Standardization of autoimmune tests: successes and challenges

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
Harmonisation of Autoantibody Testing Working Group

Dr. Joanna Sheldon
Protein Reference Unit
St. George’s Hospital
London

With thanks to
- The patients who generously donated their samples
- IFCC and IRMM
- The members of the WG-HAT
  - Ingrid Zegers (IRMM)
  - Allan Wiik
  - Pier Luigi Meroni
  - The companies for their support and participation

- Dr. Heinz Schimmel, Dr. Evanthia Monogioudi,
  Dr. Gustavo Martos-Sevilla Dr. Dana Hutu from the IRMM
- Dr. Emma Tuddenham from St. George's
Autoimmune testing..... what are we trying to do?

★ detect or quantify
★ IgG antibodies (or IgA, IgM)
★ to cell or tissue components “antigens”
★ support or exclude diagnosis
★ monitor disease
★ suggest prognosis

Antigen in tissue or purified and immobilised in e.g. ELISA

Enzyme or fluorescent conjugated anti IgG detects antibodies bound in the reaction

Bound antibody detected by addition of substrate OR fluorescence microscopy

Antibodies from the patients sample or standard or QC bind to the antigens
Detect.......... or quantify

Various substrates
• ethanol fixed neutrophils
• HEp2 cells
• Monkey kidney

Reported as
• Neg/pos
• Pattern
  • Homogeneous, speckled
  • c-ANCA or p-ANCA
• Titre or weak, strong, very strong etc.
• Subjective
• Skilled
• Hard to automate

Follow-up testing
• (more) specific
• ELISA based assays

Various substrates
• Purified
• Recombinant

Various methods
• ELISA based assays
• Multiplex assays

Reported as
• Number (concentration) with a Ref. range

Advantages and disadvantages
• Less subjective
• Easier to automate
• No standardisation
• Arbitrary values (units IU/ml, IU/L, U/ml, U/L)
• Values infer information that is not supportable
  • Patients with the same “concentration” of antibody may have completely different clinical features
  • Higher concentration worse disease is not true for many auto-antibodies
• Various ref. ranges and clinical “cut-off” values
• Marked methodological variation
Is there a problem with quantification?

Used with permission of UKNEQAS

Antibodies to myeloperoxidase, known positive sample – distribution of method means (n=38)

- Patients and clinicians move from one hospital to another
- A positive results potentially varying by 10x or 100x or 1000x is NOT SAFE
- Clinicians may not know of this variability
- Patients get different results depending on where the samples is analysed

Inappropriate interpretation of results
Inappropriate diagnosis, management or treatment

Range of method means for IgG anti MPO concentrations U/ml or IU/ml
Autoantibody testing…. the challenges

**No robust reference materials**

- **Antigen variation**
  - purified, synthetic, degraded, lot to lot variation

- **Antibody variations**
  - between patients, during disease, affinity and avidity, comparability with assay standard etc.

- **Method variation**
  - dilution, diluent, manual, automated, conjugate, capture, direct etc.

- **Detection system**
  - IgG, IgG & IgM, IgA, IgG subclasses, reactivity of detection antibody

We use arbitrary units because then all our assays look the same.

Does it really matter… it’s OK, we understand the results.

It will never work.

We have bigger worries with glucose or TSH or…

It is too complicated … and we need to use this method/analyser.
Challenge 1 – antibody

Binding of antibodies to antigens is variable – affinity and avidity

★ some patients make high affinity antibodies that bind very tightly
  • form stable complexes in vitro and in vivo
  • often are damaging e.g. through complement activation
  • are resilient to changes in temperature, ionic strength, pH etc.

★ some patients make low affinity antibodies that do not bind tightly
  • do not form very stable complexes
  • not so damaging
  • the complex can be separated by minor changes in temperature, ionic strength, pH etc.

★ the behaviour is not consistent through the disease course
★ the antibody used to “standardise” the method is unlikely to be representative of all patients auto-antibodies
★ QC materials are unlikely to be representative of patients samples
Challenge 2 – antigen

★ Purified
  • extracted from mammalian tissue
  • purification with heat, cold, salt, alcohol etc. may alter structure or denature
  • contaminated with other proteins and antigens
  • stability of preparations
  • reproducibility of preparations
  • expression of relevant antigenic epitopes

★ Synthetic
  • not necessarily identical to native (structurally or antigenically)
  • may lack important epitopes

★ Variability
  • Between manufacturers
  • Between lots
Challenge 2 – antigen e.g. proteinase 3

- 3 important epitopes
- At diagnosis, patients showed antibody reactivity to multiple parts of the molecule
- In remission, reactivity diminished
- During relapse, antibody reactivity changed e.g. from c-term to n-term
- Some patients showed multiple changes throughout their disease course
- Orientation of the proteinase 3 antigen in the assay is important

Extra text:

- Article text:
  - Epitope shift of proteinase-3 anti-neutrophil cytoplasmic antibodies in patients with small vessel vasculitis
  - D. Selga, M. Segelmark, L. Gunnarsson and T. Hellmark
  - Department of Immunology, Clinical Sciences in Lund, Lund University and Lund University Hospital, Sweden

- Journal logo:
  - The Journal of Immunology

- Article title:
  - Mapping of Conformational Epitopes on Human Proteinase 3, the Autoantigen of Wegener's Granulomatosis
  - Angelika Kuhl, Brice Kookman, Bert Utecht, Andreas Knaport, Ulf Schönsteiner, Ulrich Specks and Dieter E. Jennette
  - J Immunol 2010; 185(6):387-399; Prepublished online 7 June 2010; doi: 10.4049/jimmunol.0903887
  - http://www.jimmunol.org/content/185/1/387
Challenge 3 – method variation

Immunoassay

★ ~40 different methods for IgG anti proteinase 3 in UKNEQAS (including “in house”, “others” and “not stated”)
  ★ Manual ELISA
  ★ Automated ELISA
  ★ Automated variants of ELISA
  ★ Multiplex analysis

Various

★ sample dilution
★ Diluent – e.g. variations in ionic strength
★ “capture” – antigen specifically bound to “well” to increase sensitivity
★ “capture” – antigen specifically bound and orientated on the well to expose important epitopes and increase specificity and sensitivity
★ direct ELISAs
★ Combination of rapid (minutes) and slow (hours) methods
Challenge 4 – detection system

★ What is detected?
   ★ IgG
   ★ IgG and IgM
   ★ IgA

Possible variation in reactivity between
★ Classes of Ig
★ Subclasses of IgG
★ between standards and patient samples reacting to the detection antibody
Robust reference material for the IgG antibody to the antigen

Where to start? Likely to be more than 1 step

Antigen – may need more detailed characterisation or definition

Method – may need more detailed characterisation or definition

Detection system
IFCC/IRMM
Harmonisation of Autoantibody Testing
Working Group WG-HAT

★ Formed in 2010

★ A joint project between the IFCC and IRMM

★ Bring the excellence of the IRMM in preparation, analysis and validation of reference materials to autoimmune serology testing

★ Use similar rigorous protocols as were used on the preparation of ERM DA 470k (protein ref material)
Identified 5 analytes where the CONCENTRATION is likely to be important – IgG anti:

- Myeloperoxidase
- Proteinase 3
- Glomerular basement membrane
- Cyclic citrullinated peptide
- Cardiolipin/B2 GP1 antibodies

Define the protocol for future use
What do we expect of a lab test?
Precise, Accurate, Timely, Clinically useful, CORRECT

Easy analytes e.g. glucose, calcium, where there analyte is well defined and simple

Where we want to be for Autoimmune Serology

Where we are for Autoimmune serology

Difficult analytes e.g. proteins where defining the exact composition is complicated

Adapted from Traceability of Laboratory Test Results, Randox.
IgG anti MPO The process - briefly

- The raw material: a plasmapheresis material from a patient with antibodies to myeloperoxidase (and relevant clinical findings)
- Plasma converted into serum by the addition of protamine sulphate solution, incubation and centrifugation to remove the fibrin
- Delipidation by incubation with synthetic amorphous silica
- Dialysis against isotonic saline
- pH adjustment
- Preservatives added (sodium azide, benzamidine hydrochloride monohydrate and aprotinin)
- Sterilised through a 0.22µm filter
- 1ml serum transferred into vials under clean room conditions and lyophilised

- Evaluation process
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Explanation</th>
<th>ERM DA 476/IFCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous</td>
<td>Low and stated variability in concentration of the measurand between vials of the material</td>
<td>The uncertainty contribution for potential inhomogeneity is 0.85%</td>
</tr>
<tr>
<td>Stable</td>
<td>The material must be stable over its expected life-span</td>
<td>The material is stable e.g. during shipment (up to 2 weeks) and the on storage at -20°C and -70°C</td>
</tr>
<tr>
<td>Traceable</td>
<td>Related to a higher order reference material (usually national or international) through an unbroken chain of comparisons, all with stated uncertainty</td>
<td></td>
</tr>
<tr>
<td>Commutable</td>
<td>The characteristic of a reference material to behave in a comparable way to the samples (relevant to the intended use of the reference material)</td>
<td></td>
</tr>
<tr>
<td>Safe</td>
<td>Chemically and biologically safe (including tested as negative for HIV and Hepatitis B).</td>
<td>The raw material was tested and confirmed as negative for HIV, Hepatitis B and C</td>
</tr>
<tr>
<td>Ethical</td>
<td>Where relevant, samples from patients have been collected ethically and with appropriate agreement from the patients.</td>
<td>Consent given by patients for their material to be used</td>
</tr>
<tr>
<td>Available</td>
<td>There must be sufficient material that is readily available to relevant laboratories or companies over a time period of approx. 5-10 years. Produced with sufficient documentation to reproduce a comparable material when necessary.</td>
<td>Available from the IRMM</td>
</tr>
<tr>
<td>Certified</td>
<td>Ideally, reference material should be certified with stated uncertainties of the various characteristics</td>
<td>Certified in April 2015</td>
</tr>
</tbody>
</table>
Ideally, reference material should be certified with stated uncertainties of the various characteristics.

ERM-DA476/IFCC

- IgG anti MPO
- Certified value 84mg/L
- Uncertainty 9mg/L
IgG anti MPO

Traceable

The International Unit – only usable with WHO support

- used to compare the biological activity of different preparations of the same basic substance e.g. vitamins, hormones, vaccines etc.

- The mass or volume that constitutes one International Unit varies based on which substance is being measured.

- The WHO Expert Committee on Biological Standardisation provides a reference preparation of the agent, arbitrarily sets the number of IUs contained in that preparation, and specifies a biological procedure to compare other preparations of the same agent to the reference preparation.

- The number of IUs contained in a new substance is arbitrarily set, there is no equivalence between IU measurements of different biological agents.
  - Vitamin A: 1 IU is the equivalent of 0.3 μg retinol, or 0.6 μg beta-carotene
  - Vitamin C: 1 IU is 50 μg L-ascorbic acid

- Does the “arbitrary” International Unit meet our need for a TRACEABLE reference material? Is there anything that can?
ERM-DA470k/IFCC

★ Produced by the IRMM
★ Collaboration with Dade Behring (Marburg) and 20 laboratories across Europe
★ ERM-DA470K/IFCC distributed under strict transport guidelines to participating labs
★ Value transfer protocol detailed and strict
  ★ Storage, reconstitution, pipettes, balances, volumes, timing, operators, reagents, QC, assay performance etc.
★ Closed and open systems used for value transfer
★ Specific investigations on particular issues
IgG anti MPO Traceable

- We are measuring IgG…with specific antibody activity against myeloperoxidase

- The value assignment of IgG anti MPO was done using:
  - with dilutions of the candidate reference materials
  - Purified IgG anti MPO
    - affinity chromatography using a protein A column
    - Hi-trap column using purified human myeloperoxidase
    - Superdex 200 10/300 column
    - Confirmation of purity of material
  - Dilutions of ERM-DA470k/IFCC (CRM for IgG)

- These materials were measured under strict protocols by a variety of methods

Related to a higher order reference material (usually national or international) through an unbroken chain of comparisons, all with stated uncertainty.
IgG anti MPO Value assignment

- The affinity purified Abs or monoclonals can be assigned values that are traceable to the SI (via traceability to ERM-DA470k or UV-absorption measurements) - VITAL

- They can be used to make the values in the matrix material traceable to the SI.

- Certified values 84 mg/L (uncertainty 9mg/L)
IgG anti MPO Commutable

The characteristic of a reference material to behave in a comparable way to the samples (relevant to the intended use of the reference material)
Preliminary commutability study for Myeloperoxidase antibodies

Numerical recalibration of values for clinical samples using a conversion factor based on results for a candidate reference material (RM 5)
- good convergence for 6 out of 7 methods
- outliers remain and become more evident
  - this problem can not be solved by recalibration
The characteristic of a reference material to behave in a comparable way to the samples (relevant to the intended use of the reference material)

- Different formats of the reference material, all based on the same raw material have been tested and have been shown to be commutable for combinations of SEVEN methods
- It is expected that ERM-DA476/IFCC will be commutable for the majority of IgG anti MPO methods
- If another method is used, then commutability should be verified
The characteristic of a reference material to behave in a comparable way to the samples (relevant to the intended use of the reference material)

### Correlation coefficients 2nd commutability study

<table>
<thead>
<tr>
<th></th>
<th>Bioplex</th>
<th>Wieslab C</th>
<th>Phadia EliA</th>
<th>Euroimmuno</th>
<th>Varelisa</th>
<th>Orgentec</th>
<th>Quanta Lite</th>
<th>IMMCO</th>
<th>Biorad EIA</th>
<th>Bioflash</th>
<th>Aeskau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioplex</td>
<td>0.29</td>
<td>0.71</td>
<td>0.58</td>
<td>0.60</td>
<td>0.55</td>
<td>0.79</td>
<td>0.69</td>
<td>0.65</td>
<td>0.71</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Wieslab C</td>
<td>0.69</td>
<td>0.74</td>
<td>0.84</td>
<td>0.74</td>
<td>0.77</td>
<td>0.55</td>
<td>0.65</td>
<td>0.59</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phadia EliA</td>
<td>0.83</td>
<td>0.88</td>
<td>0.90</td>
<td>0.94</td>
<td>0.79</td>
<td>0.95</td>
<td>0.90</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euroimmuno</td>
<td>0.79</td>
<td>0.90</td>
<td>0.84</td>
<td>0.66</td>
<td>0.79</td>
<td>0.65</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varelisa</td>
<td>0.83</td>
<td>0.95</td>
<td>0.79</td>
<td>0.90</td>
<td>0.85</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orgentec</td>
<td>0.88</td>
<td>0.74</td>
<td>0.89</td>
<td>0.95</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quanta Lite</td>
<td>0.85</td>
<td>0.93</td>
<td>0.87</td>
<td>0.89</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMMCO</td>
<td>0.88</td>
<td>0.81</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biorad EIA</td>
<td></td>
<td>0.92</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioflash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.74</td>
</tr>
</tbody>
</table>
Clinical interpretation of results using cut-offs provided by manufacturers

<table>
<thead>
<tr>
<th>averages</th>
<th>W Capture</th>
<th>IMMCO</th>
<th>BioRad EIA</th>
<th>Orgentec</th>
<th>EliA_Phadia</th>
<th>QuantaLite</th>
<th>Inova</th>
<th>BIOFLASH</th>
<th>AESKULISA</th>
<th>Varelisa</th>
<th>Euroimmun</th>
<th>Bioplex2200</th>
</tr>
</thead>
<tbody>
<tr>
<td>1137.77</td>
<td>133.19</td>
<td>110.91</td>
<td>109.91</td>
<td>98.02</td>
<td>97.91</td>
<td>81.67</td>
<td>73.86</td>
<td>70.76</td>
<td>37.47</td>
<td>32.30</td>
<td>31.32</td>
<td>28.85</td>
</tr>
<tr>
<td>2540.66</td>
<td>2540.66</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
<td>331.38</td>
</tr>
<tr>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
<td>191.23</td>
</tr>
<tr>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
<td>41.02</td>
</tr>
<tr>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
<td>80.02</td>
</tr>
<tr>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
<td>57.96</td>
</tr>
<tr>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
<td>406.91</td>
</tr>
<tr>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
<td>145.72</td>
</tr>
<tr>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
<td>168.76</td>
</tr>
<tr>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
<td>92.07</td>
</tr>
</tbody>
</table>

9 samples with the same interpretation in all methods, 13 in 10 out of 11 methods
<table>
<thead>
<tr>
<th>Patient population (n) vs comparison group (n)</th>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUC/ROC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPA (86) vs non-vasculitic disease (450)20</td>
<td>IFT</td>
<td>92</td>
<td>99</td>
<td>Nd</td>
<td>Nd</td>
<td>0.96 (0.94–0.98)</td>
<td>Histological diagnosis Retrospective study</td>
</tr>
<tr>
<td></td>
<td>Direct PR3-ANCA ELISA</td>
<td>60</td>
<td>99</td>
<td>Nd</td>
<td>Nd</td>
<td>0.80 (0.76–0.83)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture ELISA</td>
<td>72</td>
<td>99.3</td>
<td>Nd</td>
<td>Nd</td>
<td>0.86 (0.82–0.89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA</td>
<td>96</td>
<td>98.5</td>
<td>Nd</td>
<td>Nd</td>
<td>0.96 (0.94–0.98)</td>
<td></td>
</tr>
<tr>
<td>GPA (232) vs inflammatory diseases (661)23</td>
<td>IFT</td>
<td>77.9</td>
<td>90.9</td>
<td>73</td>
<td>88</td>
<td>93</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA</td>
<td>80.4</td>
<td>97.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPA (59*) vs inflammatory and infectious diseases (585)30</td>
<td>Hn-hr PR3-ANCA ELISA</td>
<td>94</td>
<td>99 (predefined)</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Histological diagnosis Retrospective study</td>
</tr>
<tr>
<td></td>
<td>Capture ELISA</td>
<td>66</td>
<td></td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Direct (hn) PR3-ANCA</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPA (34) vs SLE (65)21</td>
<td>Direct PR3-ANCA</td>
<td>97.1</td>
<td>98.4</td>
<td>Nd</td>
<td>Nd</td>
<td>0.999 (0.947–1.00)</td>
<td>Clinical diagnosis Retrospective study</td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPA (40) vs RA or SLE (20)22</td>
<td>IFT</td>
<td>62.5</td>
<td>45–55</td>
<td>95–100</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>Direct PR3-ANCA (n=5 kits)</td>
<td>60–62.5</td>
<td>60–62.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture ELISA (n=2 kits)</td>
<td>60</td>
<td>60–62.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA (n=4 kits)</td>
<td>60–62.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPA (40) vs RA or SLE (20)12</td>
<td>IFT</td>
<td>82.5</td>
<td>95–100</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Histological diagnosis Retrospective study</td>
</tr>
<tr>
<td></td>
<td>Direct MPO-ANCA ELISA (n=8 kits)</td>
<td>62.5–85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture ELISA (n=2 kits)</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA (n=1 kit)</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPA (55) vs suspected vasculitis (175)23</td>
<td>IFT</td>
<td>69.1</td>
<td>100</td>
<td>93.4–96.4</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>Direct PR3-ANCA ELISA (n=2 kits)</td>
<td>61.8–72.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture ELISA (n=2 kits)</td>
<td>70.9–72.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchor ELISA (n=3 kits)</td>
<td>61.8–72.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other assays (n=2)</td>
<td>72.7–74.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*47 of 59 patients in the GPA group had a cytoplasmic ANCA pattern on IFT. Abbreviations: ANCA, antineutrophil cytoplasmic antibody; AUC, area under the curve; GPA, granulomatosis with polyangiitis; hn, human native; hr, human recombinant; IFT, indirect immunofluorescence technique; MPA, microscopic polyangiitis; MPO, myeloperoxidase; Nd, not determined; NPV, negative predictive value; PPV, positive predictive value; PR3, proteinase 3; RA, rheumatoid arthritis; ROC, receiver operating characteristics; SLE, systemic lupus erythematosus.
We can improve the numbers….

- Introduction and adoption of traceable commutable reference materials should reduce the variability in the values for autoantibody measurements.

- It will not solve the inherent variability in the values given by certain patient samples in different methods.

- It should help identify methodological outliers and guide investigation and improvements.
Standardization in autoimmune testing
IFCC/JRC-IRMM WG-HAT

Successes
★ huge advances
★ defined processes for making CRM for autoantibodies
★ further materials in progress

Challenges
★ introducing the materials
★ evaluate the impact e.g. on patient and EQA
★ consider further harmonisation or better definition of:
  ★ antigen type/source, method, detection system