.48
	5.0 - 7.5	0.027	0.058	2.14	1.25 - 3.65
	7.5 - 15.0	0.027	0.085	3.16	1.95 - 5.12
	15.0 - 45.0	0.025	0.188	7.49	4.90 - 11.45
	45.0 - ≥ 200	0.023	0.291	12.51	8.25 - 18.97
TF RF IgA	< 10.0	0.900	0.467	0.52	0.47 - 0.58
	10.0 – 14.0	0.025	0.038	1.50	0.81 - 2.79
	14.0 – 27.0	0.026	0.131	5.01	3.21 - 7.81
	27.0 - 62.4	0.023	0.178	7.66	4.93 - 11.90
	62.4 - ≥ 214	0.025	0.186	7.39	4.83 - 11.31
R RF	< 14.6	0.900	0.362	0.40	0.35 - 0.46
	14.6 - 17.3	0.024	0.023	0.93	0.44 - 1.97
	17.3 - 25.5	0.025	0.095	3.79	2.35 - 6.13
	25.5 - 57.2	0.025	0.128	5.09	3.24 - 8.00
	57.2 - ≥ 130.0	0.025	0.392	15.58	10.53 - 23.05
D RF	< 10.9	0.900	0.352	0.39	0.34 - 0.45
	10.9 - 16.9	0.024	0.070	2.90	1.72 - 4.89
	16.9 - 24.6	0.025	0.065	2.60	1.53 - 4.39
	24.6 - 57.9	0.025	0.158	6.29	4.07 - 9.73
	57.9 - ≥ 120.0	0.025	0.354	14.08	9.48 - 20.91
C RF IgM	< 12.3	0.899	0.427	0.47	0.42 - 0.53
	12.3 - 17.2	0.025	0.055	2.20	1.27 - 3.81
	17.2 - 26.5	0.025	0.058	2.30	1.33 - 3.96
	26.5 - 126.0	0.025	0.234	9.29	6.15 - 14.03
	126.0 - ≥ 600.0	0.025	0.226	8.99	5.94 - 13.60
C RF IgA	< 11.2	0.899	0.455	0.51	0.45 - 0.56
	11.2 - 17.4	0.024	0.078	3.21	1.93 - 5.34
	17.4 - 38.7	0.026	0.148	5.68	3.68 - 8.78
	38.7 - 85.3	0.025	0.121	4.79	3.03 - 7.57
	85.3 - ≥ 600.0	0.025	0.198	7.89	5.18 - 12.02

	Interval	Fraction of controls	Fraction of patients	LR	95% CI
O RF IgA	< 21.9	0.900	0.558	0.62	0.57 - 0.68
	21.9 - 34.0	0.025	0.065	2.60	1.53 - 4.39
	34.0 - 62.5	0.024	0.058	2.38	1.38 - 4.13
	62.5 - 226.7	0.025	0.146	5.79	3.72 - 9.01
	226.7 - ≥ 500.0	0.025	0.173	6.89	4.48 - 10.59
A RF	< 10.0	0.915	0.427	0.47	0.42 - 0.52
	10.0 - 24.6	0.009	0.035	3.77	1.69 - 8.43
	24.6 - 45.4	0.025	0.078	3.10	1.87 - 5.12
	45.4 - 107.6	0.025	0.188	7.49	4.90 - 11.45
	107.6 - ≥ 200	0.025	0.271	10.78	7.19 - 16.18
O RF	< 16.3	0.900	0.352	0.39	0.34 - 0.45
	16.3 - 19.7	0.025	0.048	1.90	1.07 - 3.37
	19.7 - 28.4	0.024	0.101	4.15	2.57 - 6.70
	28.4 - 60.0	0.025	0.126	4.99	3.17 - 7.86
	60.0 - ≥ 120.0	0.025	0.374	14.88	10.04 - 22.05
BC RF	< 16.8	0.899	0.354	0.39	0.34 - 0.45
	16.8 - 21.0	0.028	0.055	1.98	1.15 -3.39
	21.0 - 30.0	0.023	0.085	3.67	2.22 - 6.07
	30.0 - 69.8	0.024	0.148	6.12	3.91 - 9.56
	69.8 - ≥ 120.0	0.025	0.357	14.18	9.55 - 21.05
S RF	< 18.0	0.903	0.362	0.40	0.35 - 0.46
	18.0 - 22.0	0.024	0.047	1.95	1.06 - 3.59
	22.0 - 32.0	0.025	0.091	3.63	2.19 -6.02
	32.0 - 75.1	0.023	0.138	5.98	3.71 - 9.66
	75.1 - ≥ 90.0	0.025	0.362	14.42	9.56 - 21.74

	Interval	Fraction of controls	Fraction of patients	LR	95% CI
TF ACPA	< 3.3	0.902	0.299	0.33	0.29 - 0.39
	3.3 - 6.3	0.063	0.050	0.79	0.49 - 1.29
	6.3 - 9.5	0.009	0.018	1.89	0.72 - 4.92
	9.5 - 27.7	0.009	0.045	4.85	2.26 - 10.42
	27.7 - 79.8	0.006	0.085	15.28	6.46 - 36.1
	79.8 − 325.0	0.010	0.210	28.31	13.83 - 57.93
	325.0 - ≥340.0	0.000	0.290	104.24	33.33 - 326.07
R ACPA	< 3.5	0.955	0.362	0.38	0.33 - 0.43
	3.5 - 11.1	0.009	0.005	0.54	0.12 - 2.45
	11.1 - 36.1	0.010	0.025	2.45	1.05 - 5.73
	36.1 - 103.9	0.009	0.073	7.82	3.85 - 15.90
	103.9 - 230.4	0.006	0.121	21.57	9.30 - 50.00
	230.4 - ≥500.0	0.010	0.410	40.44	22.21 - 73.64
Sv ACPA	< 4.3	0.898	0.352	0.39	0.34 - 0.45
	4.3 - 14.1	0.066	0.030	0.46	0.25 - 0.83
	14.1 - 26.6	0.010	0.005	0.49	0.11 - 2.20
	26.6 - 83.8	0.009	0.048	5.12	2.40 - 10.92
	83.8 - 147.8	0.006	0.065	11.68	4.85 - 28.17
	147.8 - 398.0	0.010	0.230	30.33	14.85 - 61.93
	398.0 - ≥ 3200.0	0.000	0.270	97.95	31.29 - 306.68
I ACPA	< 2.0	0.904	0.281	0.31	0.27 - 0.37
	2.0 - 4.7	0.061	0.090	1.49	1.01 - 2.21
	4.7 - 7.7	0.010	0.010	0.98	0.31 - 3.06
	7.7 - 31.7	0.009	0.085	9.17	4.57 - 18.38
	31.7 - 43.4	0.006	0.035	6.29	2.43 - 16.26
	43.4 - 236.8	0.010	0.270	36.73	18.08 - 74.62
	236.8 - ≥320.0	0.000	0.220	79.98	25.46 - 251.27
O ACPA	< 5.8	0.900	0.314	0.35	0.30 - 0.40
	5.8 - 10.6	0.064	0.055	0.86	0.54 - 1.37
	10.6 - 14.7	0.010	0.025	2.45	1.05 - 5.73
	14.7 - 60.4	0.009	0.098	10.51	5.30 - 20.86
	60.4 - 137.7	0.006	0.058	10.33	4.24 - 25.19
	137.7 - 926.0	0.010	0.180	23.93	11.63 - 49.25
	926.0 - ≥1000.0	0.000	0.270	97.06	31.00 - 303.91

|	Interval	Fraction of controls	Fraction of patients	LR	95% CI
A ACPA	< 1.0	0.913	0.314	0.34	0.30 - 0.40
	1.0 - 2.70	0.051	0.043	0.83	0.49 - 1.42
	2.7 - 4.50	0.010	0.020	1.96	0.79 - 4.84
	4.5 - 25.4	0.009	0.113	12.13	6.17 - 23.84
	25.4 - 53.1	0.006	0.073	13.03	5.45 - 31.15
	53.1 - 188.2	0.007	0.190	24.94	12.13 - 51.25
	188.2 - ≥196.0	0.003	0.250	89.87	28.66 - 281.74
E ACPA	< 3.0	0.901	0.319	0.35	0.31 - 0.41
	3.0 - 6.9	0.063	0.055	0.87	0.55 - 1.39
	6.9 - 9.9	0.010	0.015	1.47	0.55 - 3.95
	9.9 - 21.0	0.010	0.035	3.43	1.57 - 7.50
	21.0 - 37.5	0.005	0.070	15.10	5.87 - 38.83
	37.5 - 187.7	0.010	0.280	37.07	18.25 - 75.28
	187.7 - ≥200.0	0.000	0.230	81.78	26.04 - 256.81
B ACPA	< 1.5	0.912	0.334	0.37	0.32 - 0.43
	1.5 - 2.9	0.054	0.055	1.02	0.62 - 1.68
	2.9 - 5.5	0.009	0.022	2.45	0.95 - 6.29
	5.5 - 35.1	0.010	0.094	9.35	4.67 - 18.74
	35.1 - 127.5	0.005	0.066	13.21	5.08 - 34.35
	127.5 - ≥300.0	0.010	0.430	42.65	22.76 - 79.92
S ACPA	< 1.1	0.904	0.273	0.30	0.26 - 0.36
	1.1 - 3.0	0.061	0.072	1.17	0.75 - 1.83
	3.0 - 5.6	0.010	0.022	2.20	0.88 - 5.53
	5.6 - 32.8	0.010	0.086	8.53	4.23 - 17.22
	32.8 - 57.8	0.005	0.069	13.76	5.31 - 35.67
	57.8 - ≥200.0	0.010	0.480	47.60	25.45 - 89.02

- Defining thresholds for antibody levels and assigning test result interval-specific LRs allows to align clinical interpretation of RF and ACPA assays.

- Serological weight factors for RA classification can be improved by taking into account the nature of the antibody (RF versus ACPA), the antibody level and combined positivity.
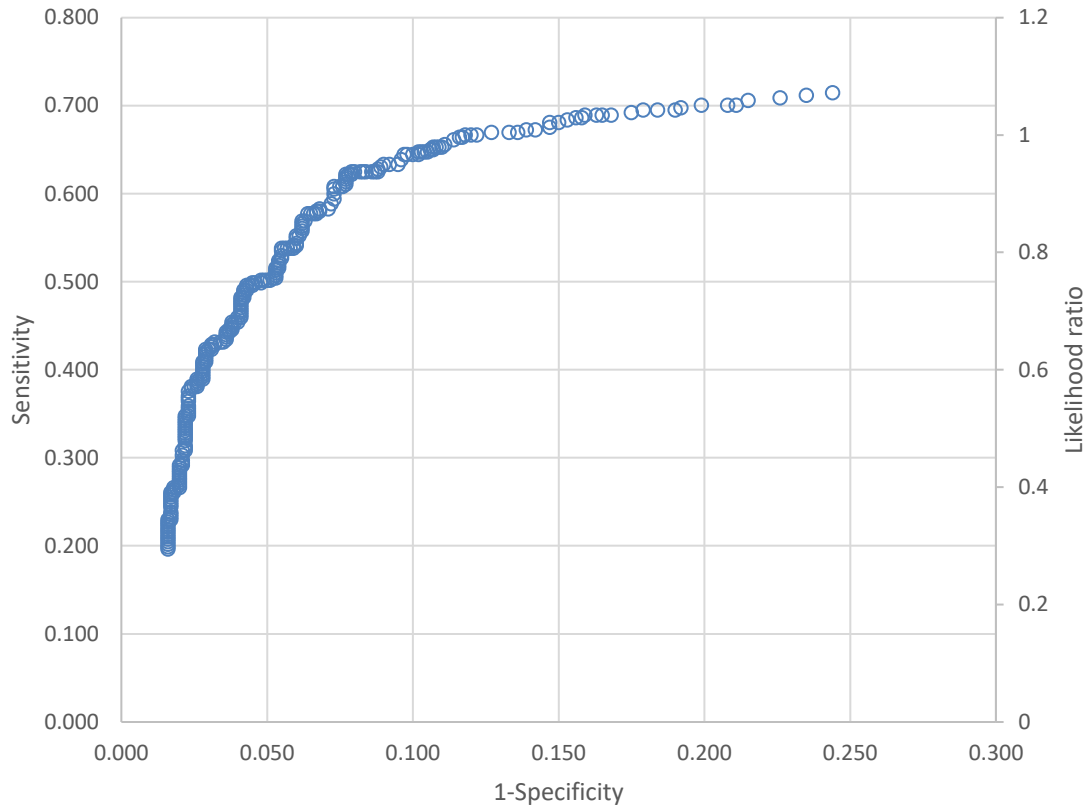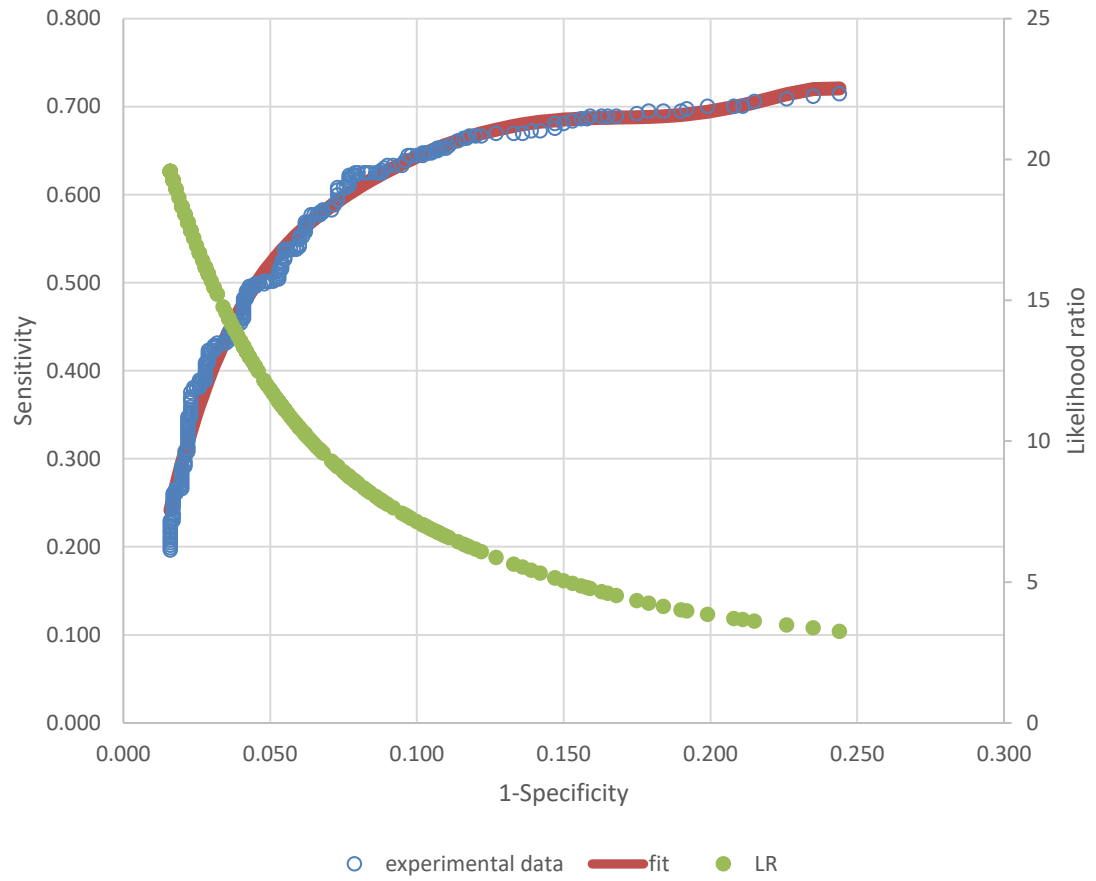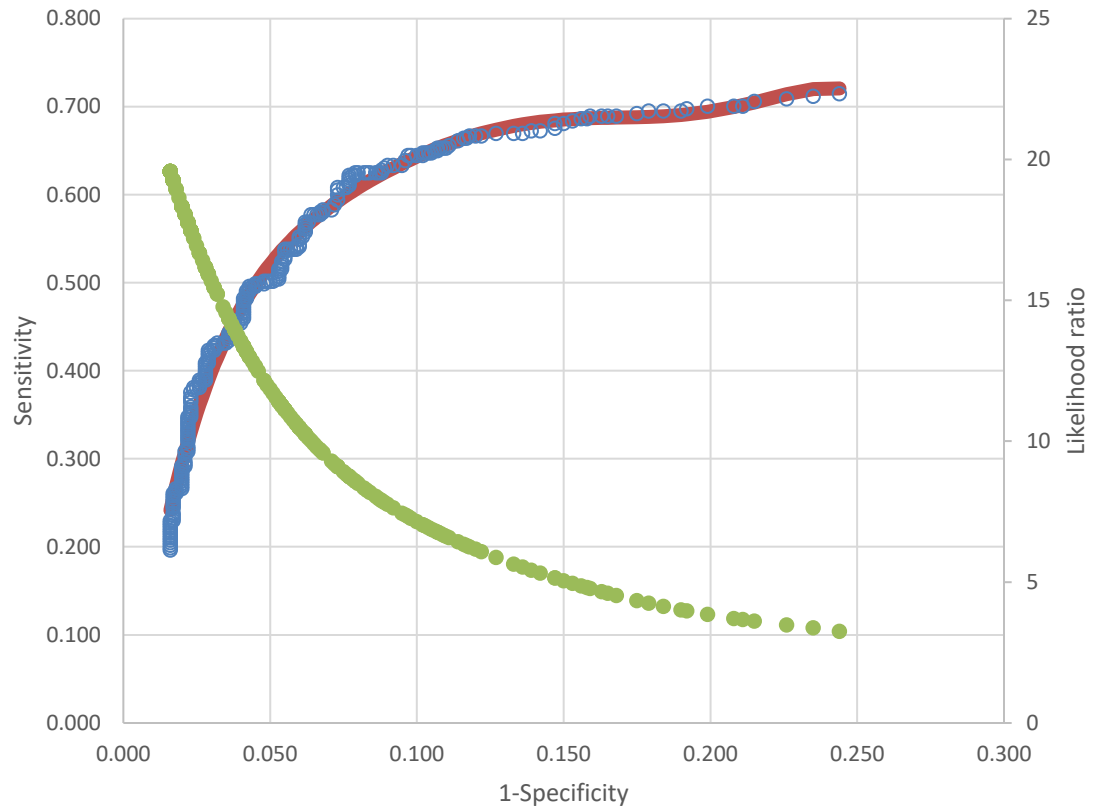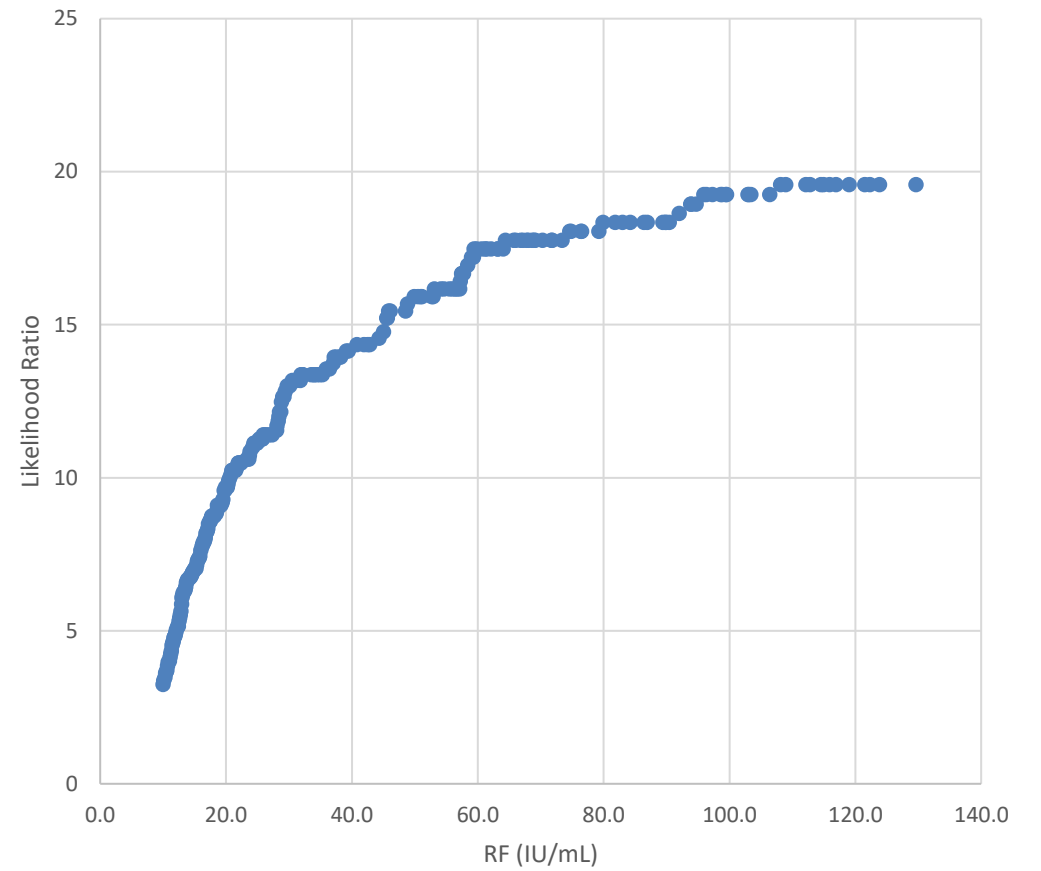
Roche RF
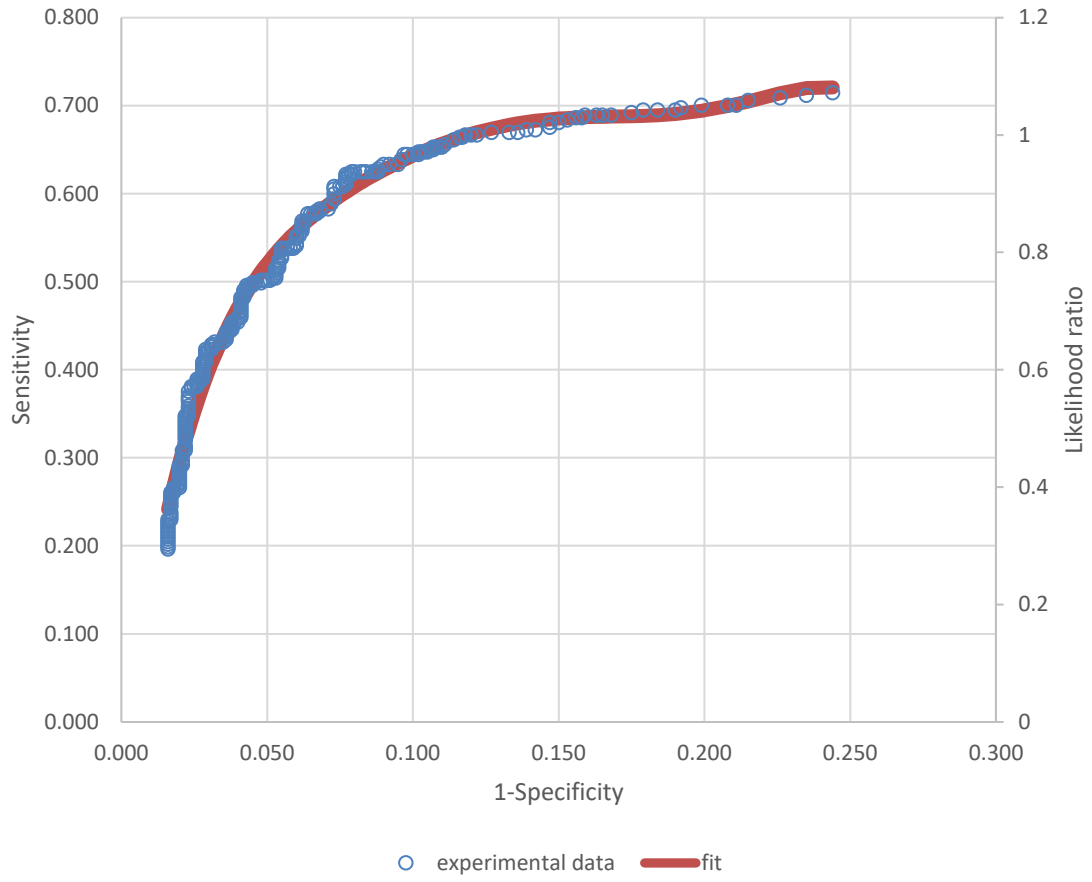
Roche RF

Roche RF
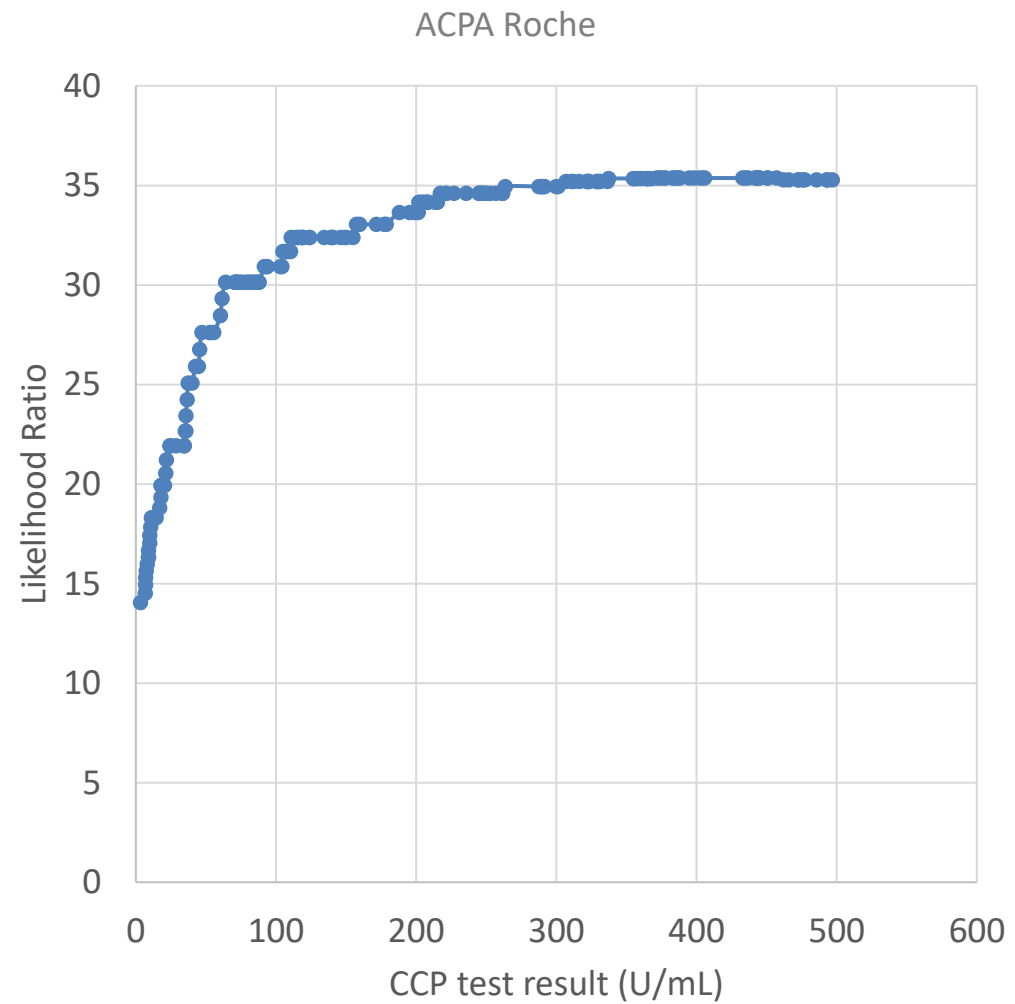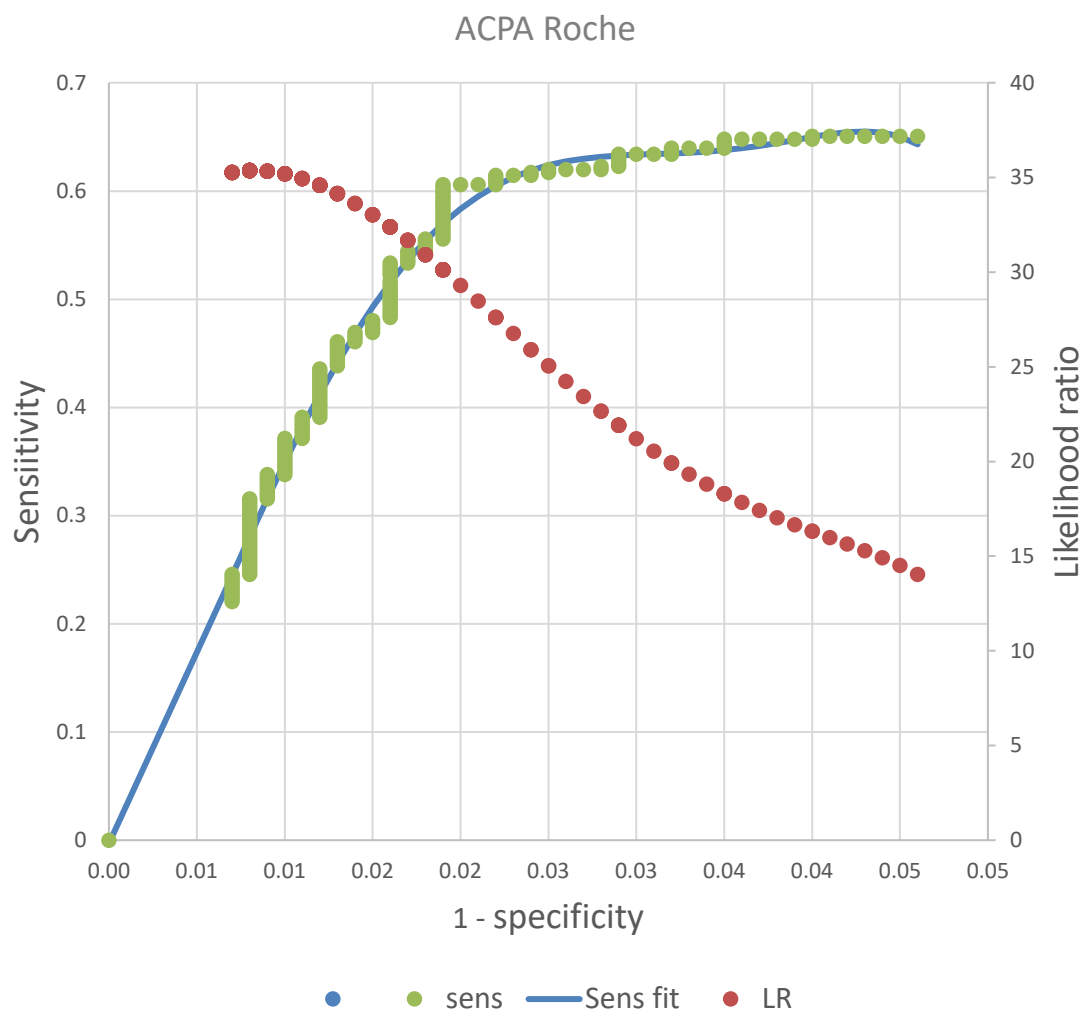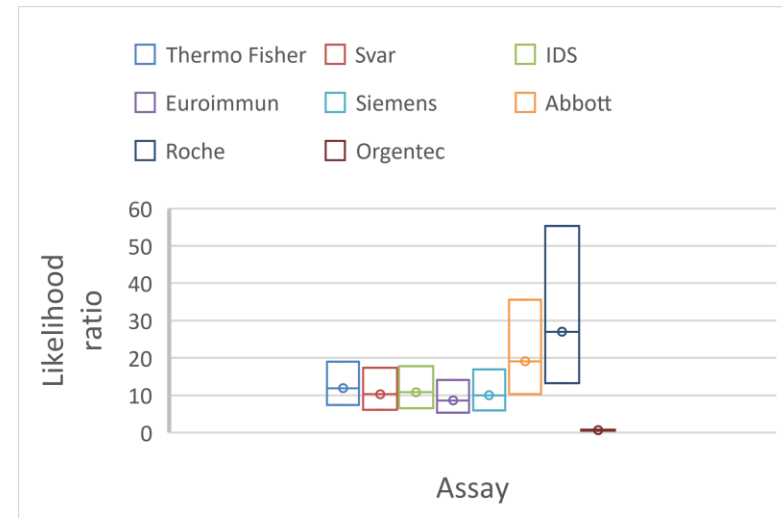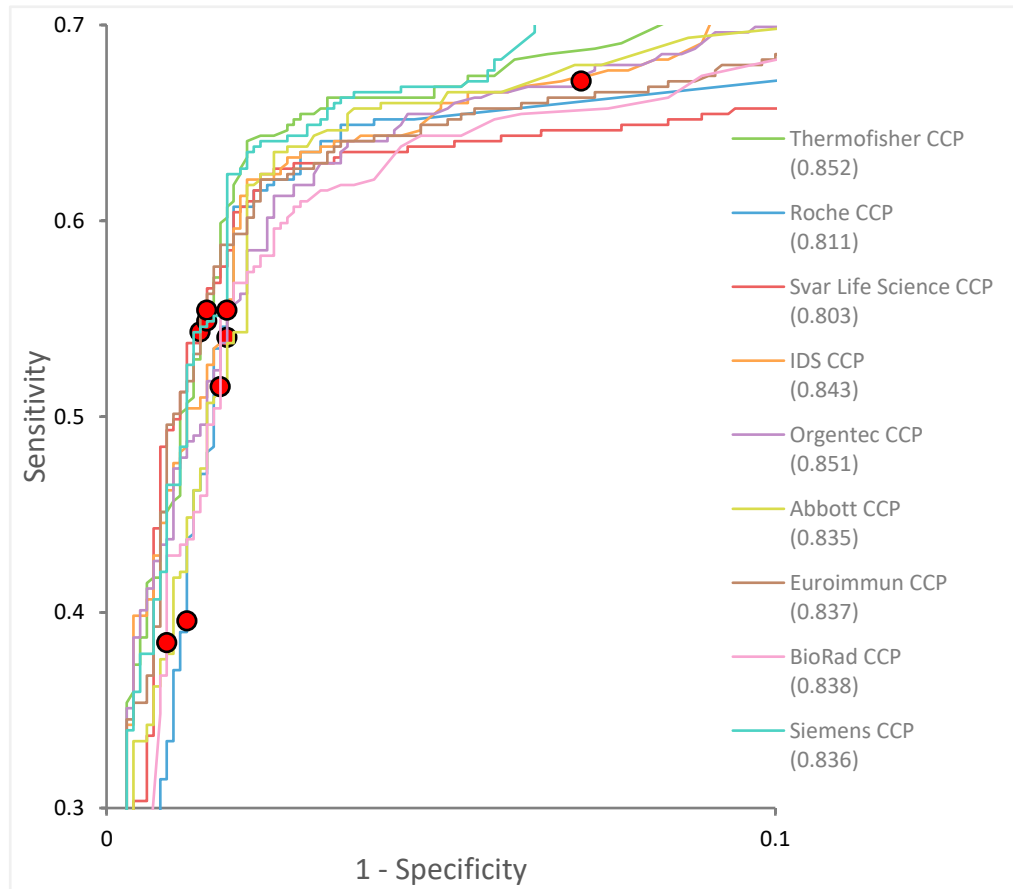
	LR	95% CI		LR	95% CI
Thermo Fisher	11.9	7.4 - 19	Abbott	19.1	10 - 36
Svar	10.3	6.1 - 17	Roche	27	13 - 55
IDS	10.8	6.5 - 18	Orgentec	0.61	0.5 - 0.7
Euroimmun	8.6	5.3 - 14			
Siemens	10	5.9 - 17			

Van Hoovels et al. ARD 2022

CELIAC DISEASE

Thermo Fisher EliA
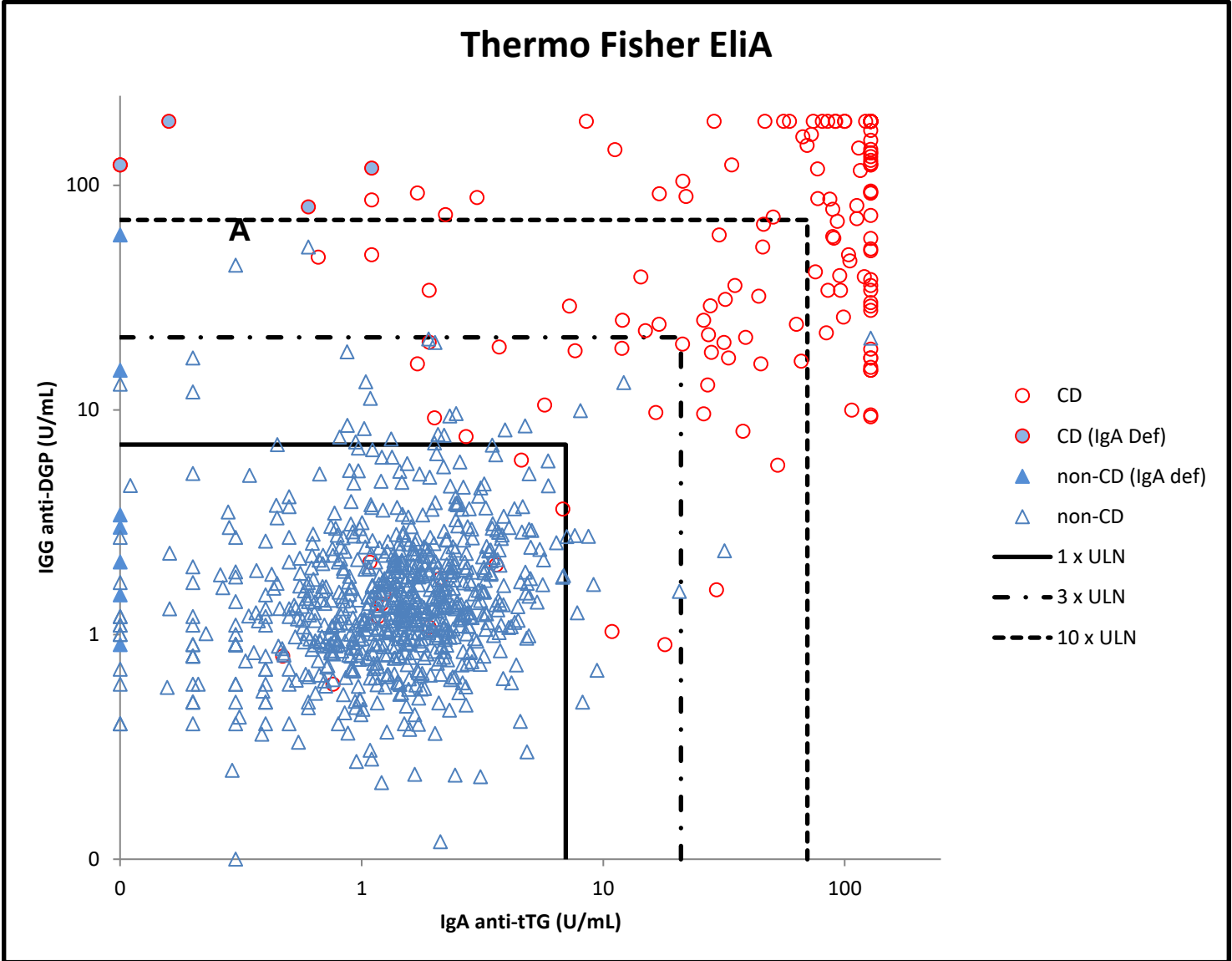
CD (n=156)
Diseased controls (n=974)

Oyaert et al.
Clin Chem Lab Med. 2015;53:1537-46

IgA anti-tTG

IgA anti-tTG

Post-test probability vs Pre-test probability

- <20 CU
- 20-60 CU
- >60-200 CU
- >200 CU

Oyaert et al.
Clin Chem Lab Med. 2015;53:1537-46

IgG DGP

Likelihood (left axis): non-CD, CD; Likelihood ratio (right axis): LR

- neg: 0.12
- 20-60: 9
- >60-200: 100
- >200

IgG DGP

Post-test probability vs Pre-test probability

Legend: <20 CU, 20-60 CU, >60-200 CU, >200 CU
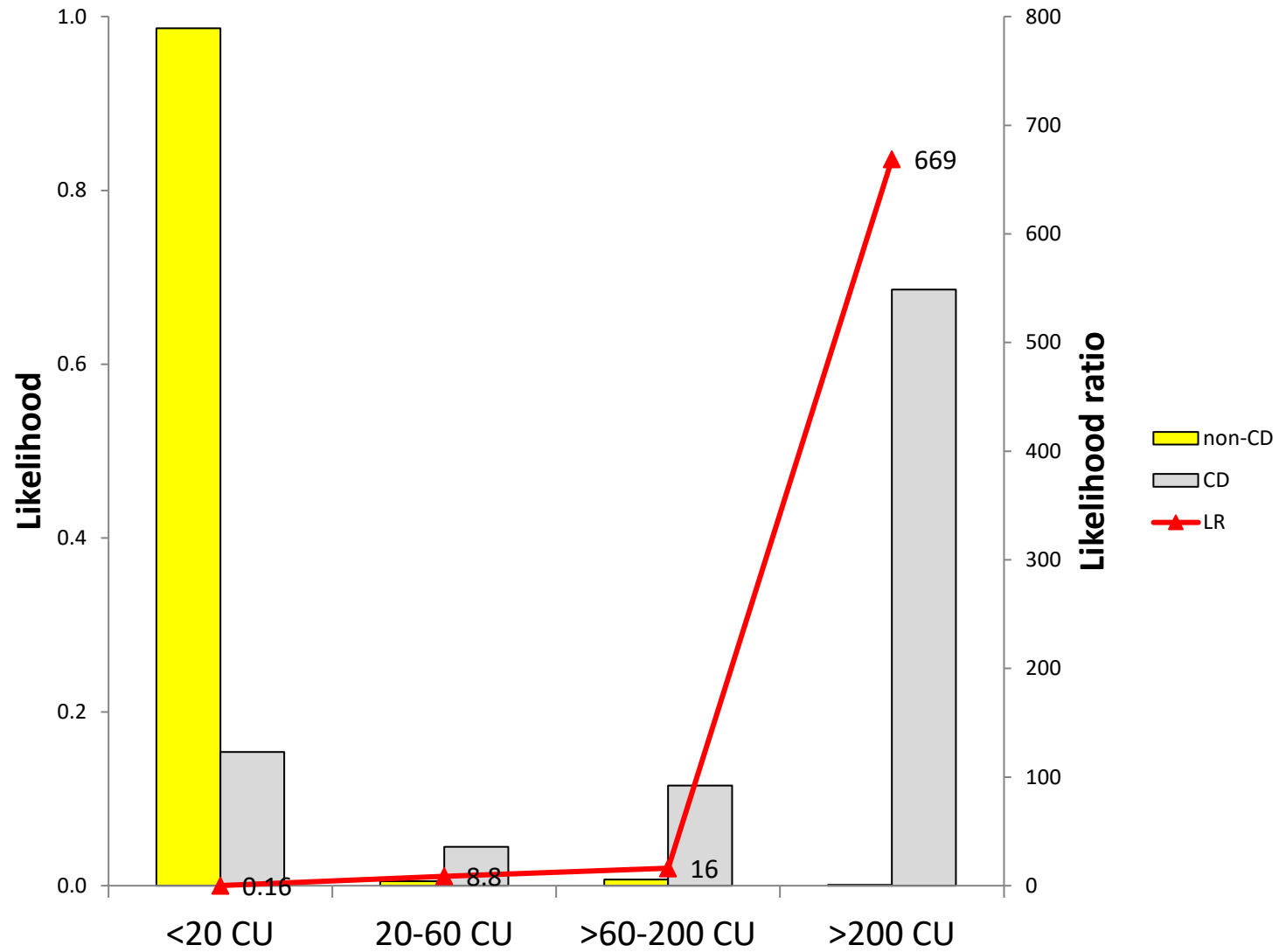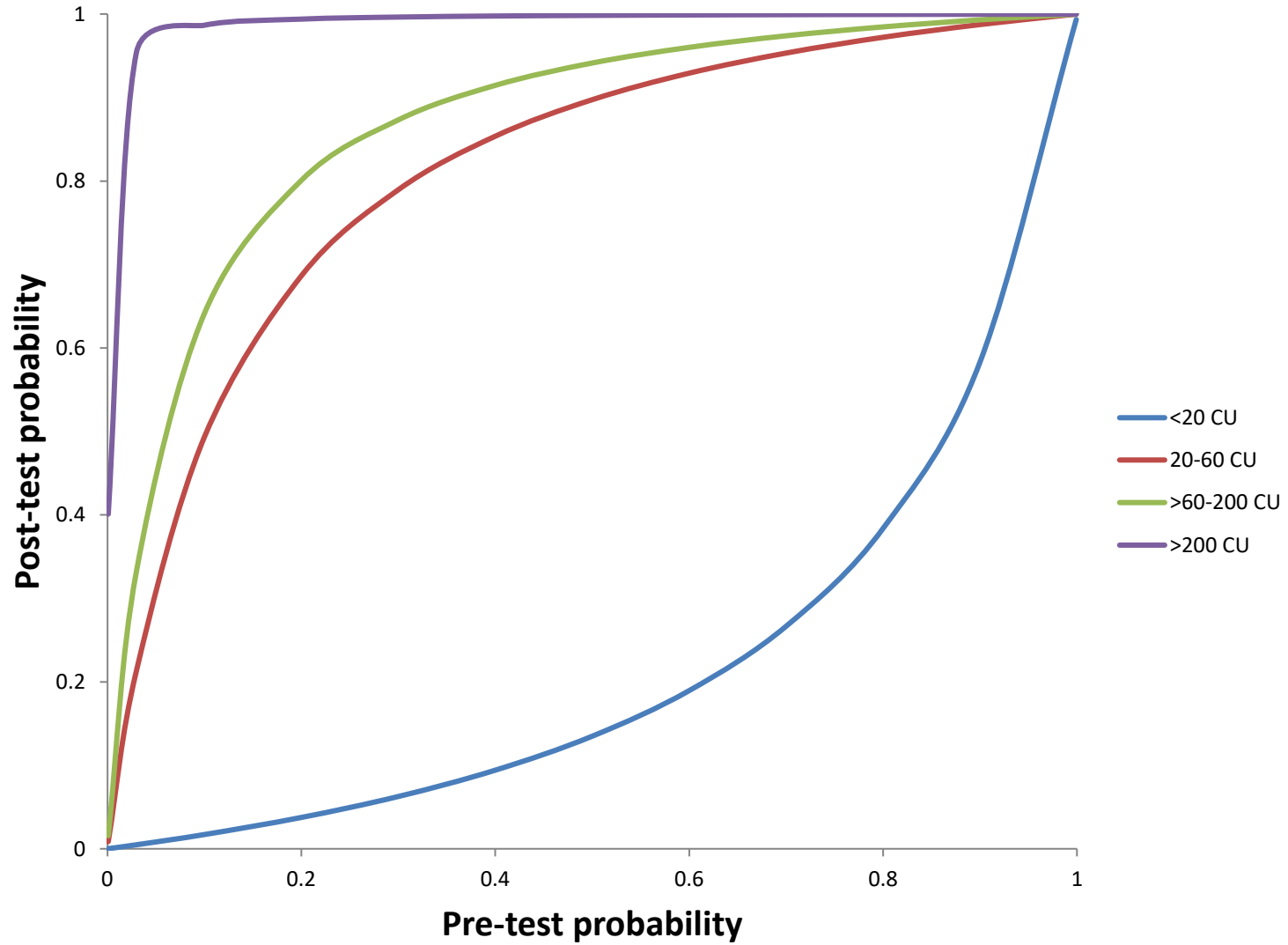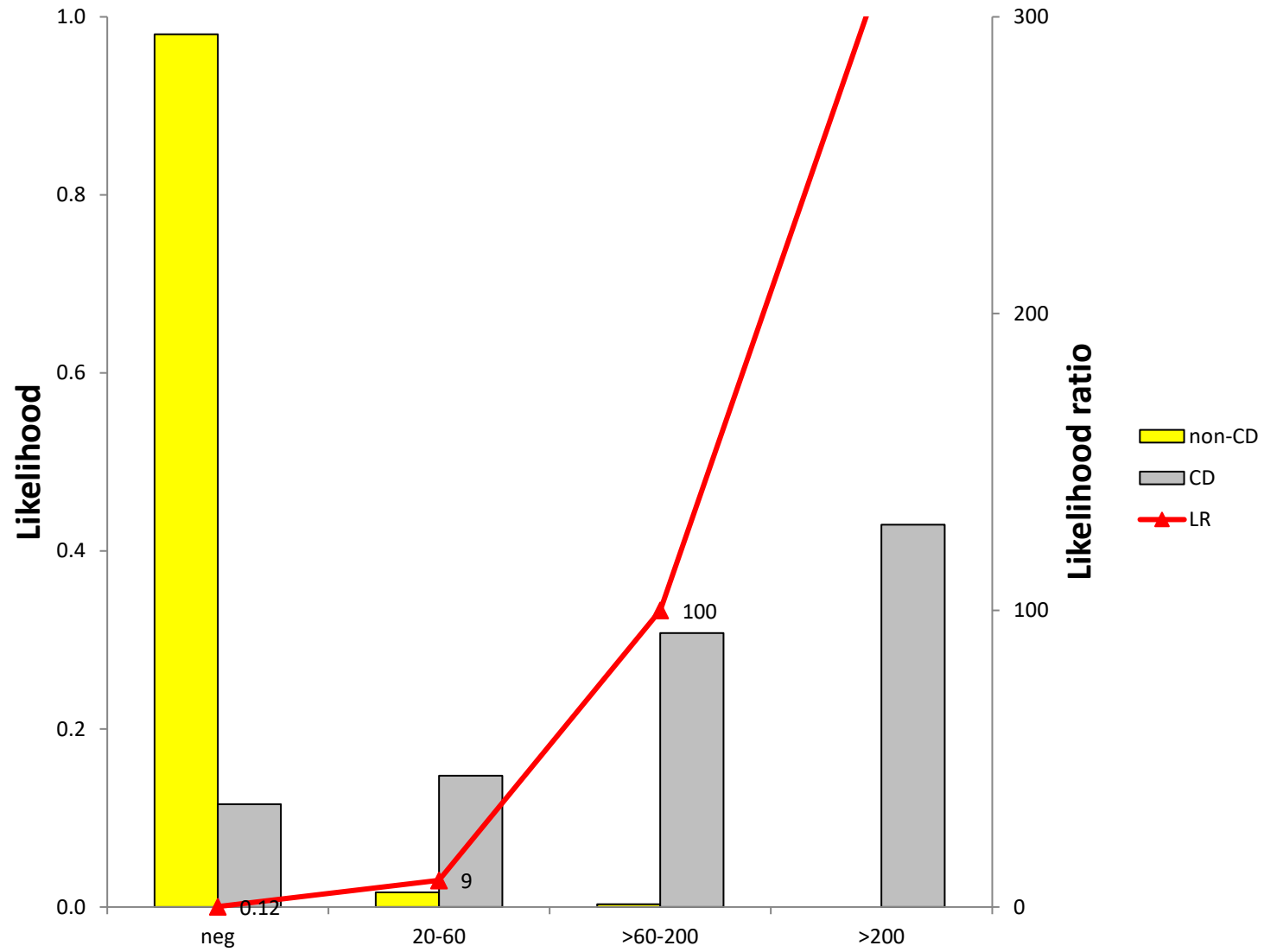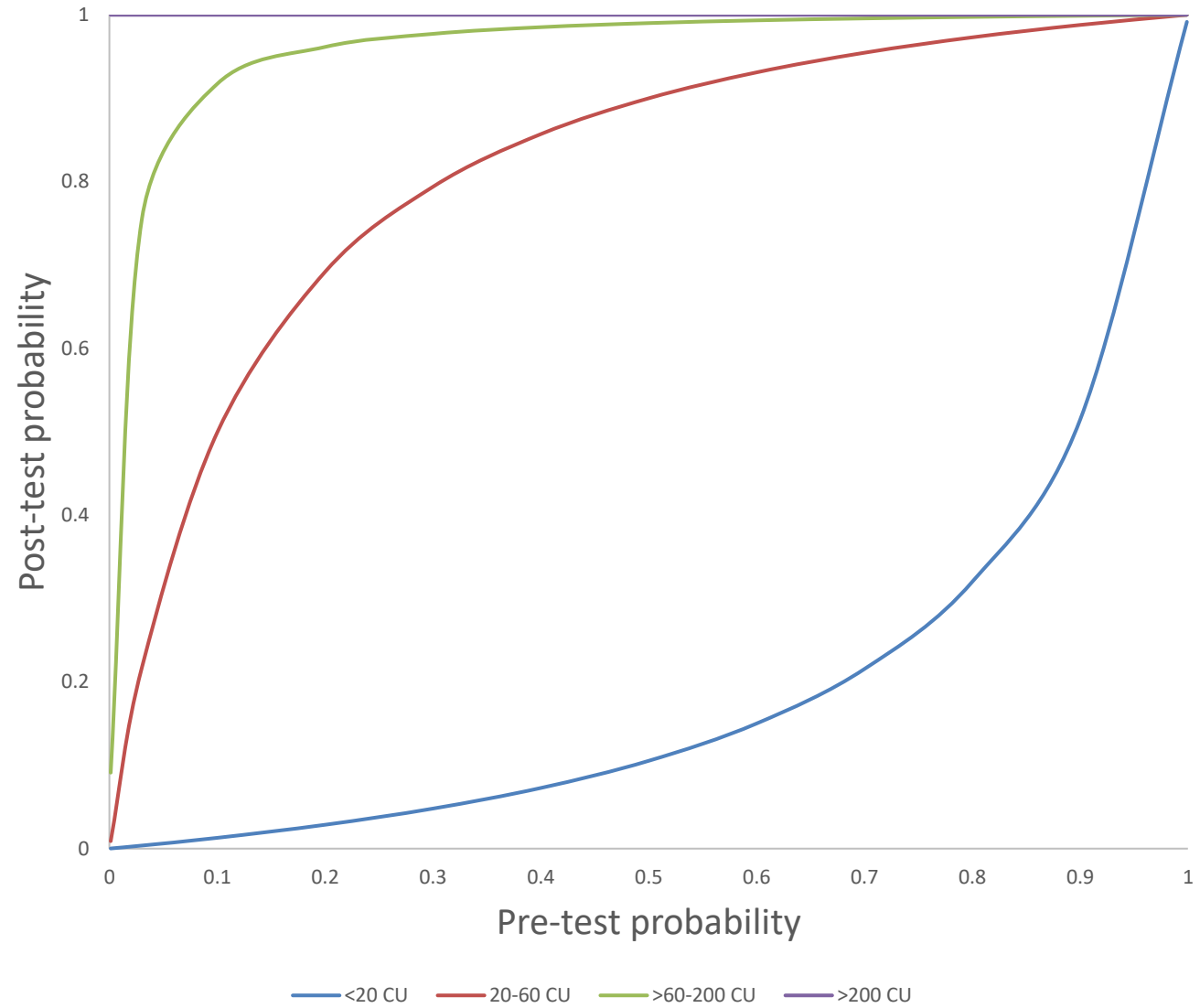
The pretest probability of celiac disease

- ✓ in the general population: **1%** .
- ✓ in patients suspected of celiac disease who underwent intestinal biopsy: **7%**.
- ✓ In patients with a Marsh class I lesion: **20%**.

- ✓ In patients with gastrointestinal complaints: **6%**
- ✓ In patients with weight loss, failure to thrive, or small stature: **14%**
- ✓ in patients with anemia or iron deficiency: **14%**
- ✓ in patients with malabsorption: **9%.**

IgA anti-tTG

Bogaert L et al. Autoimmun Rev. 2020;19:102513.
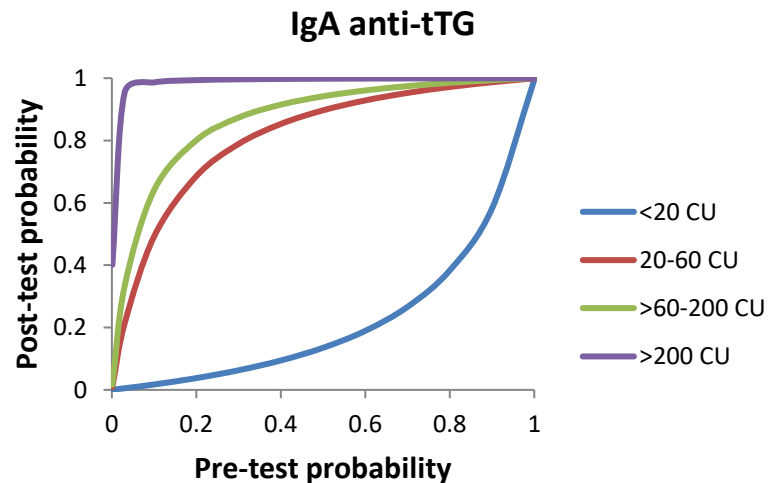
CD children <16 years: n=77
Controls: n=610
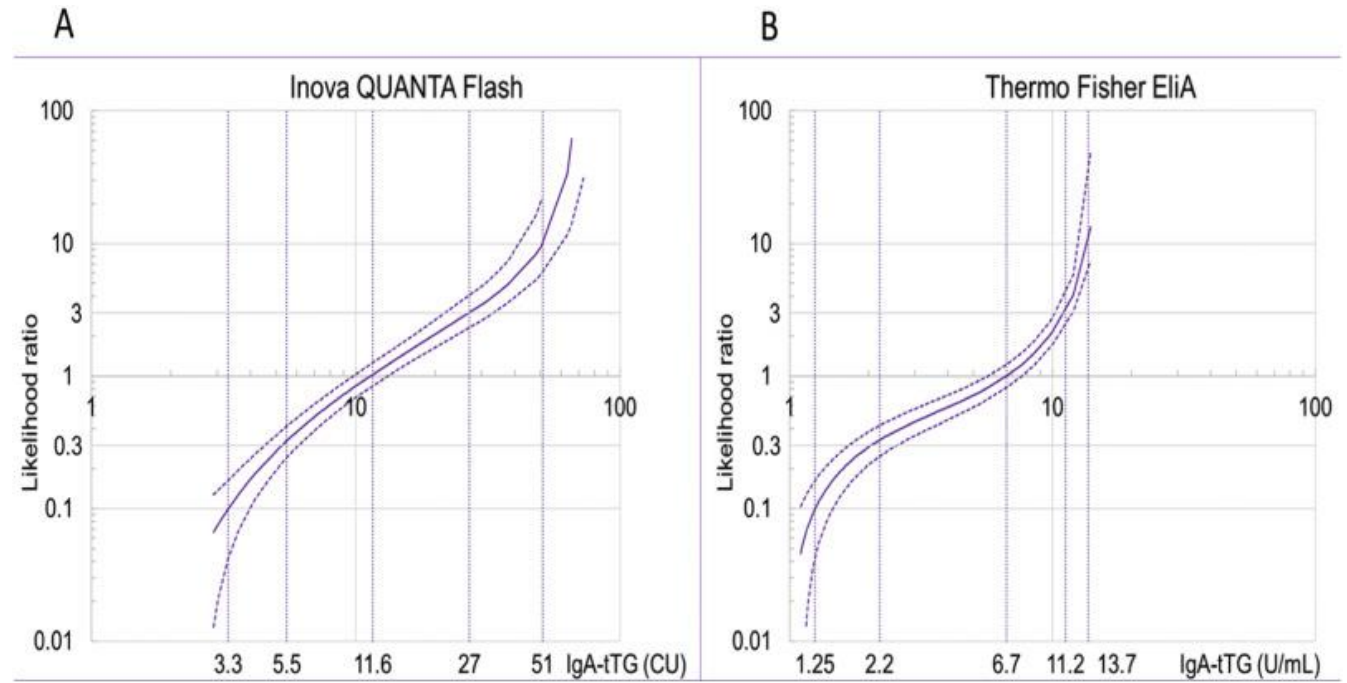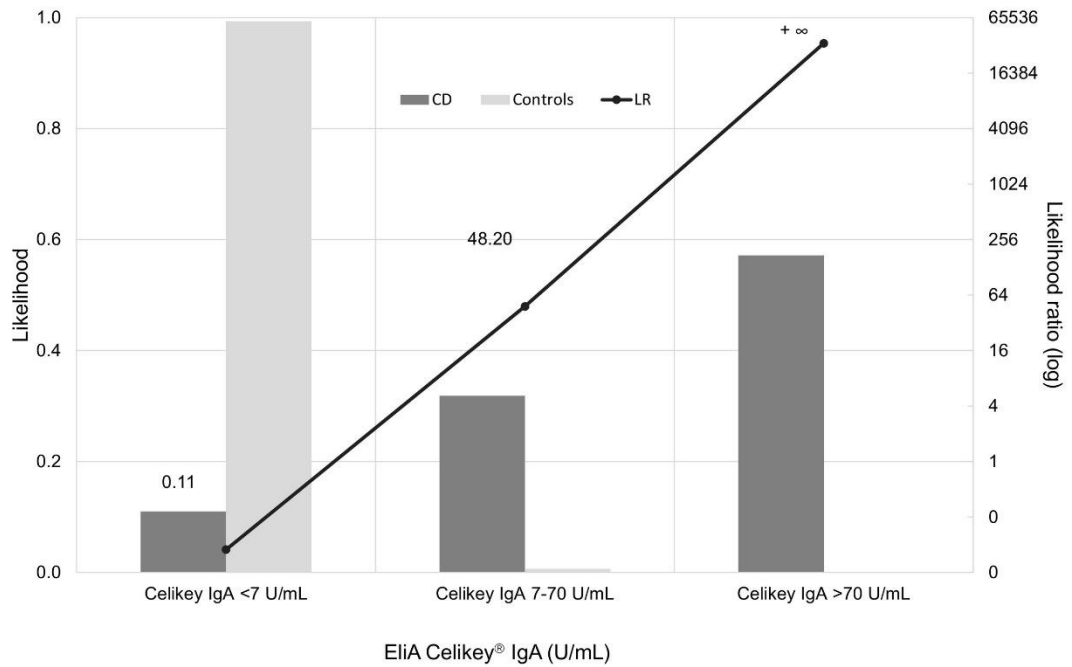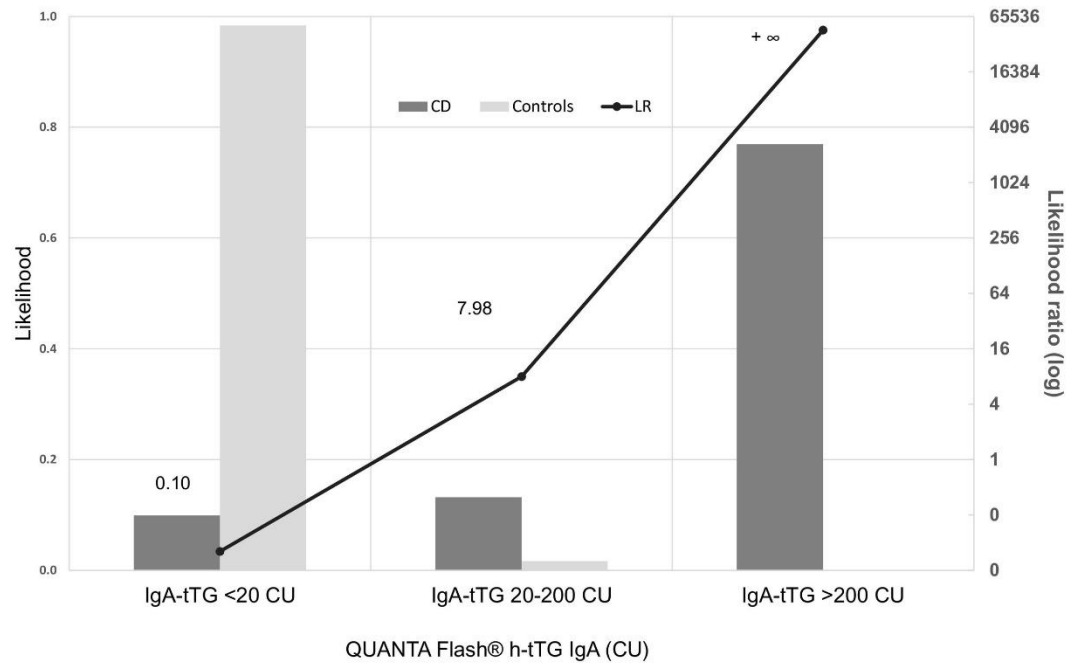
ALLERGY TESTING

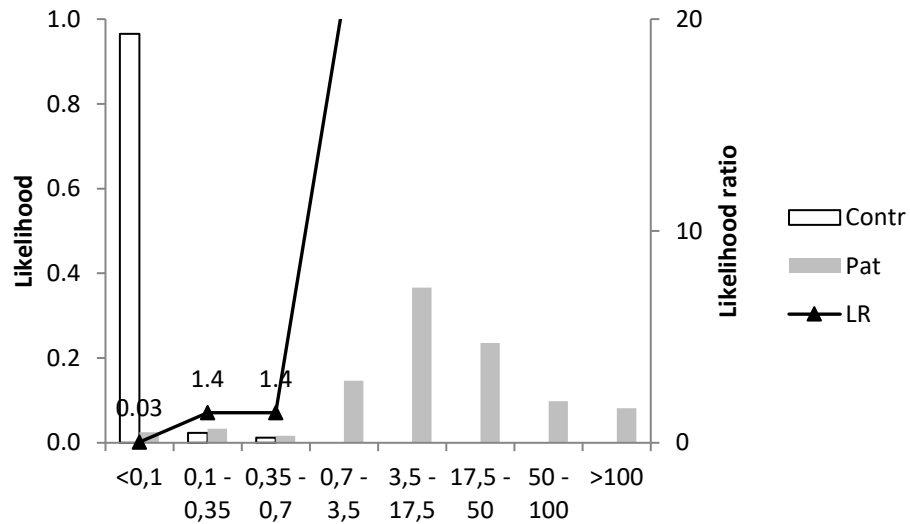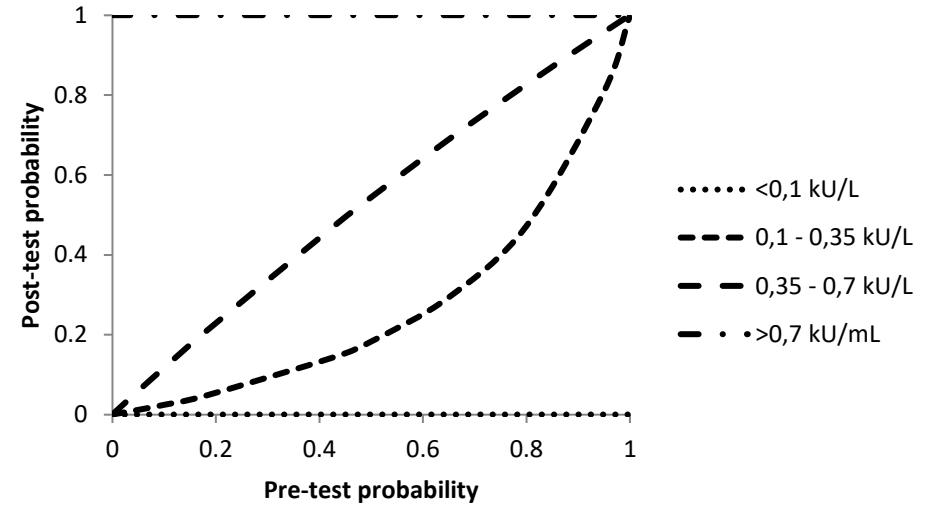| Clinical history | + | + | - | - |
Skin prick testing	+	-	+	-
Grass pollen	135	2	4	161
Birch pollen	123	0	9	173
	allergic	possible allergy	sensitization	Non-allergic

Birch pollen t3
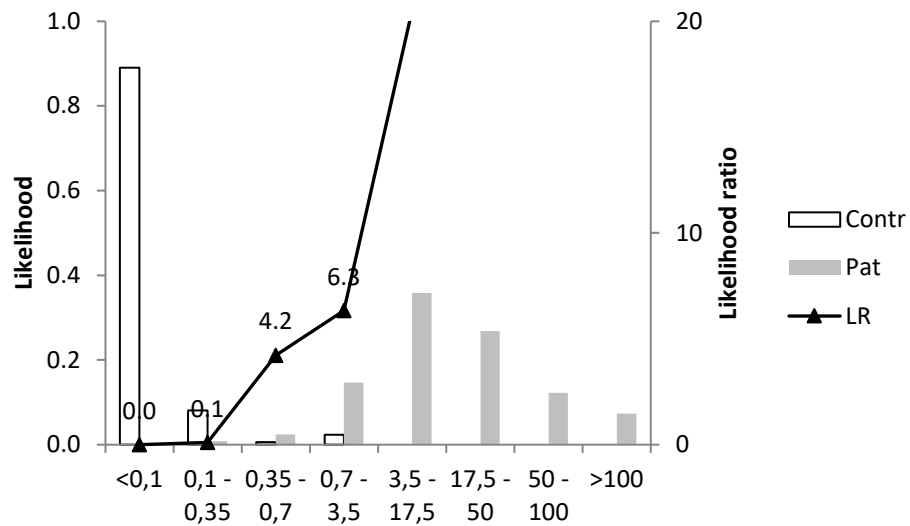
Grass pollen mixture gx3
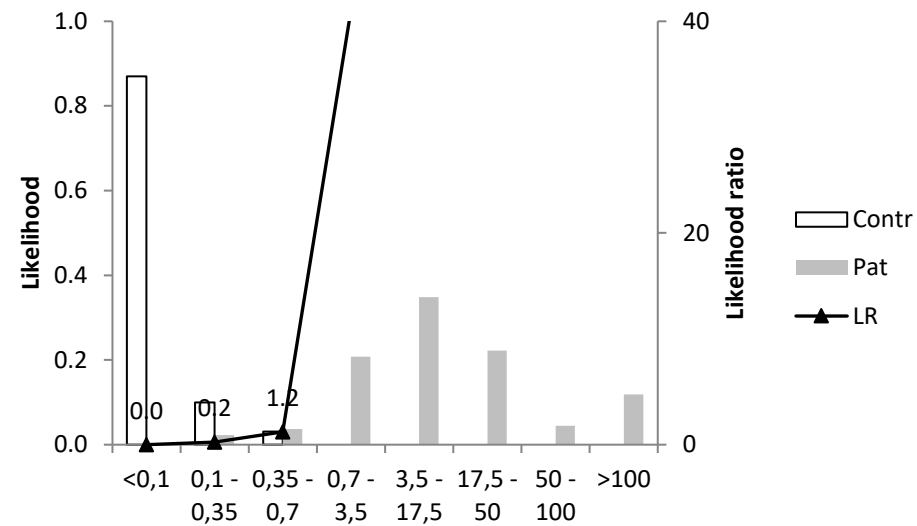
rBet v1

rPhl p1,5

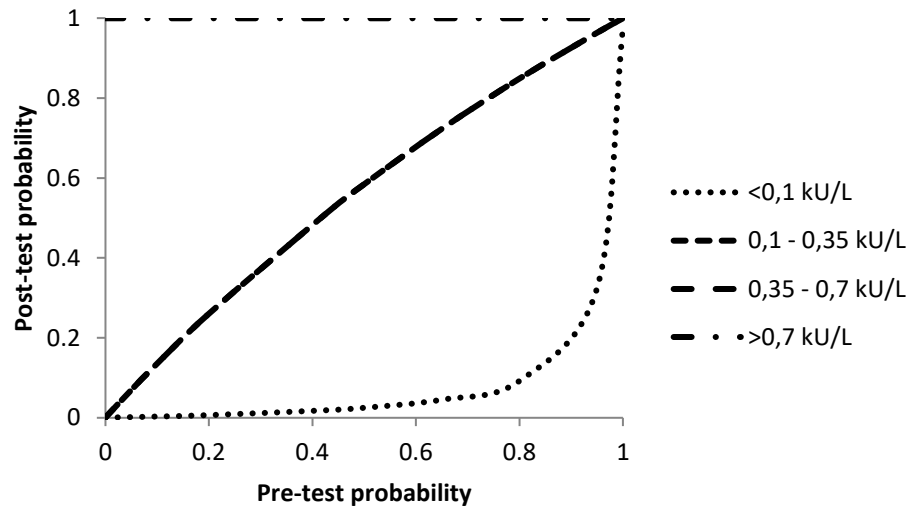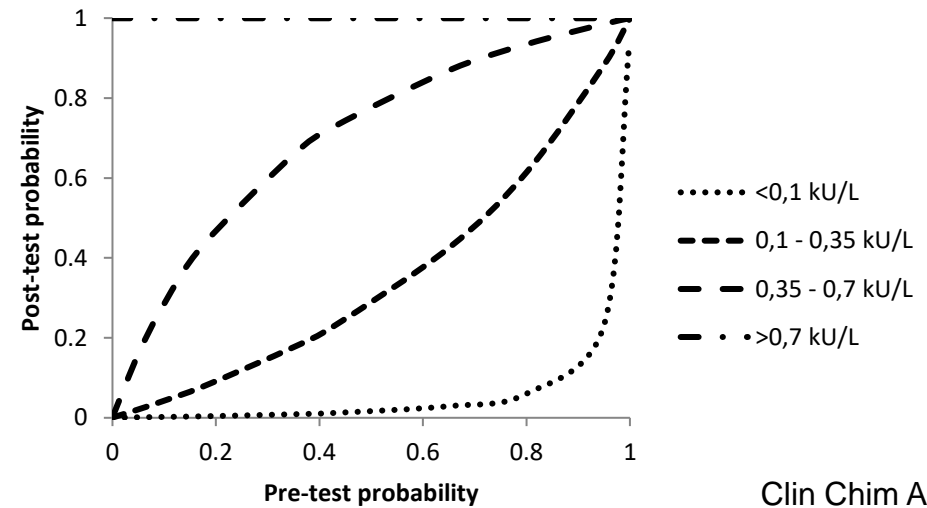| Allergen	sIgE (kU/L)				
	<0.1	**0.1-0.35**	**0.35-0.7**	**0.7-3.5**	**>3.5-17.5**
grass pollen mixture	0.00	0.22 (0.07-0.75)	1.19 (0.35-4.03)	∞	∞
rPhl p 1,5	0.02 (0.004-0.06)	0.4 (0.11-1.44)	3.6 (0.73-17.44)	∞	∞
birch pollen	0.00	0.1 (0.01-0.75)	4.22 (0.44-40.09)	6.33 (2.19-18.24)	∞
rBet v 1	0.03 (0.01-0.08)	1.41 (0.36-5.52)	1.41 (0.20-9.85)	∞	∞

Birch pollen t3

Post-test probability vs Pre-test probability

- ⋯⋯⋯ <0,1 kU/L
- ─ ─ ─ 0,1 - 0,35 kU/L
- ─ ─ 0,35 - 0,7 kU/L
- ─ · ─ 0,7 - 3,5 kU/L
- ─── >3,5

Grass pollen mixture gx3

Post-test probability vs Pre-test probability

- ⋯⋯⋯ <0,1 kU/L
- ─ ─ ─ 0,1 - 0,35 kU/L
- ─ ─ 0,35 - 0,7 kU/L
- ─ · ─ >0,7 kU/mL

rBet v1

Post-test probability vs Pre-test probability

- ⋯⋯⋯ <0,1 kU/L
- ─ ─ ─ 0,1 - 0,35 kU/L
- ─ ─ 0,35 - 0,7 kU/L
- ─ · ─ >0,7 kU/L

r Phl p1,5

Post-test probability vs Pre-test probability

- ⋯⋯⋯ <0,1 kU/L
- ─ ─ ─ 0,1 - 0,35 kU/L
- ─ ─ 0,35 - 0,7 kU/L
- ─ · ─ >0,7 kU/L

Van Hoeyveld et al.
Clin Chim Acta. 2015;450:46-50

Pre-test Probability	Allergen	Post-test probability				
		<0.1 kU/L	0.1-0.35 kU/L	0.35-0.7 kU/L	0.7-3.5 kU/L	>3.5 kU/L
		Grass pollen allergy				
17.6%	GP	0%	5%	20%	100%	100%
	rPhl p 1,5	0.3%	8%	43%	100%	100%
44%	GP	0%	15%	48%	100%	100%
	rPhl p 1,5	1%	24%	74%	100%	100%
		Birch pollen allergy				
9.7%	BP	0%	1%	31%	40%	100%
	rBet v 1	0.3%	13%	13%	100%	100%
40%	BP	0%	6%	74%	81%	100%
	rBet v 1	1.6%	48%	48%	100%	100%

Abrams EM, Chan ES, Portnoy J.
Evolving Interpretation of Screening and Diagnostic Tests in Allergy.
J Allergy Clin Immunol Pract. 2021;9:4183-4191.

Conclusions

- We illustrated how likelihood ratios depend on antibody level for a number of immune-mediated diseases

- Such knowledge may help with the interpretation of a specific test result

- Clinical laboratories might consider to provide likelihoods ratios or probability data for autoantibody test result intervals

Acknowlegdments