

ANCA

Xavier Bossuyt

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ANCA

granulomatosis with polyangiitis (**GPA**)

(formerly known as Wegener's granulomatosis)

microscopic polyangiitis (**MPA**)

eosinophilic granulomatosis with polyangiitis (EGPA)

(formerly known as Churg-Strauss syndrome)

Clinical manifestations

Ear, nose, throat

Lower respiratory tract and lung

Nervous system

Kidney

Skin

Ocular

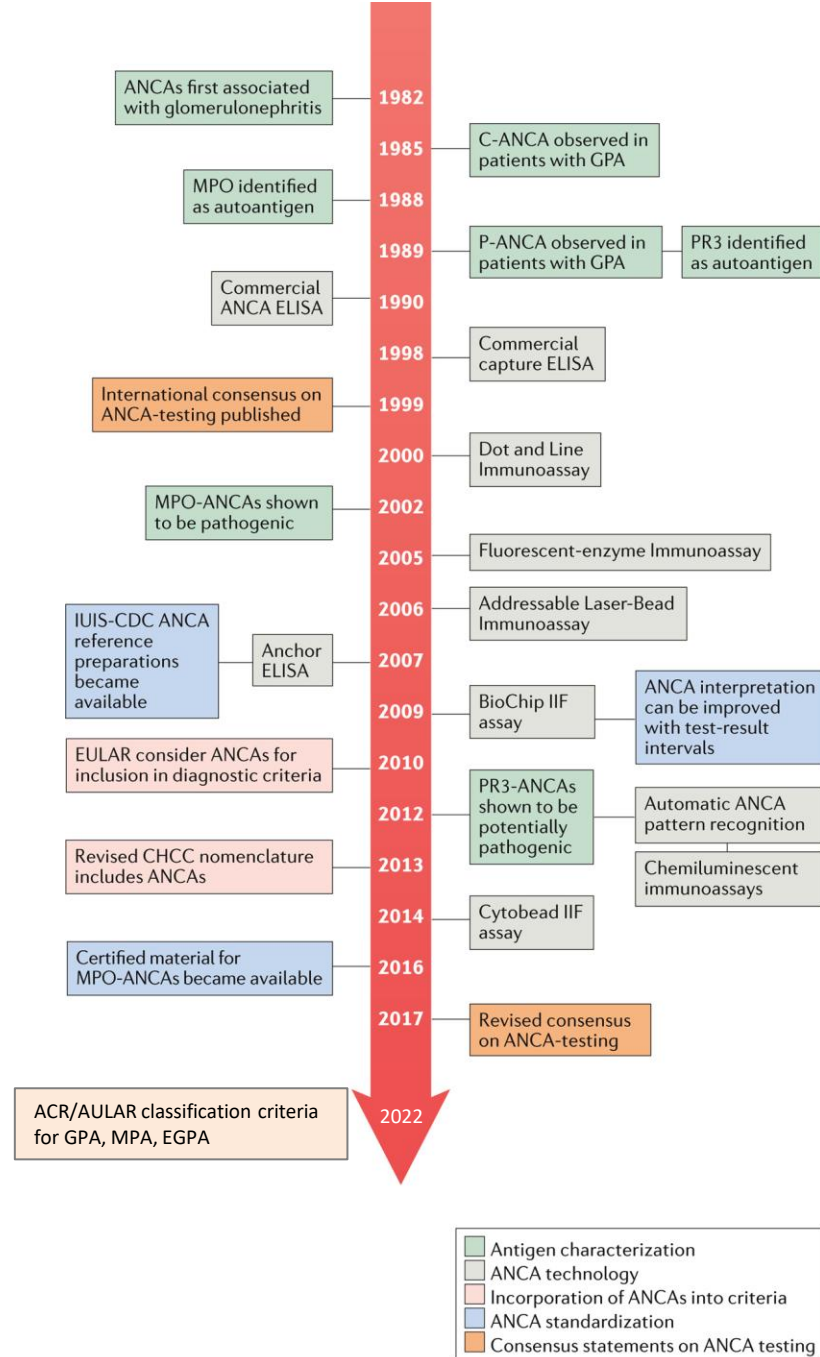
Cardiovascular

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

Bossuyt X, Cohen Tervaert JW, Arimura Y, Blockmans D, Flores-Suárez LF, Guillevin L, Hellmich B, Jayne D, Jennette JC, Kallenberg CGM, Moiseev S, Novikov P, Radice A, Savige JA, Sinico RA, Specks U, van Paassen P, Zhao MH, Rasmussen N, Damoiseaux J, Csernok E.

Position paper: Nat Rev Rheumatol. 2017

Historical landmarks of ANCA-testing in small vessel vasculitis



EUVAS Study

Diagnosis	Bad Bramstedt	Copenhagen	Leuven	Maastricht	Total
Controls	189	237	246	252	924
GPA	40	38	40	68	186
MPA	26	8	15	16	65
Total	255	283	301	336	1175

IIF Copenhagen: ethanol-fixed mixture of neutrophils and lymphocytes (Staten Serum Institut, Denmark). (Wiik, Rasmussen, Wieslander 1993)

IIF Bad Bramstedt: ethanol-fixed neutrophils, formalin-fixed neutrophils and HEp-2 cells

Euro-Diagnostica: *second generation* capture PR3- and MPO-ANCA ELISA.

Euroimmun: *third generation* anti-PR3 and *first generation* MPO-ANCA ELISA.

Orgentec: *third generation* anti-PR3 and *first generation* anti-MPO ELISA.

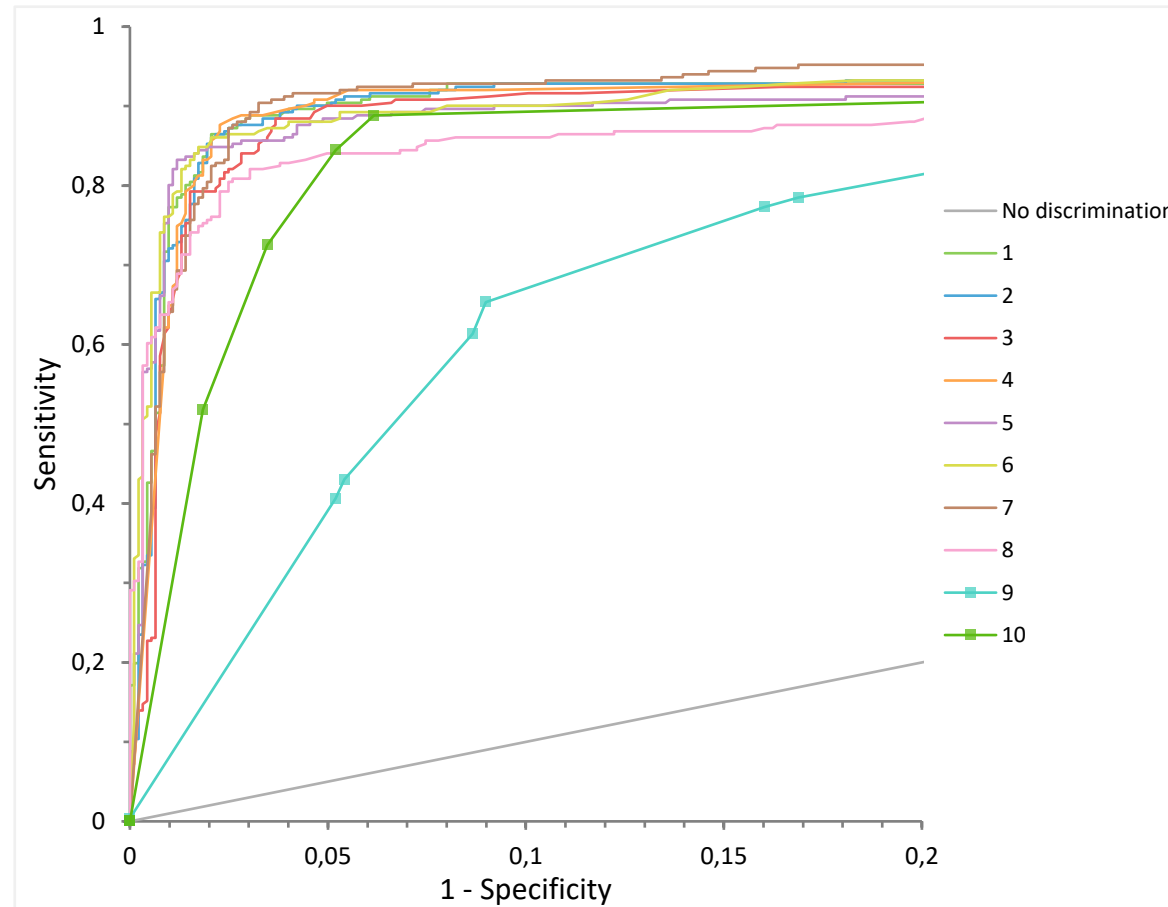
Inova Diagnostics: QuantaLite ELISA / QuantaFlash CLIA.

Bio-Rad: multiplex flow immunoassay (BioPlex 2200 Vasculitis kit)

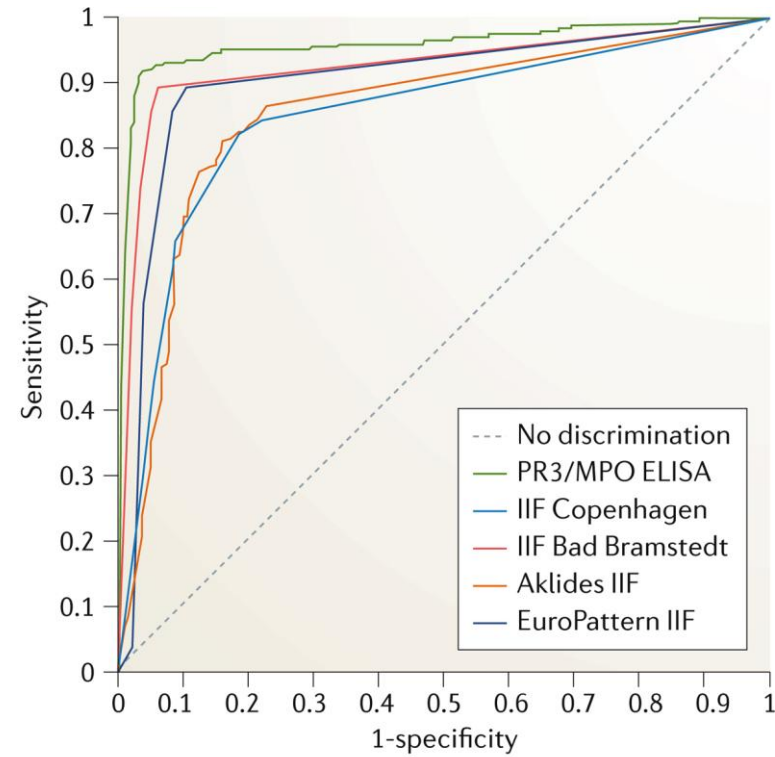
Thermo-Fisher Scientific: *third generation* EliA PR3^S and MPO^S FEIA.

Medipan/Generic Assays: CytoBead ANCA assays.

Receiver operating characteristics curve for eight antigen-specific immunoassays and two IIF methods (Copenhagen and Bad Bramstedt) for ANCA detection.



Comparison of the receiver operating characteristics curves for different ANCA assays



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Csernok, E., et al. *Autoimmun. Rev.* **15**, 736–741 (2016)

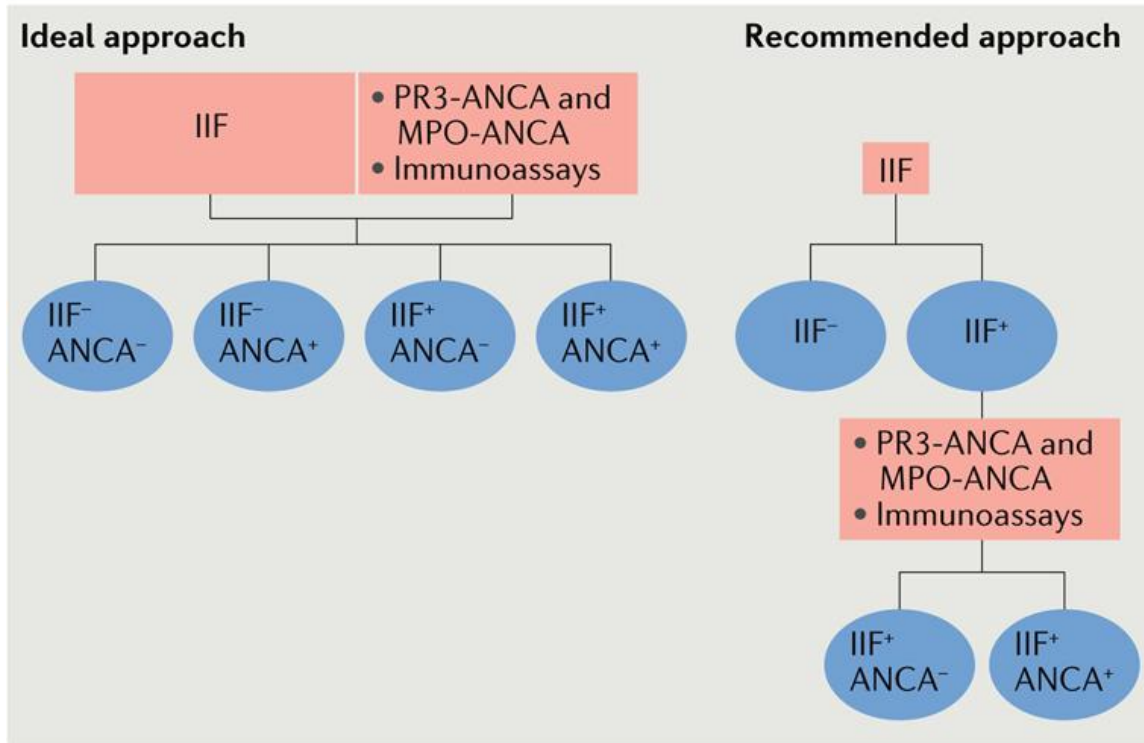
Table 1 | **Comparison of the specificity and sensitivity for different ANCA assays**

Study population	IIF		Immunoassay	
	C-ANCA	P-ANCA	PR3-ANCA	MPO-ANCA
Specificity in disease controls				
Hagen <i>et al.</i> (n = 184)	95%	81%	86–89%	91%
Damoiseaux <i>et al.</i> (n = 924)	97–98%	81–96%	98–99%	96–99%
Sensitivity in ‘newly diagnosed’ GPA				
Hagen <i>et al.</i> (n = 97)	64%	21%	65–67%	24%
Damoiseaux <i>et al.</i> (n = 186)	65–77%	11–15%	77–81%	9–12%
Sensitivity in ‘newly diagnosed’ MPA				
Hagen <i>et al.</i> (n = 44)	23%	58%	25–27%	58%
Damoiseaux <i>et al.</i> (n = 65)	5–6%	85–89%	5–9%	71–88%

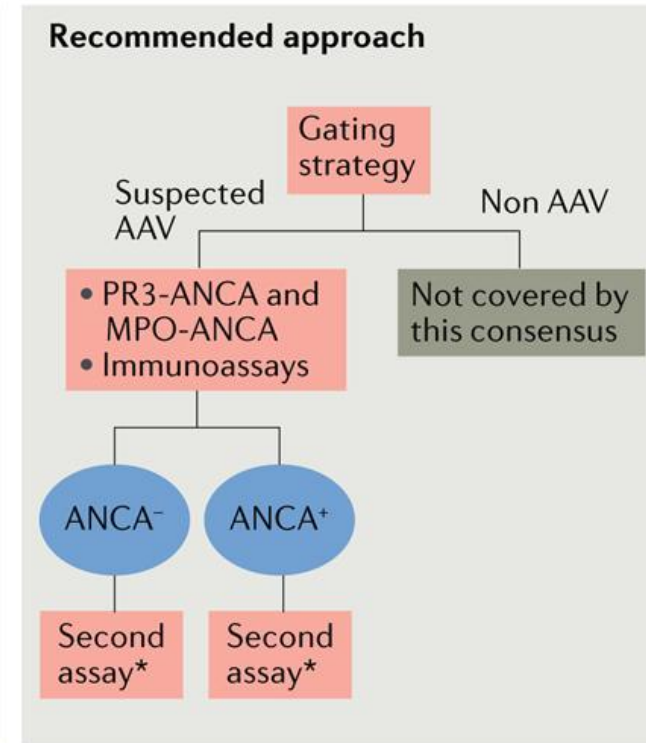
This table compares the specificity and sensitivity of indirect immunofluorescence (IIF) and antigen-specific immunoassays for newly diagnosed patients with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) as reported by Hagen *et al.* (the basis for the 1999 consensus)²⁵ and Damoiseaux *et al.* (the basis for the revised 2017 consensus)⁶. Hagen *et al.*²⁵ used one IIF method, three different proteinase 3 (PR3)-anti-neutrophil cytoplasmic antibody (ANCA) enzyme-linked immunosorbent assays (ELISAs) and one myeloperoxidase (MPO)-ANCA ELISA, whereas Damoiseaux *et al.*⁶ performed IIF using two methods at two different sites and eight different PR3-ANCA and eight different MPO-ANCA antigen-specific enzyme immunoassays. AAV, ANCA-associated vasculitis. C-ANCA, cytoplasmic ANCA staining pattern; P-ANCA, perinuclear ANCA staining pattern.

Visual representation of the 1999 recommendations and revised 2017 recommendations

a 1999 consensus



b 2017 consensus



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International recommendations for ANCA detection in GPA and MPA:

Recommendation 1

A gating policy for requesting an ANCA test is advisable and adherence to clinical guidelines for ANCA testing is recommended.

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- Glomerulonephritis, especially *rapidly progressive glomerulonephritis*
- Pulmonary haemorrhage, especially *pulmonary renal syndrome*
- Cutaneous vasculitis with systemic features
- Multiple lung nodules
- Chronic *destructive disease* of the *upper airways*
- Long-standing sinusitis or otitis
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- Mononeuritis multiplex or other peripheral neuropathy
- Retro-orbital mass
- Scleritis

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Recommendation 2

High-quality antigen-specific assays for proteinase 3 (PR3)-ANCAs and myeloperoxidase (MPO)-ANCAs should be used as the primary screening method for ANCA.

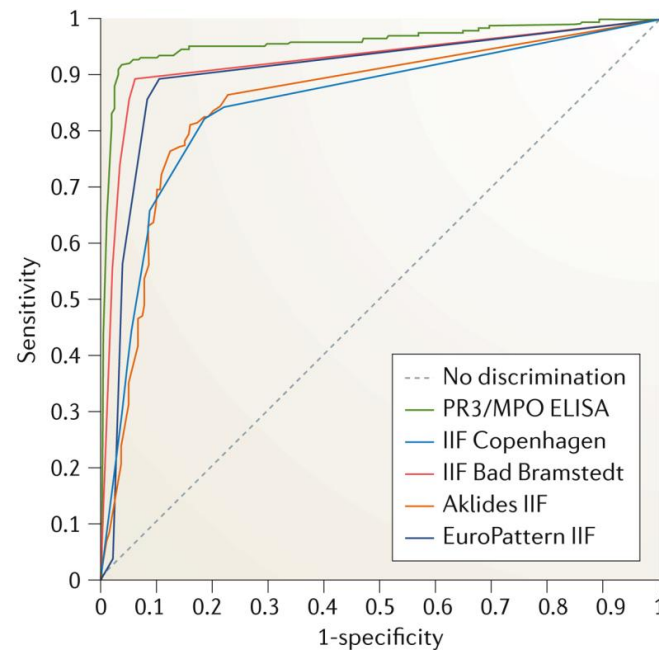
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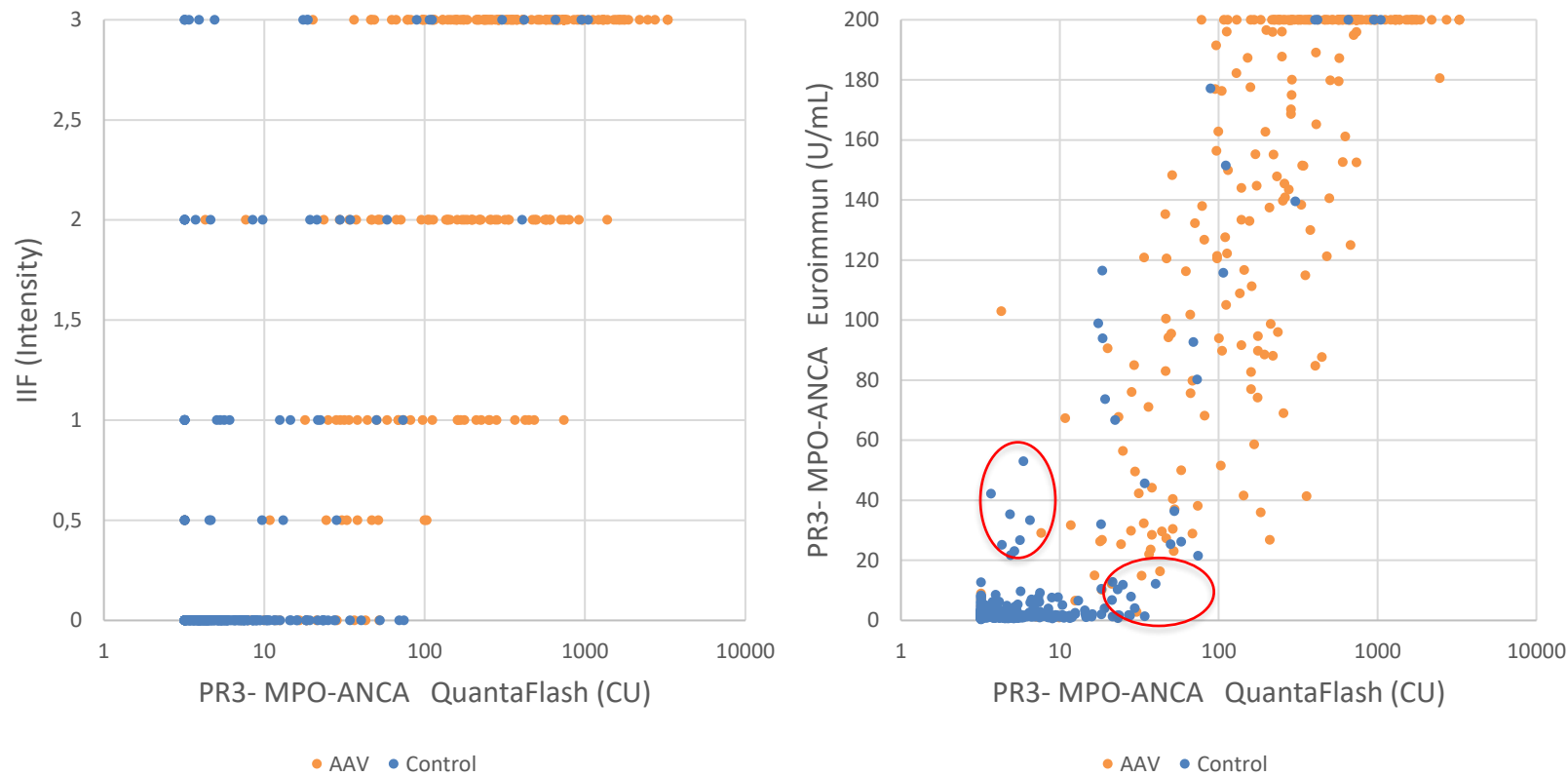
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Recommendation 3

If results for both PR3-ANCAs and MPO-ANCAs are negative, and there is still a strong suspicion of small-vessel vasculitis, then use of other immunoassays and/or indirect immunofluorescence (IIF), or referral to an experienced laboratory is recommended. Performing a second assay or IIF can also marginally increase the specificity in cases of low-positive test results.



Recommendation 3

If results for both PR3-ANCAs and MPO-ANCAs are negative, and there is still a strong suspicion of small-vessel vasculitis, then use of other immunoassays and/or indirect immunofluorescence (IIF), or referral to an experienced laboratory is recommended. Performing a second assay or IIF can also marginally increase the specificity in cases of low-positive test results.

International recommendations for ANCA detection in GPA and MPA:

Recommendation 4

A diagnosis of ANCA-associated vasculitis (AAV) cannot be excluded on the basis of negative PR3-ANCA and MPO-ANCA results.

Recommendation 5

A positive PR3-ANCA and/or MPO-ANCA result only contributes to the diagnostic work-up for AAV and is not diagnostic by itself.

Recommendation 6

Taking into account antibody levels improves clinical interpretation.

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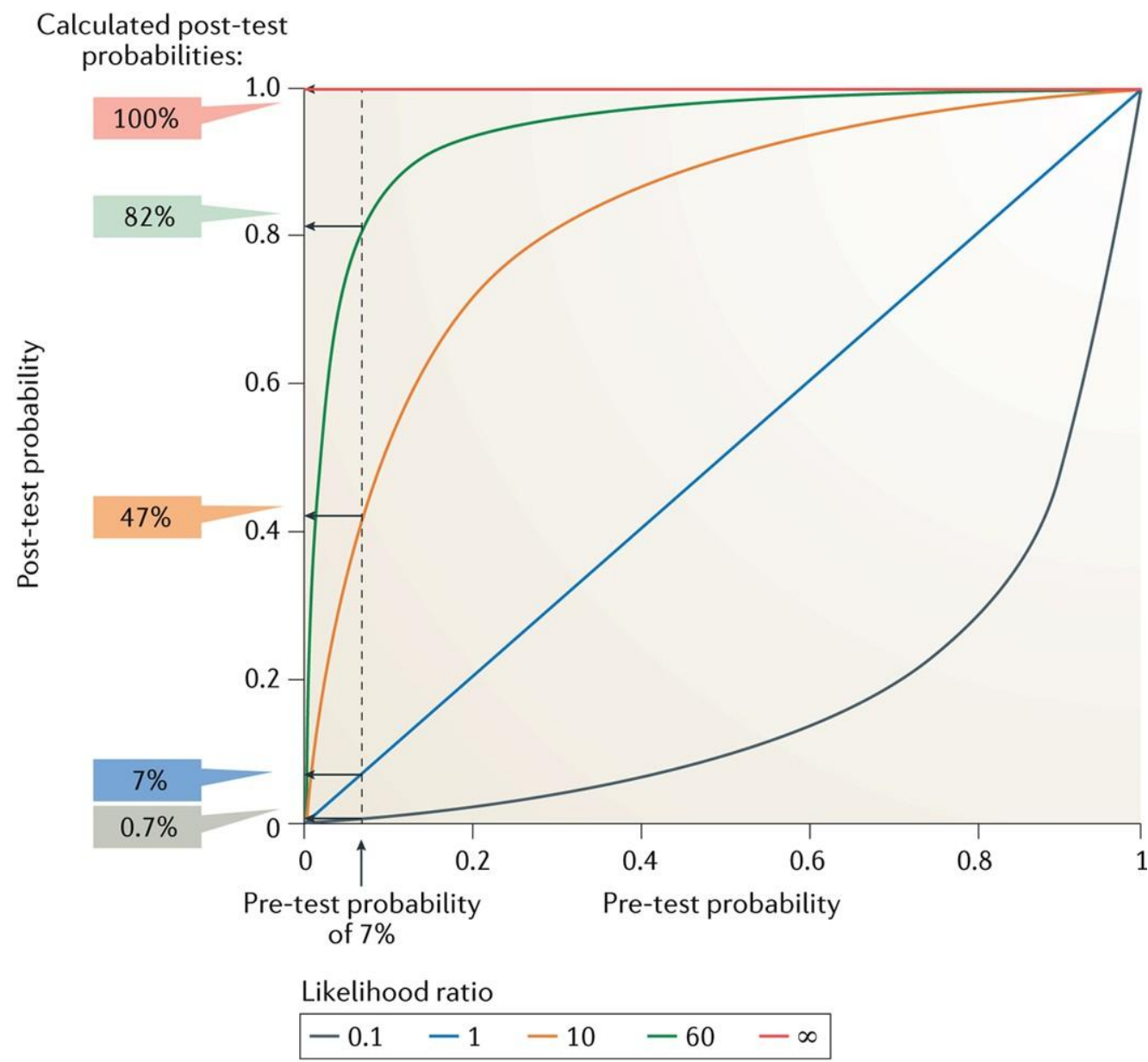
Manufacturer Cutoff	Interval	Fraction of patients	Fraction of controls	LR	95% CI
QuantaLite - Inova	0,0 - 10,0	0,096	0,949	0,10	0,07 to 0,15
20 Units	10.1 - 19,0	0,040	0,026	1,50	0,74 to 3,16
	19,0 - 37,0	0,092	0,014	6,51	3,34 to 12,67
	37,0 - 159,1	0,602	0,011	55,59	29,76 to 103,81
	159,1 - 250,0	0,171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

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]

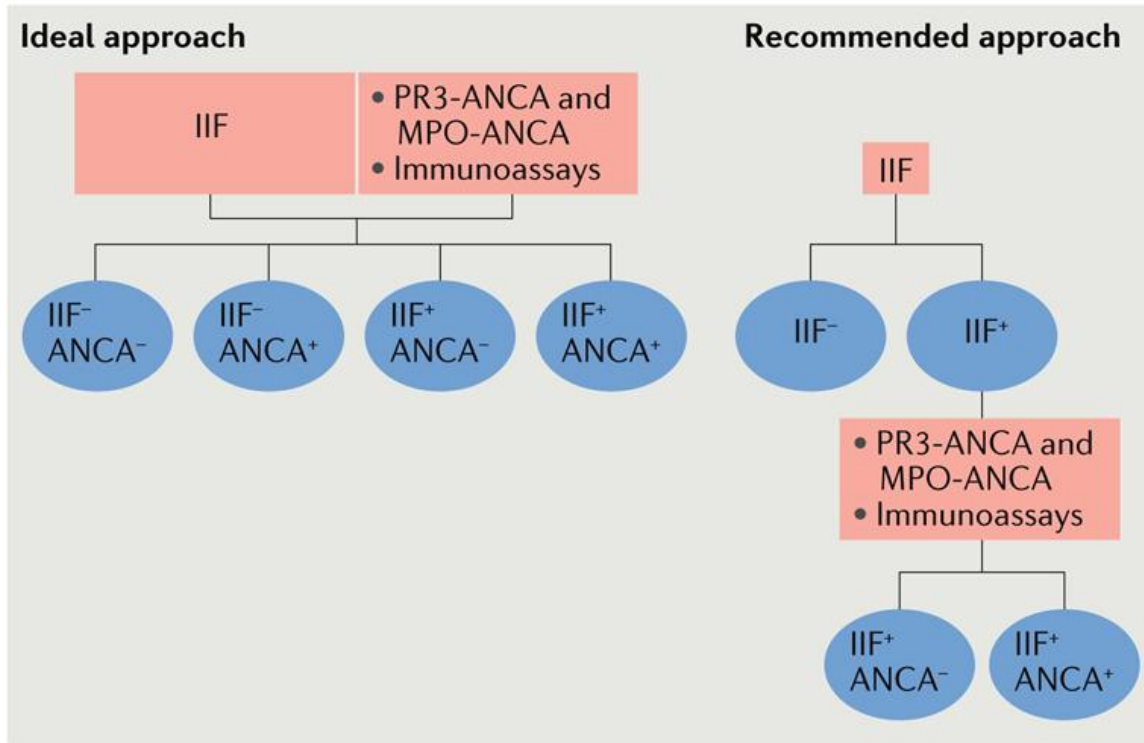
- ☐ sinus: radiographic evidence of mucosal thickening involving one or more sinuses
- ☐ pulmonary: radiographic presence of pulmonary infiltrates or nodules, or both
- ☐ glomerulonephritis: urinalysis demonstrating hematuria and red blood cell casts

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

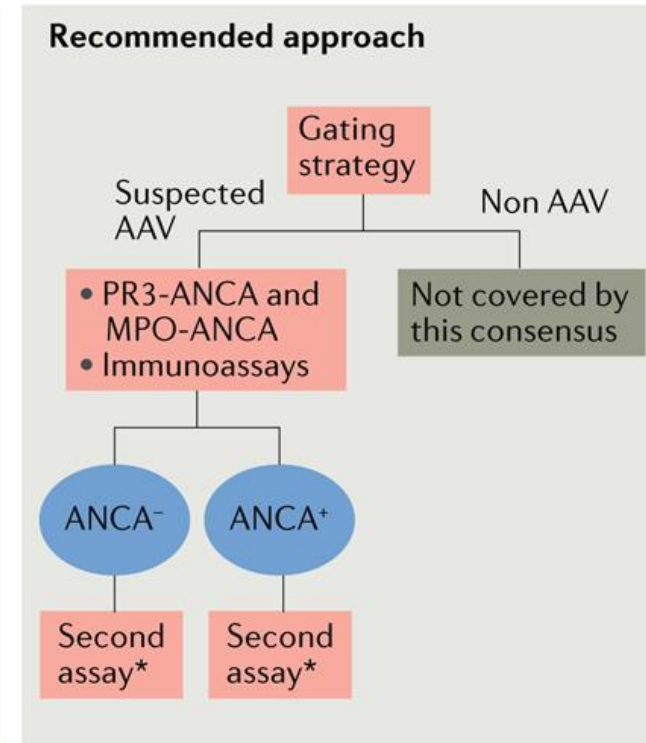


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	68,2 - 180,0	0,291	0,000	∞	33,42 to ∞

ANCA: clinical suspicion AAV

PR3-ANCA

MPO-ANCA

LR reporting

ANCA: follow-up

PR3-ANCA or MPO-ANCA

ANCA: IBD

IIF

Box 2: Clinical indications for ANCA testing

- Glomerulonephritis, especially rapidly progressive glomerulonephritis
- Pulmonary haemorrhage, especially pulmonary renal syndrome
- Cutaneous vasculitis with systemic features
- Multiple lung nodules
- Chronic destructive disease of the upper airways
- Long-standing sinusitis or otitis
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ANCA: clinical suspicion AAV

ANCA identification

PR3-ANCA <0.6 U/mL

<2: negative, 2-3: equivocal, >3: positive

MPO-ANCA 12.0 U/mL

<3.5: negative, 3.5-5: equivocal, >5 positive

Likelihood ratio for ANCA-associated vasculitis at diagnosis 11.8

(*Rheumatology* 2017; 56:1533-1541) (95% CI: 6.6-21.1)

CONSIDERATIONS

IBD

atypical P-ANCAs: UC: 50%–67%
CD: 6%–15%
diseased controls: <11%.

P-ANCA for UC: large meta-analysis (3841 UC and 4019 CD patients)
sensitivity: 55,3%
specificity: 88.5%
LR: 4.8 (*modest clinical utility*)

ASCA: CD: 40%-60%
UC: 4%-14%
controls: <5% of controls

ASCA(+)/P-ANCA(-): associated with CD
ASCA(-)/P-ANCA(+): associated with UC

European evidence-based consensus on UC:

“In view of the current limited sensitivity of these markers, their routine use for the diagnosis of UC and for therapeutic decisions is not clinically justified”

Infection and ANCA

Infective endocarditis can mimic ANCA-associated glomerulonephritis
Patients with infective endocarditis can develop ANCA

ANCA (by IIF) in 18%-24% of patients with infective endocarditis
4%-12% PR3-ANCA, 4%-6% MPO-ANCA, some being double pos (PR3- and MPO-ANCA).

Infections such as infective endocarditis, hepatitis C infection, tuberculosis, etc., should be excluded before establishing a diagnosis of AAV and starting immunosuppressive therapy.

EUVAS study was performed in Europe where the prevalence of infections such as malaria, leprosy and tuberculosis is lower than in other regions.

Drug-induced AAV

levamisole-adulterated cocaine-induced AAV: MPO-ANCA, PR3-ANCA, HNE-ANCA, ANA

In 30 cases of AAV associated to cocaine use:

all had MPO-ANCA

50% had PR3-ANCA.

double positivity (MPO-ANCA and PR3-ANCA) is characteristic

hydralazine-induced AAV

MPO-ANCA can be found together with HNE-ANCA, lactoferrin-ANCA, and ANA

propylthiouracil-mediated AAV

High titers of MPO-ANCA

32-41% of propylthiouracil-treated patients develop ANCA (also PR3- and HNE-ANCA) without symptoms

minocycline-induced AAV

P-ANCA (~80 % of cases): MPO-, HNE-, BPI, lactoferrin, cathepsin ANCA, ANA

Most patients with drug-induced AAV have MPO-ANCA, which can be in combination with antibodies to other neutrophilic cytoplasmic proteins and with ANA

STANDARDIZATION

2007: human reference sera for MPO- and PR3-ANCA: CDC under auspices of IUIS.

Plasmapheresis from a single donor.

Assigned value: 100 IU/mL for PR3-ANCA and 100 IU/mL for MPO-ANCA.

At least four companies use it for calibration:

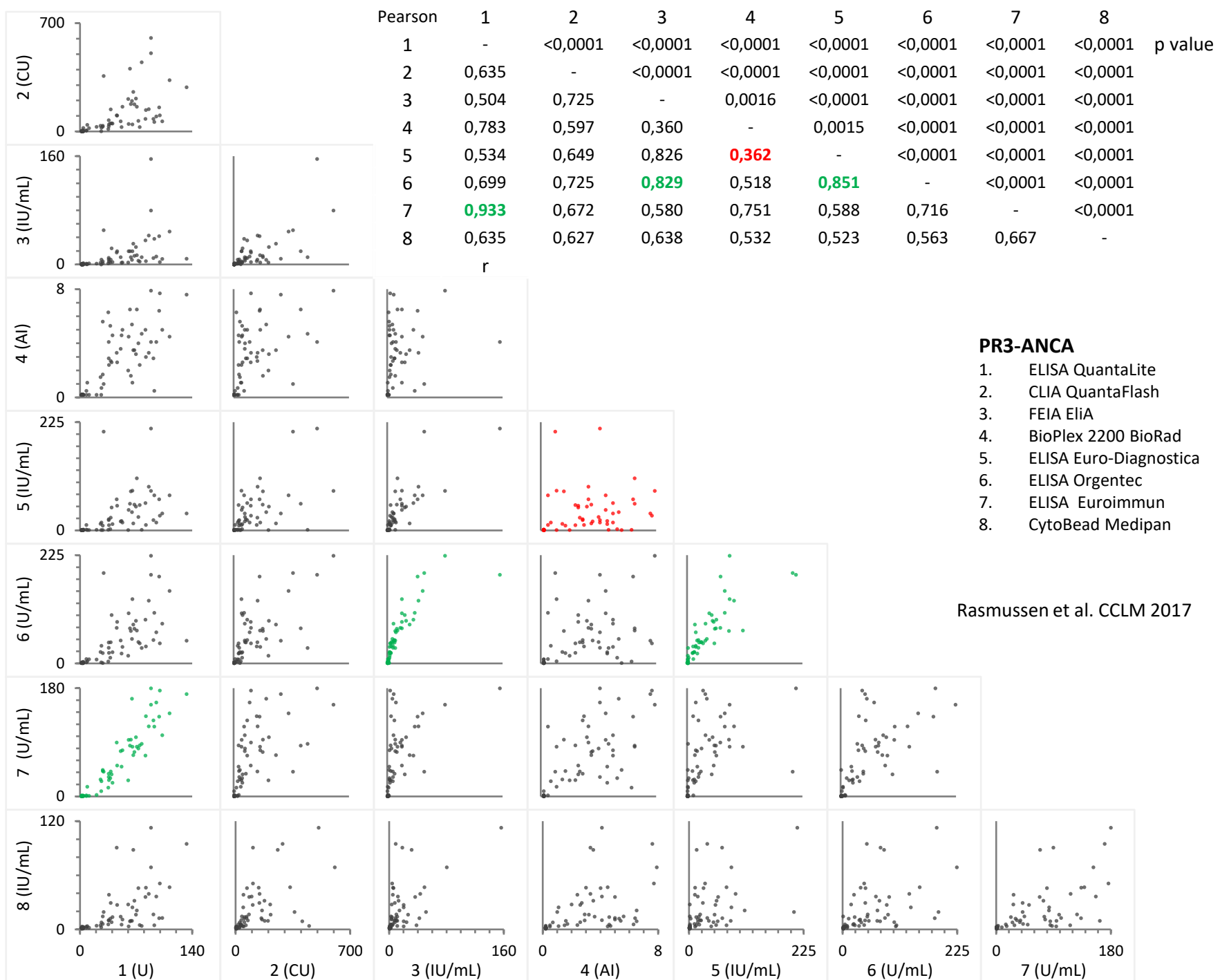
- ✓ second generation ANCA ELISA's of Wieslab (Euro-Diagnostica)
- ✓ third generation ANCA FEIA (Thermo-Fisher)
- ✓ cytobead IIF assay (Medipan)
- ✓ CLIA (Inova).

2016: MPO-ANCA Certified Reference Material: Institute for Reference Materials and Measurements

Plasmapheresis sample from a single patient.

Assigned value: 270 µg/L for PR3-ANCA and 84 µg/L for MPO-ANCA

*Cave: autoantibodies are not uniform and differ in terms of:
composition of IgG subclass, avidity, glycosylation, and epitope recognition.
May impact on standardization.*



Acknowledgments

Bio-Rad

Euro-Diagnostica

Euroimmun

Inova Diagnostics

Medipan/Generic Assays

Orgentec

Thermo-Fisher Scientific

J-W Cohen Tervaert, E Csernok, J Damoiseaux, N Rasmussen

D Blockmans, B Hellmich, P van Paassen

Y Arimura, F Flores-Suárez, L Guillevin, , D Jayne, JC Jennette, CM Kallenberg,

S Moiseev, P Novikov, A Radice, JA Savige, A Sinico, U Specks, M Zhao

