Voorbij de grenzen van een kwalitatieve test

(Sectie HIM)

SKML jaarcongres
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# Clinical significance of autoantibodies
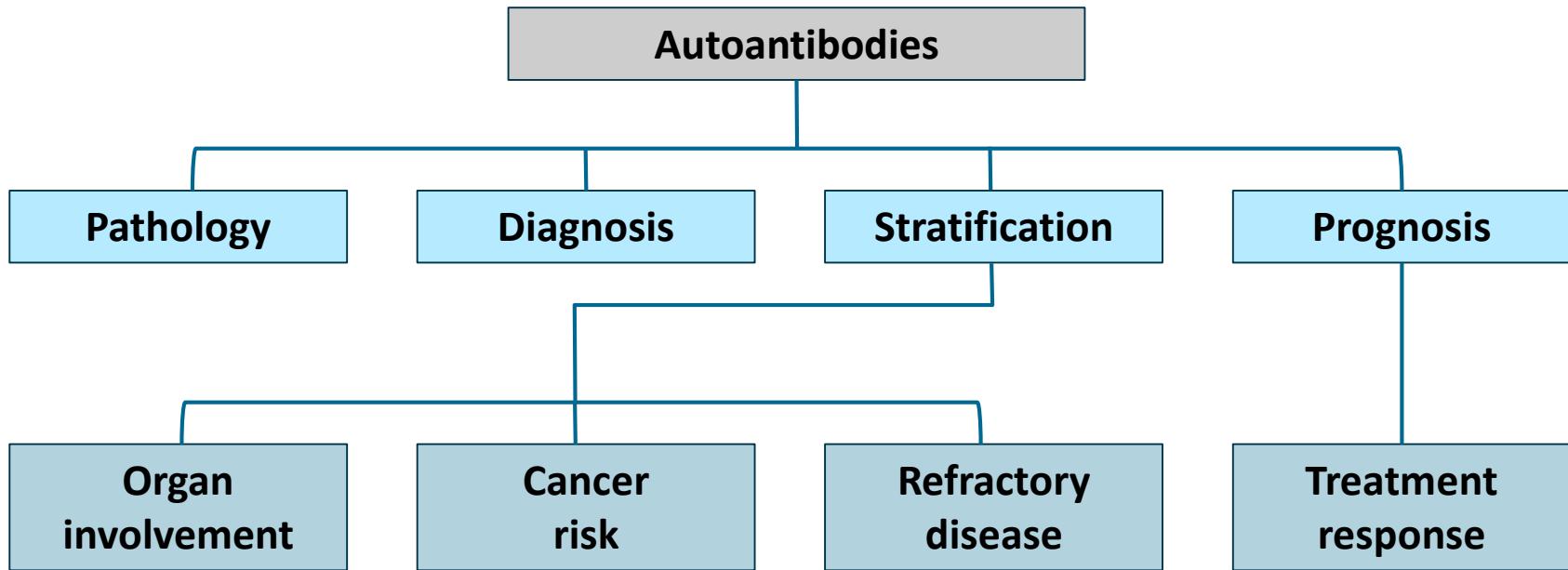
Autoantibody testing… what do we expect?

Patient

(Auto)antibody

(Auto)antigen

Method
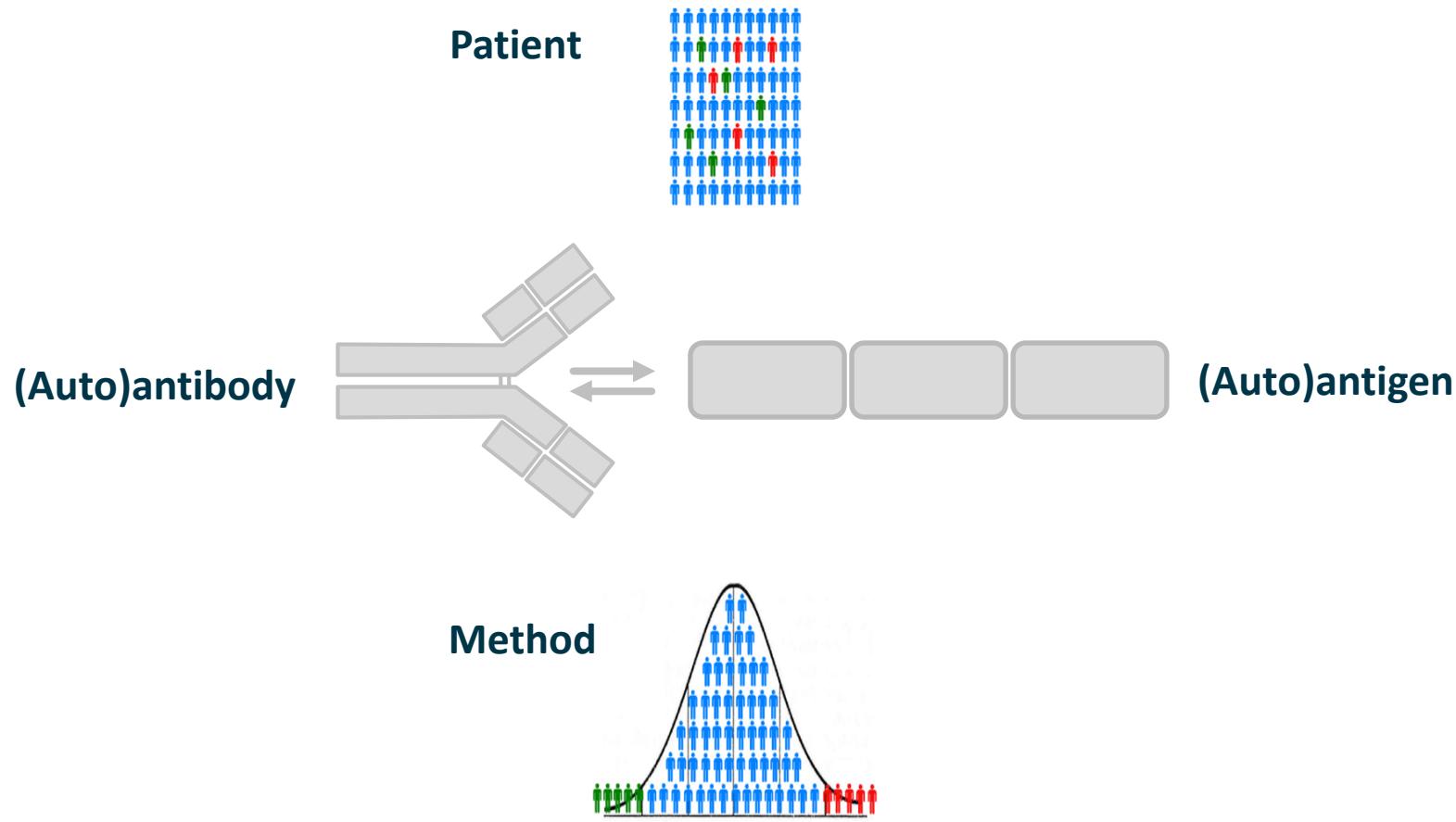
Overall reliability

- **Meaningful results**
- **No false positives, no false negatives**
- **Reproducibility:**
  - ➤ **same result day to day, month to month, year to year**
  - ➤ **same result if the sample is analysed in another lab**
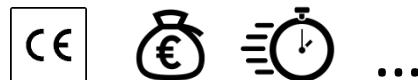
Autoantibody testing... reality check

Patient

(Auto)antibody ⇌ (Auto)antigen

Method

Anti dsDNA Kit Control

Anti dsDNA Pt Control

Batch #1: mean titer 62.2 AU, CV 10.7%
Batch #6: mean titer 82.5 AU, CV 5.8%
Batch #7: mean titer 76.6 AU, CV 4.5%
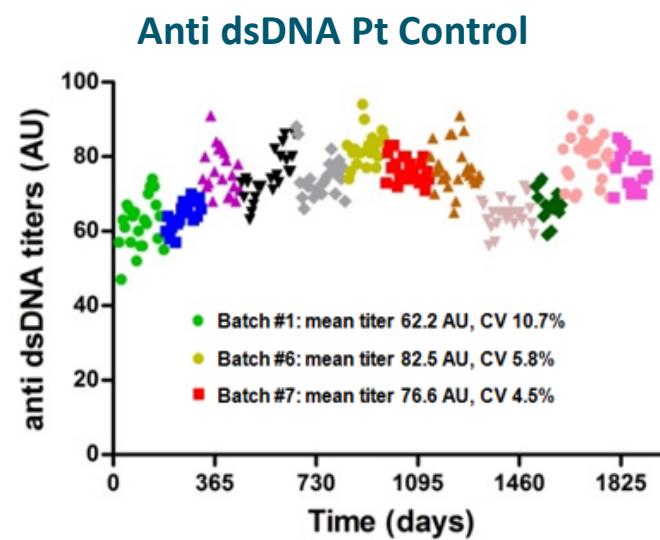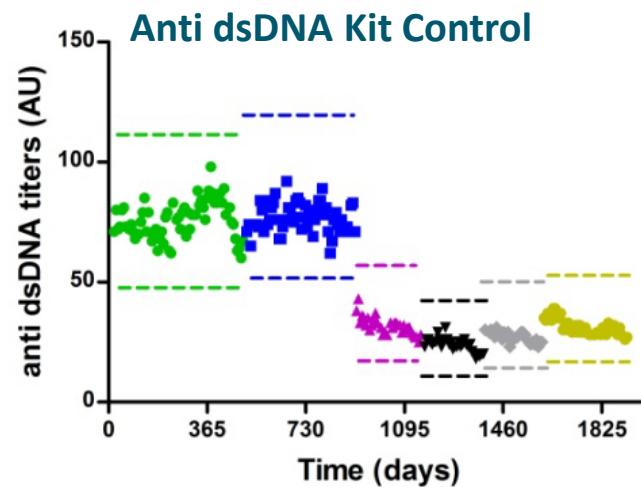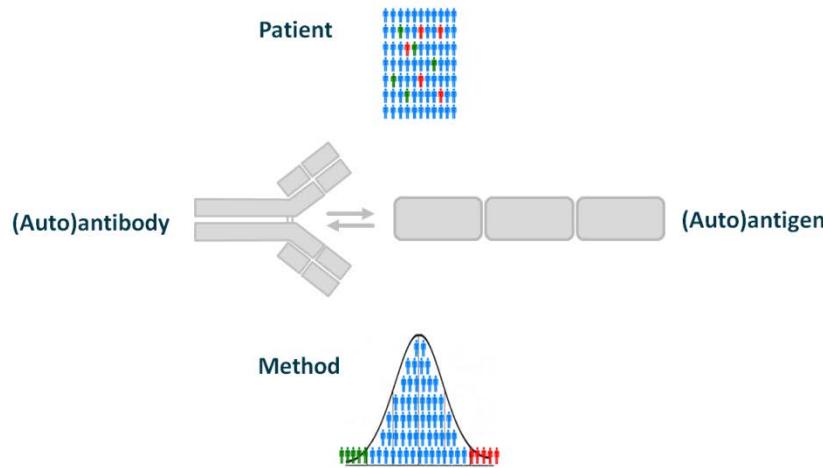
Standardisation of autoantibody testing... the challenges

Patient-variation
- Pre-clinical/Diagnostic/Follow-up
- Heterophillic Ab interference
- Treatment interference

Antibody-variation
- Isotype
- Subclass
- Affinity/avidity
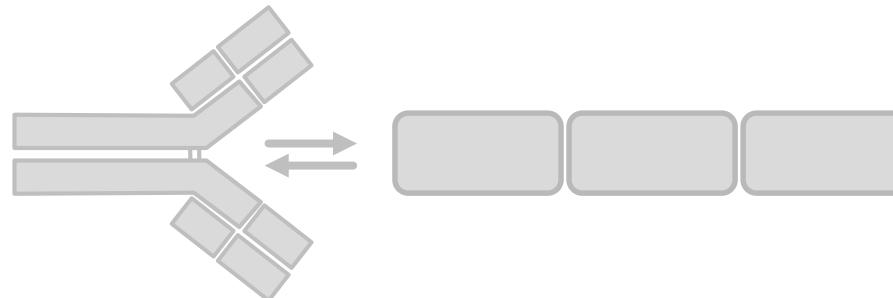
Antigen-variation
- Human/xeno
- Purified/synthetic
- Complex/protein/subunit
- Stability
- Co-factor needed
- Lot-to-lot variation

Absence of robust reference materials

Method-variation
- Different immuno-assays
  - Dilution
  - Diluent
  - Capture/direct
  - Qualitative vs (semi) quantitative
- Different detection systems
  - Conjugate
  - Manual/automated
  - Qualitative vs (semi)quantitative

Turbidimetry

Nephelometry
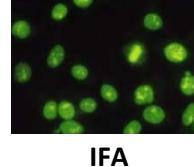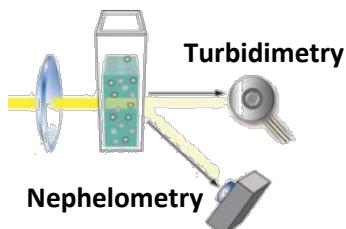
Immunodiffusion

Bead assay

ELISA

IFA
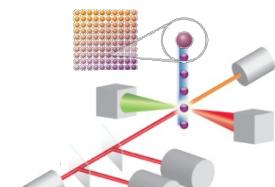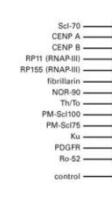
Blot
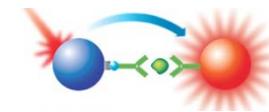
Luminescence

# The dilemma of choosing your reference material…

Test Black: IgM ab's
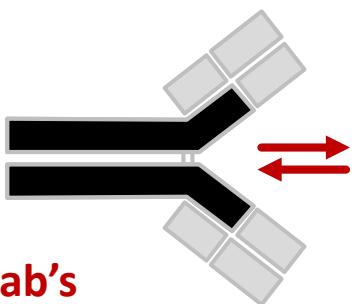
Test Blue: anti-human-Ag

Test Red: High affinity ab's
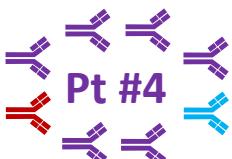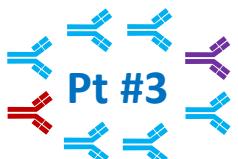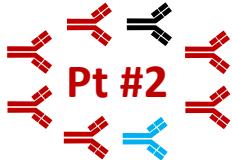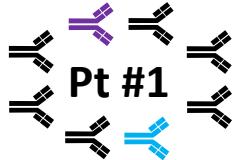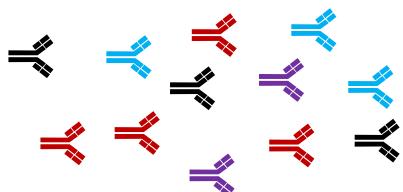
Test Purple: anti-domain-X

Pt #1

Pt #2

Pt #3

Pt #4

$R_{individual\ samples} = 0.56$
$R_{pooled\ samples} = 0.96$

- • 32 individual samples
- • 4 pools of 8 samples
- • 2 pool of 16 samples
- • 1 pool of 32 samples
- ◆ Relares pool

RF test B (AU)

RF test A (AU)

Falkenburg et al. CCLM 2018

Jacobs and Bossuyt. CCLM 2018

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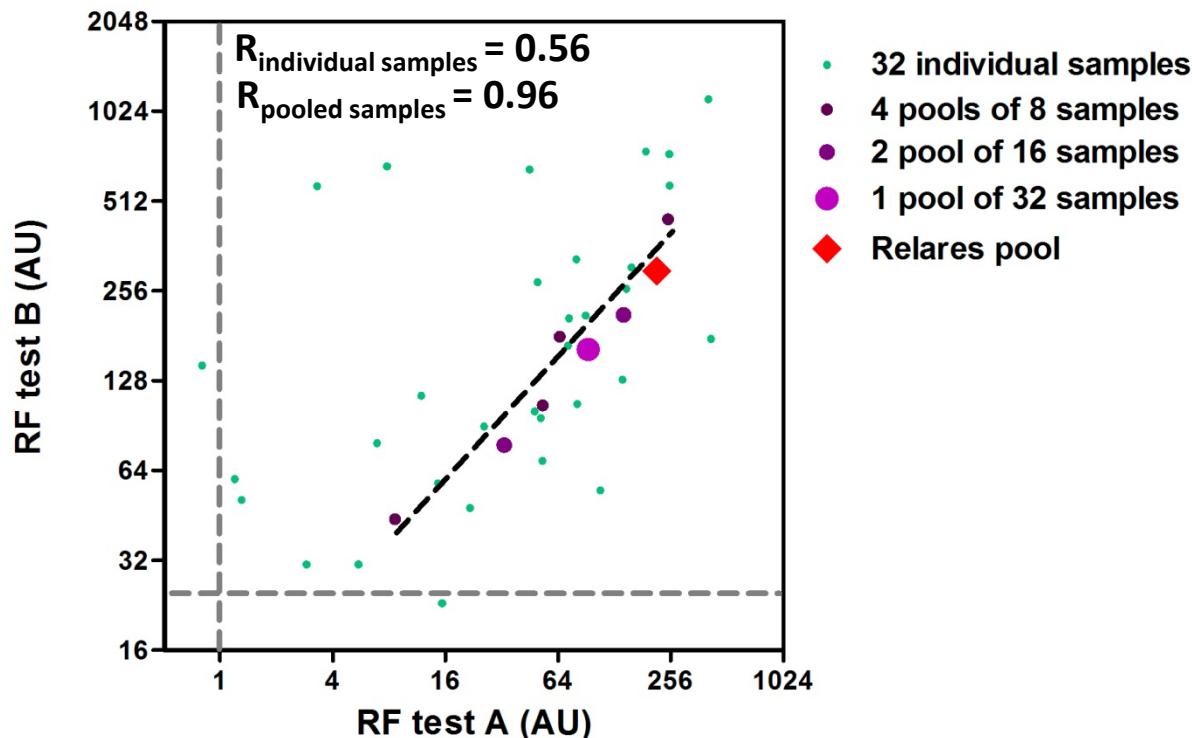
Practical reality of autoimmune diagnostics: quantitative elements

'Strong positive' ('>titer', 'xULN')

'Negative-test' | **'Positive-test'**

A

B

C

— no disease
---- disease
A 100% sensitivity
B most accurate
C 100% specificity

Frequency

TN

Δ

TP

Test Results
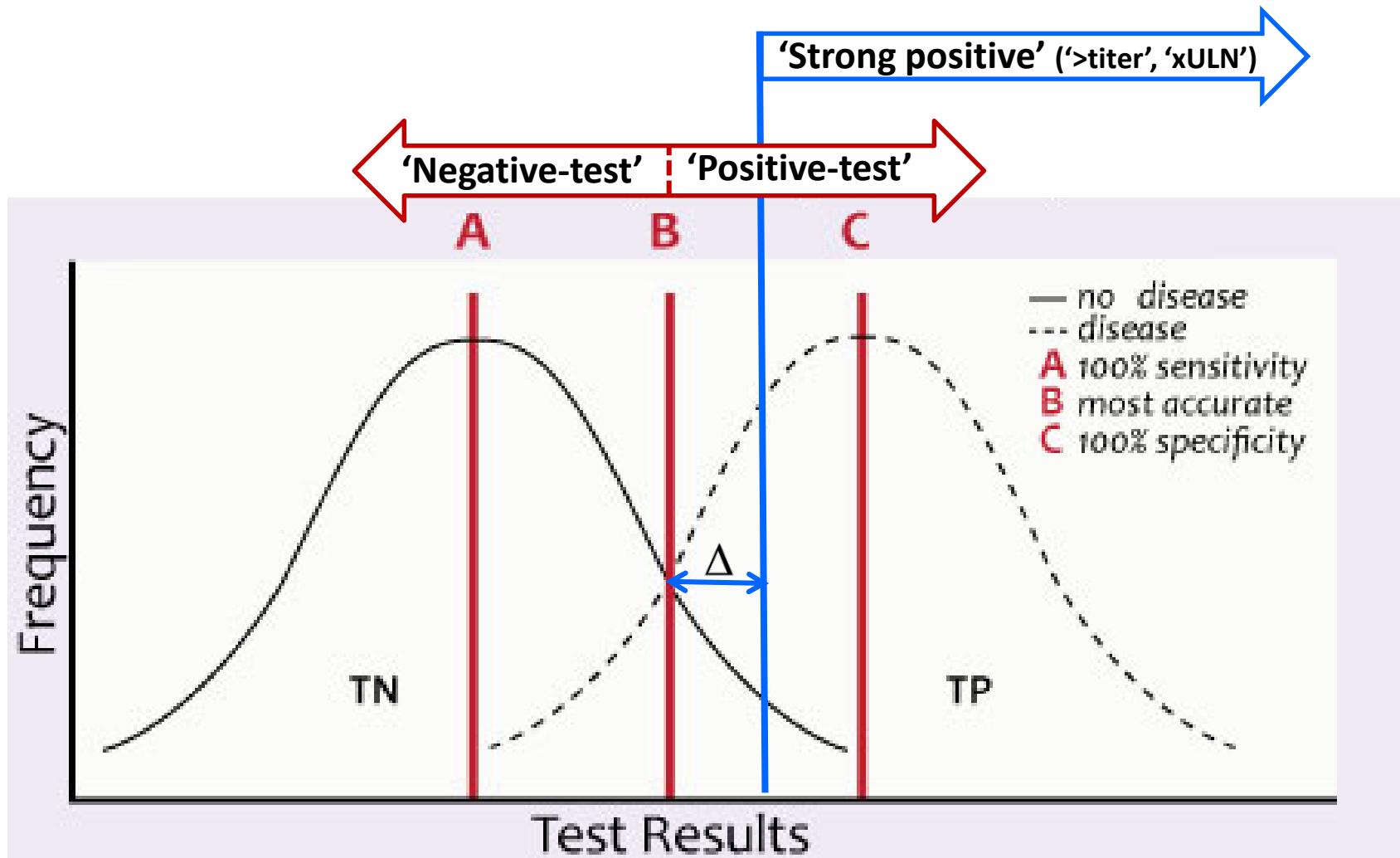
AID diagnosis = Clinical Features (score) AND Laboratory results (score)

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

|	Score
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
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
<6 weeks	0
≥6 weeks	1

≤1 ULN

>1≤3 ULN

>3 ULN

Performance assays has impact on ACR/EULAR classification of RA

WHO IgM RF standard = 25 IU/mL

CDC ACPA reference = 100 IU/mL

Van Hoovels et al. Ann Rheum Dis 2018

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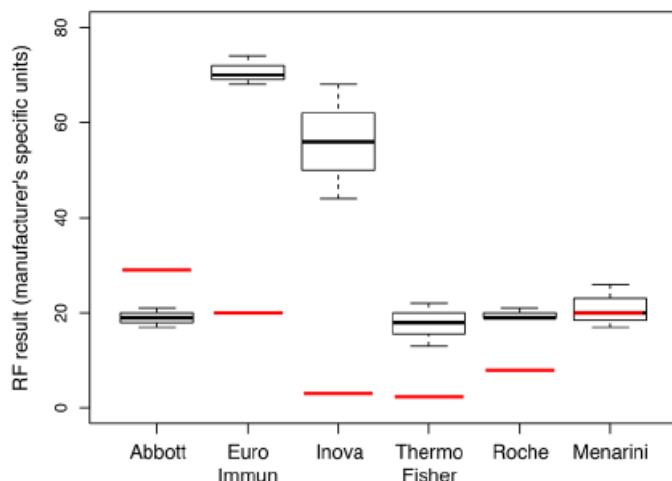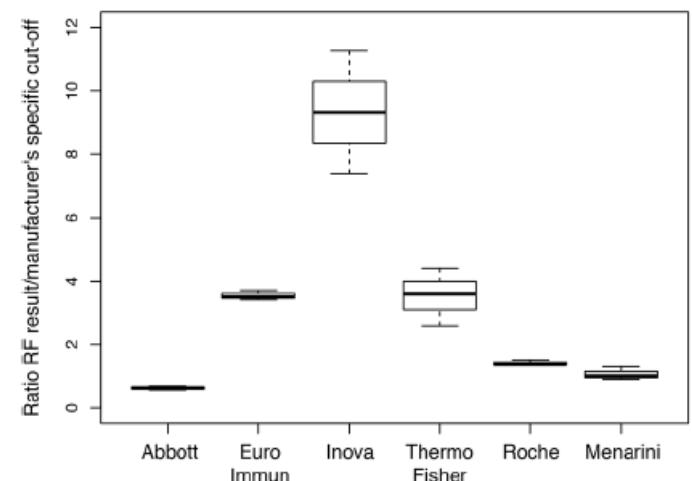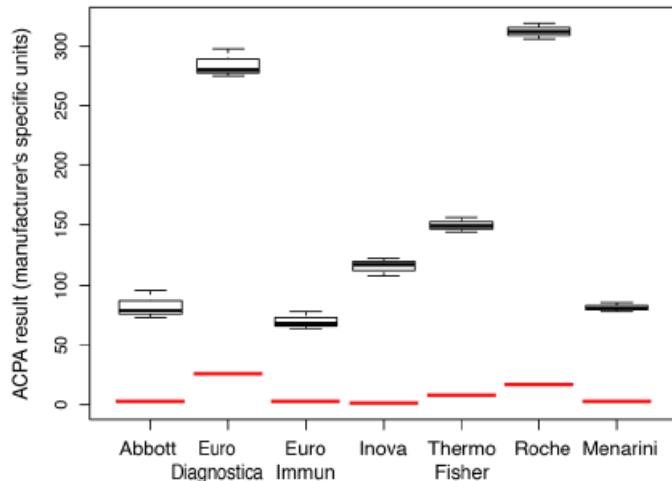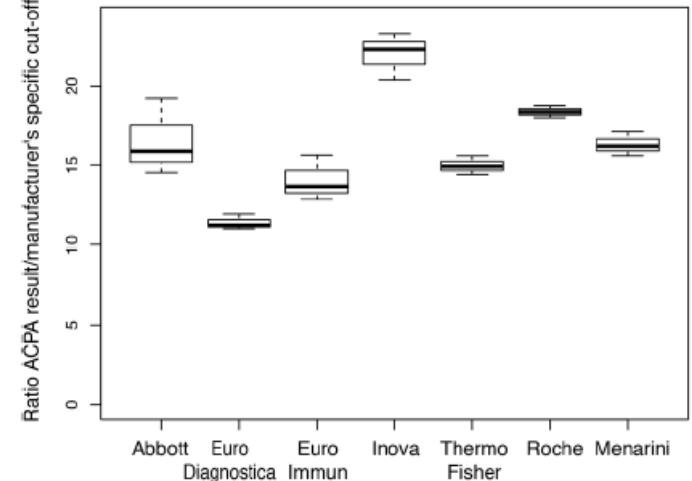
Reality of RF measurements in SKML EQA

negatief   dubieus   zwak pos   sterk pos
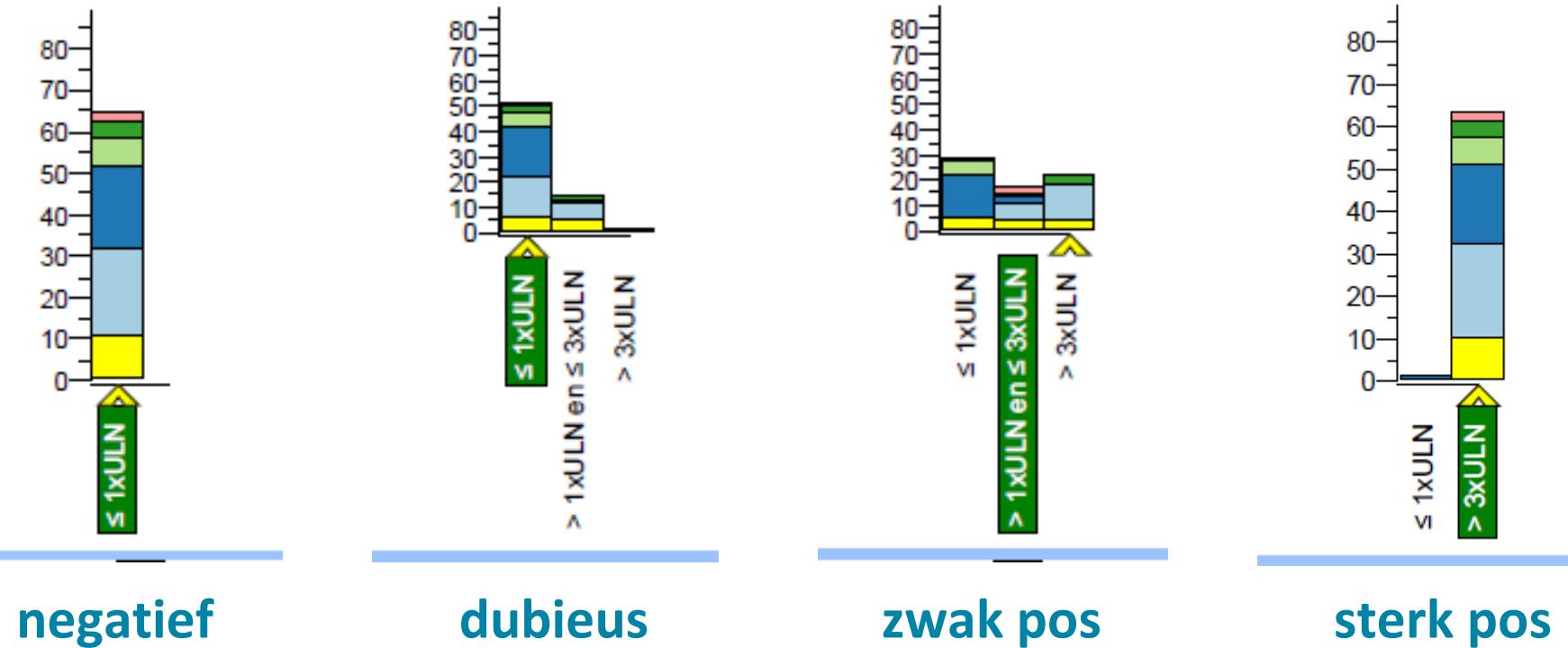
Legenda

- Onbekend
- EIIA IgM (FEIA)
- Turbidimetrisch
- Nefelometrisch
- ELISA
- Overig

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Coeliac disease guideline 2012, pediatric cases

Child/adolescent with symptoms suggestive of CD

Anti-TG2/tTG IgA and total IgA

Anti-TG2/tTG positive

Anti-TG2/tTG negative → Not CD

Consider further testing if:
IgA deficiency
Low gluten intake
High suspicion

Anti-TG2/tTG, >10x ULN

Anti-TG2/tTG, <10x ULN

EMA (or DGPA) & HLA DQ8/DQ2*

Not available

Gastroduodenoscopy with duodenal biopsies

EMA pos
HLA pos

EMA pos
HLA neg

EMA neg
HLA neg

EMA neg
HLA pos

Marsh 0-1

Marsh 2 or 3

CD

Consider:
False neg HLA biosies

Consider:
False pos anti-TG2/tTG

Consider:
Potential CD
False pos serology
False neg biopsy
Follow-up serology/biosy

CD

GFD
& FU

GFD
& FU

Transglutaminase IgA tests are not standardized at xULN

EliA (U)

Quanta Flash (CU)

24/183 (13%) of samples with < 100 U/ml (<10x ULN) in Phadia FEIA test, were > 200 CU (>10x ULN) in QUANTAflash CIA IgA anti-tTG

Unpublished data Bontkes/Norman

Variability in x ULN for top 6 IgA anti-tTG testing methods in UK NEQAS

Mean ULN +/− 1 SD for the top 6 methods by users

- Method 1
- Method 2
- Method 3
- Method 4
- Method 5
- Method 6

y-axis: Manufacturer x ULN (mean) for assay
x-axis: Sample
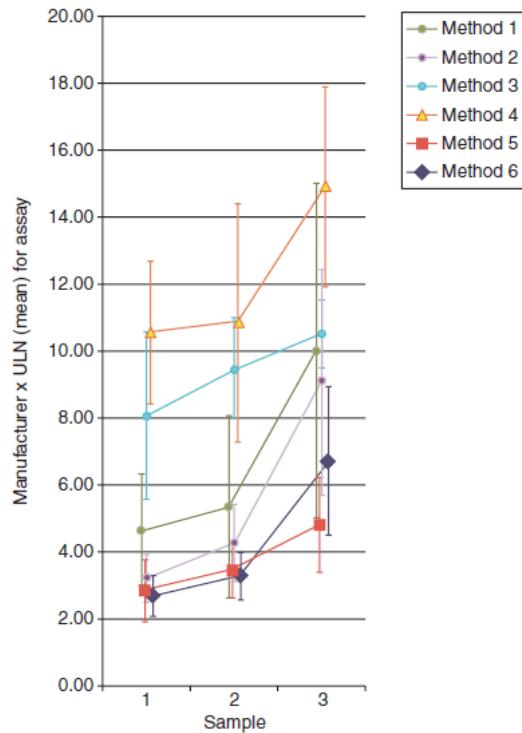
Egner et al, JPGN, 2012; 55:733-735

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xULN TGA cut-off should be established for each method

CD tTGA IgA
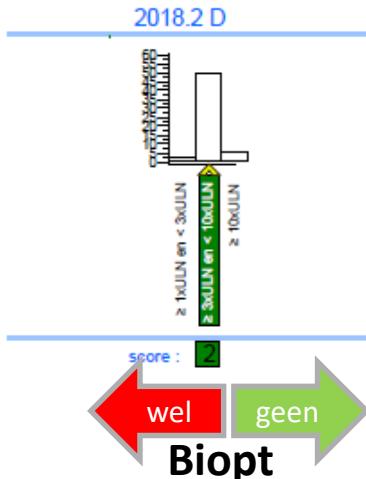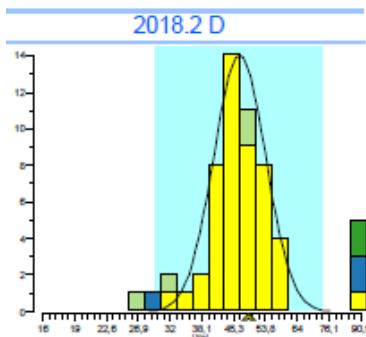
Correlation between Marsh ≥2 and different ULN of CLIA assay

TABLE 2. Correlation between different antitissue transglutaminase immunoglobulin A cut offs and presence of duodenal mucosa damage suggestive for celiac disease (Marsh ≥2)

	Anti-tTG IgA cut off	Marsh ≥2	PPV %	NPV %	LR+	LR−
Children	200 CU (10× ULN[*])	119/130	92	78	7.71	0.21
	560 CU (28× ULN)	85/86[†]	99	62	59.84	0.43
	1000 CU (50× ULN)	78/78	100	55	+∞	0.58
Adult	200 CU (10× ULN[*])	134/143	94	82	28.84	0.38
	350 CU (17.5× ULN)	110/112	98	78	94.69	0.48
	400 CU (20× ULN)	106/106	100	77	+∞	0.5

CU = chemiluminescent units; LR = likelihood ratio; NPV = negative predictive value; PPV = positive predictive value; ULN = upper limit of normal.
[*]Cut off suggested by ESPGHAN 2012 to avoid duodenal biopsy.
[†]One patient with anti-tTG IgA 960 CU, EMA 1:320; Marsh 1 on a small biopsy fragment.

Previtali, JPGN 2018:66

Values CLIA:
202 CU
308 CU

When cut-off Previtali is used (>28x ULN): biopsies should be taken, which is in agreement with the FEIA assay at >10x ULN

score : 2

wel — geen

Biopt

Legenda

| FEIA Phadia | CLIA |
| ELISA | Luminescentie |

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# Analytical issues of monoclonal FLC measurements
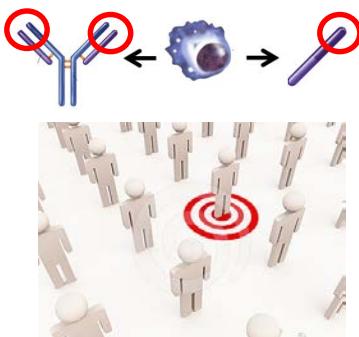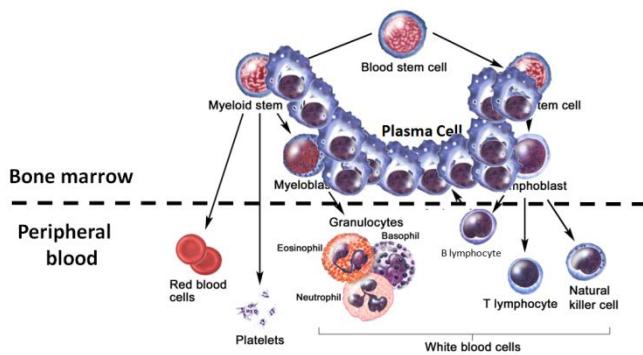
Variation in:

- AA sequence and size
- Charge (pI range 4.5 – 9)
- Glycosylation
- Polymerisation
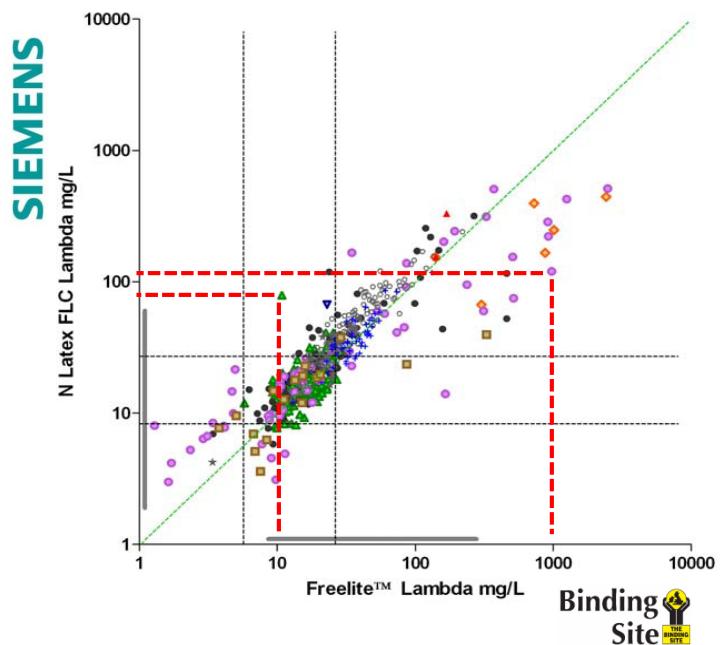
> - Both assays report results in mg/L
> - Which result is correct?
> - Patients switching from hospital…

Tate et al. Clin Biochem Rev 2009
Hoedemakers et al. Clim Chem Lab Med 2011
**Jacobs et al. Clin Chem Lab Med 2016**

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The importance of FLC standardisation/harmonisation

FLC ratio

2015.2 A

N Latex   Freelite

skml
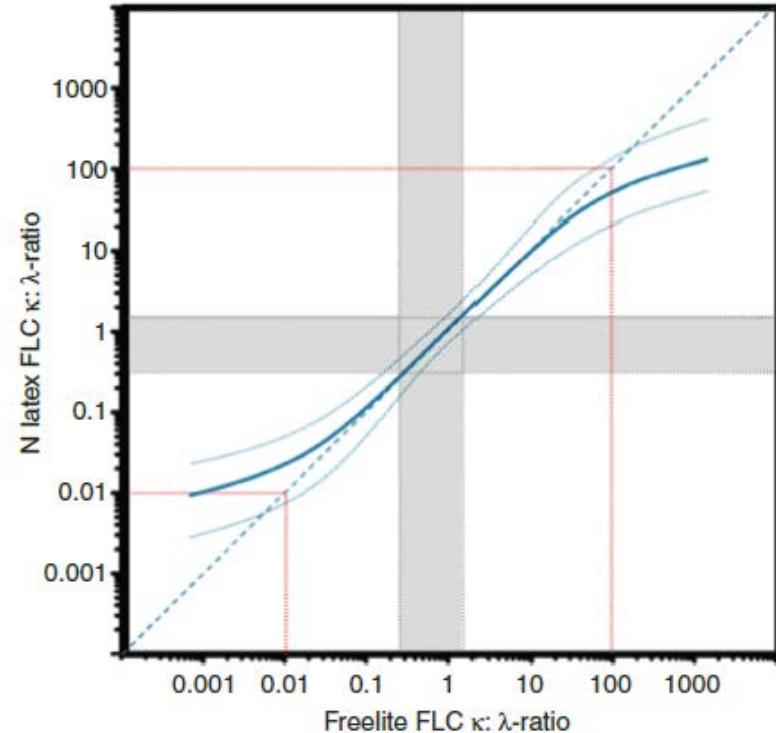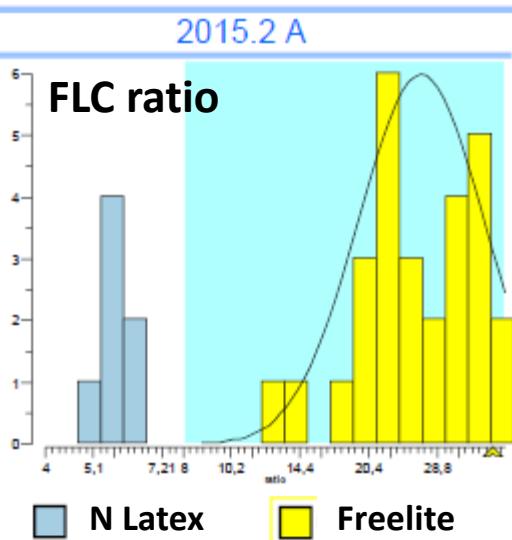
Newly Added Criteria To Diagnose MM

Clonal bone marrow plasma cells ≥10% or plasmacytoma plus one of these:	
	2-y Incidence of Organ Damage, %
Clonal marrow plasma cells ≥60%	95
Ratio of involved to uninvolved serum free light chain ≥100	80[a]
≥2 focal bone lesions ≥5 mm on MRI	70-80

Freelite FLC-ratio : N Latex FLC-ratio

100 ~ 30

Rajkumar et al. Lancet Oncology 2014

Jacobs, Tate & Merlini. Clin Chem Lab Med 2016

Bossuyt et al. Clin Chem Lab Med 2018

Conclusions

(Auto)antibody   **(Auto)antigen**
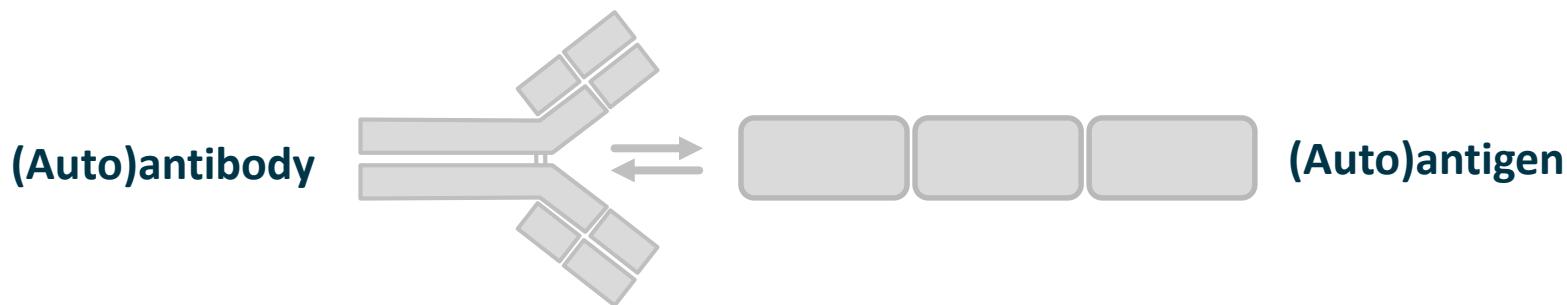
- **AID mostly developed/calibrated to be qualitative (Pos/Neg)**
- **Increasing number of 'quantitative elements' in guidelines**
- **AID mostly non-harmonized at these 'quantitative cut-offs'**
- **EQA useful tool to create awareness of differences between methods**

Thank you

Section Humoral Immunology
All board members, all coordinators.
Hetty Bontkes
Marco Schreurs
Cas Weykamp

Autoimmune diagnostics = Personalized diagnostics