DIASYS DIAGNOSTIC SYSTEMS

Challenges in developing IVD tests for innovative biomarkers: what an IVD manufacturer needs for accurate measurements

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IVDR (EU) 2017/746

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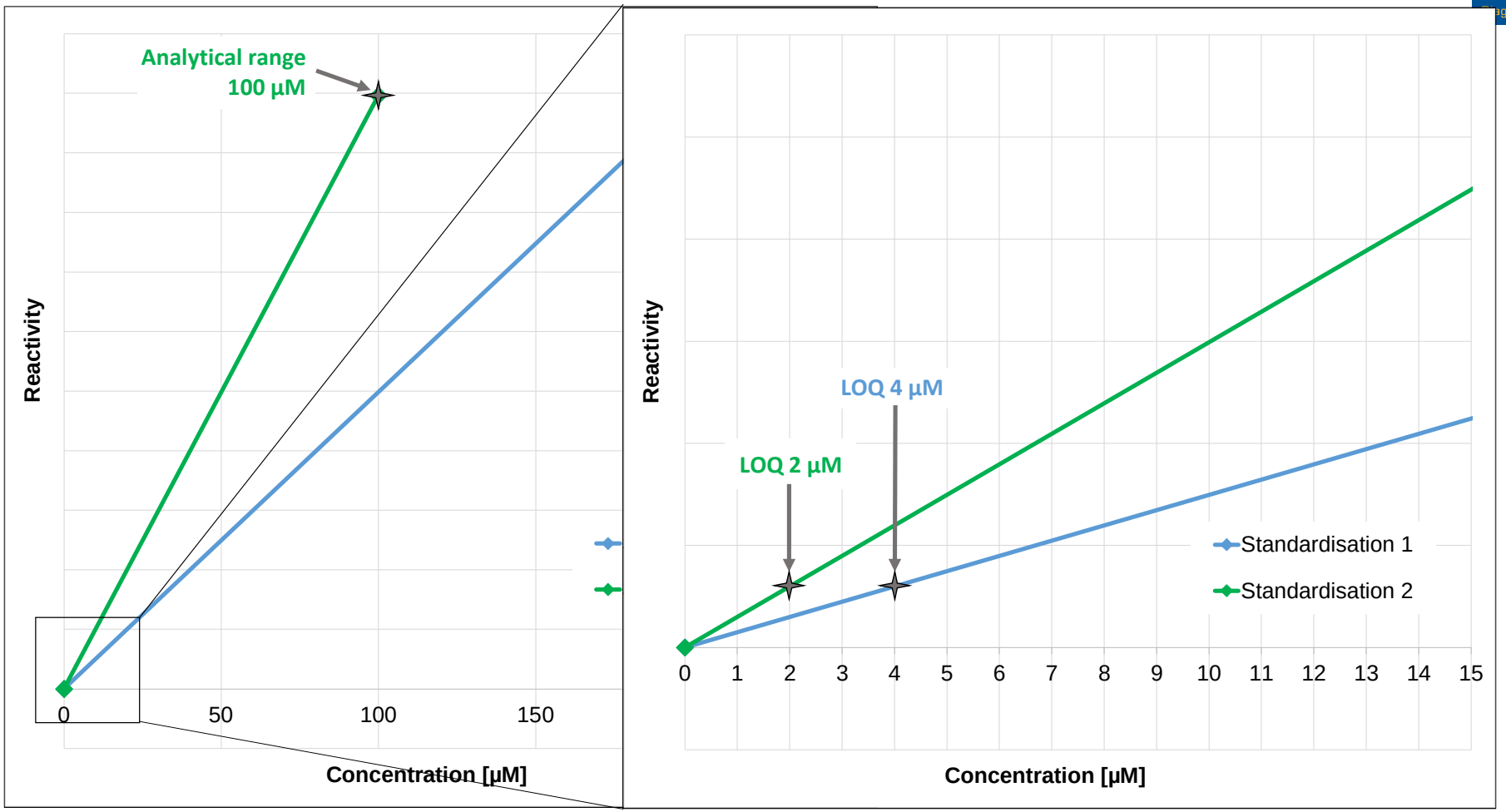
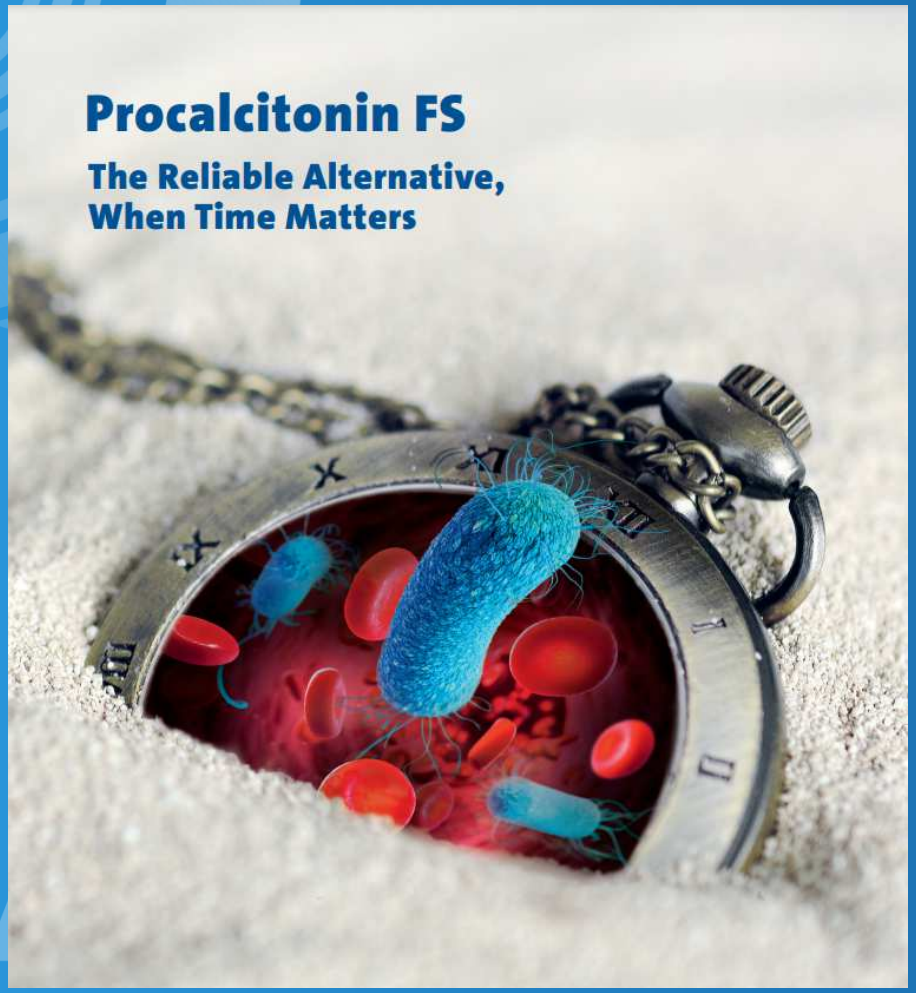
Annex I – Chapter II “Requirements regarding performance, design and manufacture”

9.3 Where the performance of devices depends on the use of calibrators and/or control materials, the metrological traceability of values assigned to calibrators and/or control materials shall be assured through suitable reference measurement procedures and/or suitable reference materials of a higher metrological order. Where available, metrological traceability of values assigned to calibrators and control materials shall be assured to certified reference materials or reference measurement procedures.

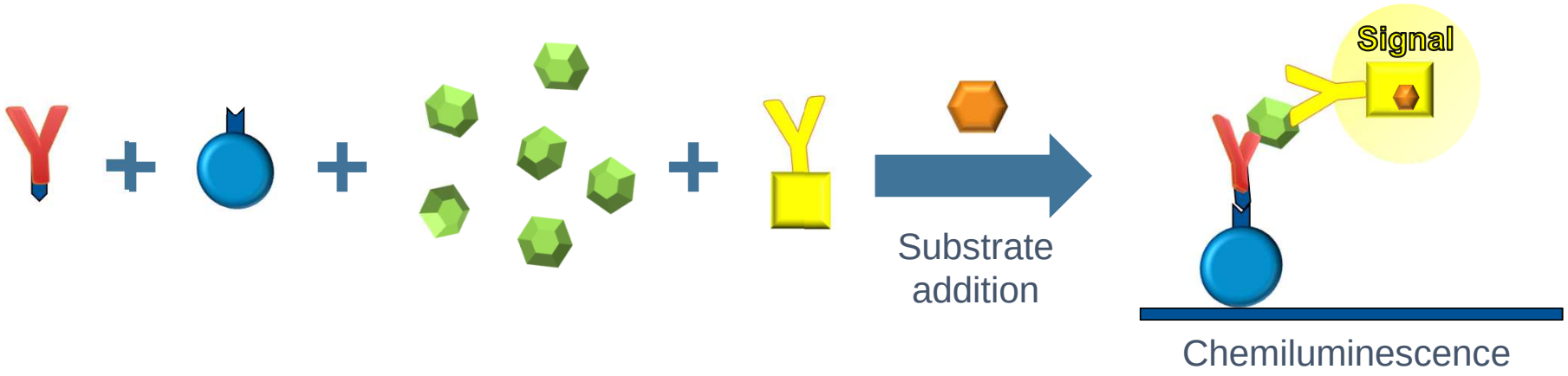
Direct effects of a (missing) standardisation



Procalcitonin



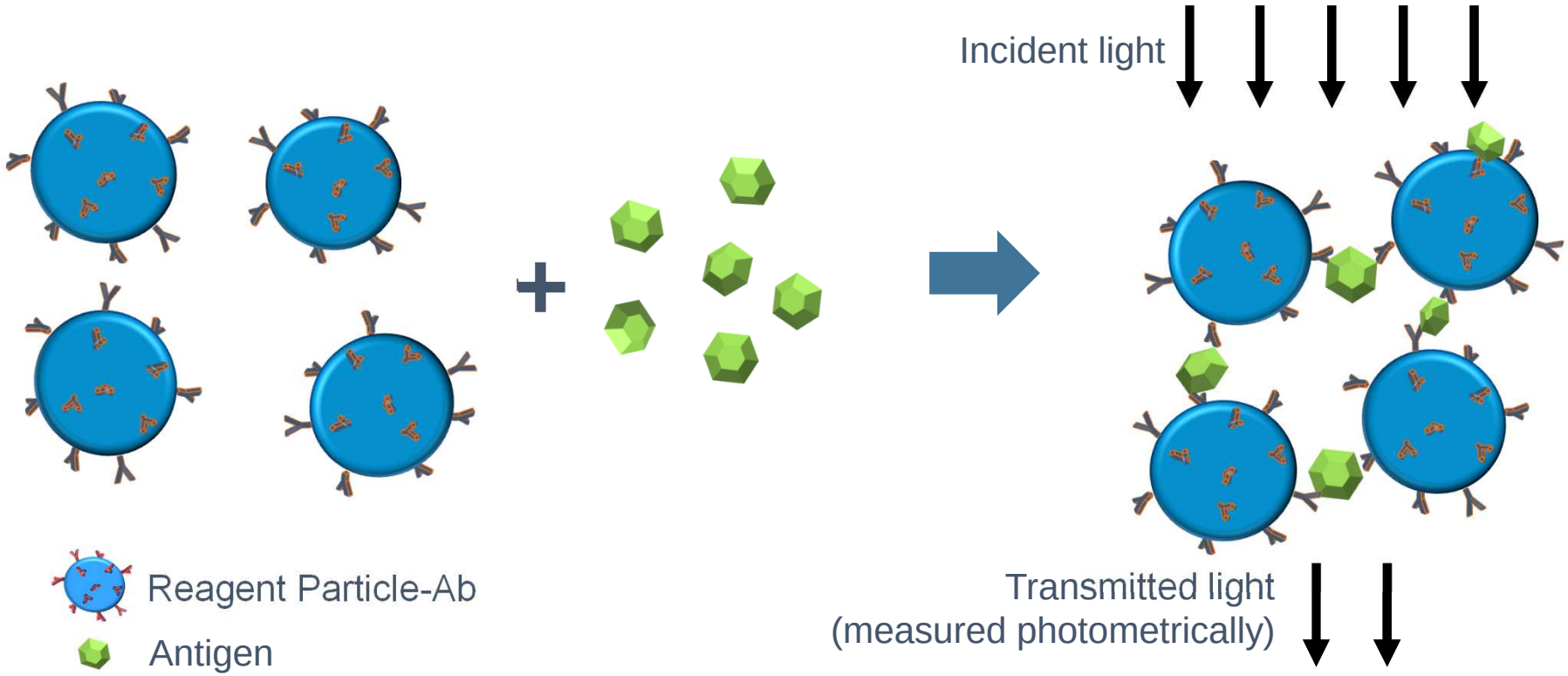
Chemiluminescence Immunoassay (CLIA) technology



- Biotin-labelled antibody
- Streptavidin labelled-magnetic beads
- Antigen
- Detection system labelled-antibody (Acridinium-ester, Ruthenium, Luminol, etc.)
- Substrate

Courtesy of Leoni Wey

Particle Enhanced Turbidimetric Immunoassay (PETIA) technology



Courtesy of Leoni Wey

CLIA vs. PETIA – Example PCT

Parameter	CLIA	PETIA
Precision	++ (CV% ca. 2% @0.49 µg/L)	+ (CV% ca. 7% @0.45 µg/L)
Functional sensitivity (LOQ)	++ (0.06 µg/L ca.)	+ (0.2 µg/L ca.)
Analytical range (Δ linearity-LOQ)	++ (up to 100 µg/L)	+ (50 µg/L)
Interferences	++	+
Stability	++	++
Cost structure	High	Low
Sample volume	High (average ca. 100 µL)	Small (ca. 10-15 µL)
Waste production	Disposable materials	No disposable materials needed
Applicability	Low (instrument specific)	Very high (every photometric system)
Time-to-result	Longer (18-40 min)	Short (10 min)

Influence of the Antibody – pAbs vs. mAbs

BRAHMS PCT Sensitive Kryptor

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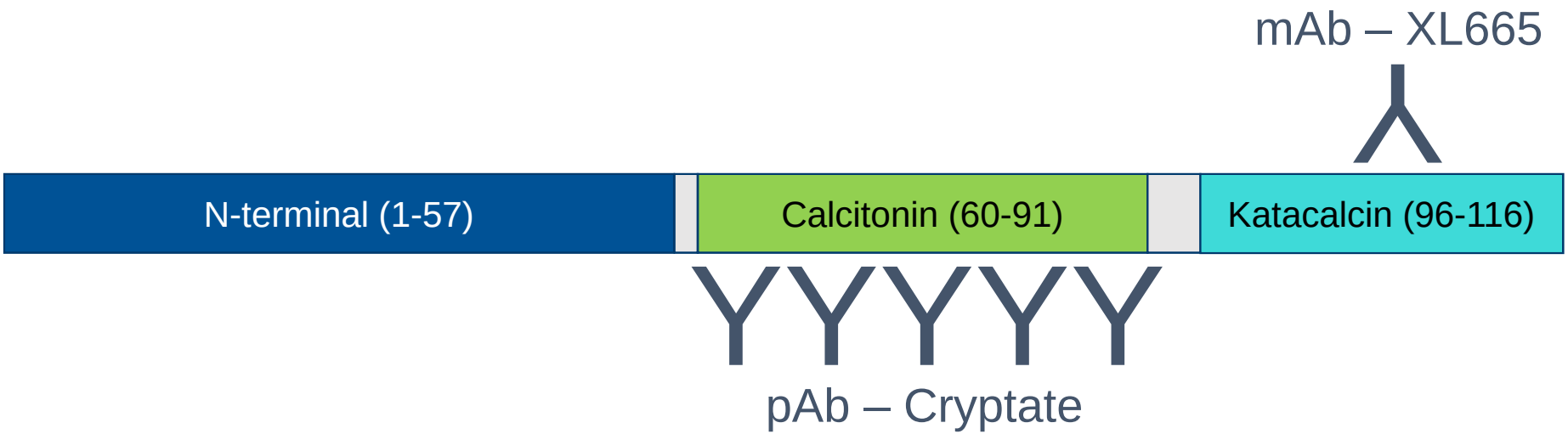

Thermo-BRAHMS package insert

Influence of the Antibody – pAbs vs. mAbs

DiaSys PCT FS (PETIA)

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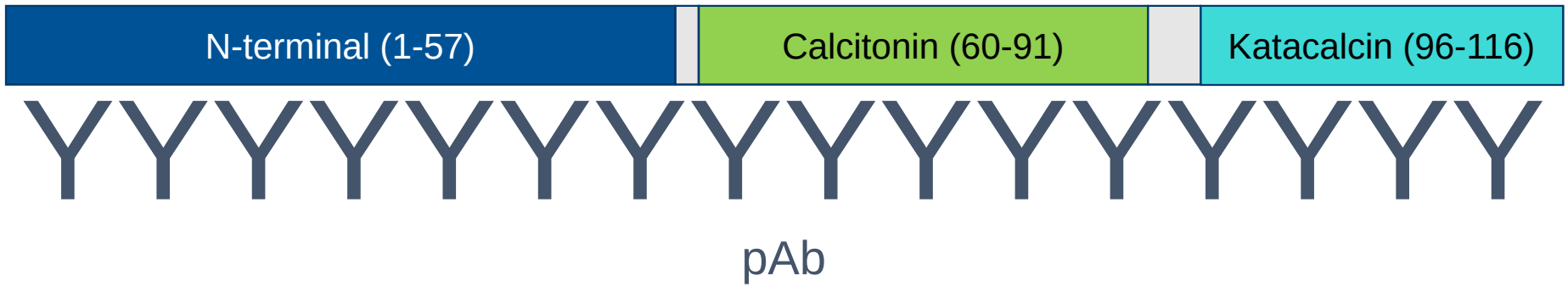

Influence of the Antibody – pAbs vs. mAbs

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Diagnostic Systems

Architect BRAHMS PCT (Abbott)

mAb (mouse) – Acridinium (tracer)

(23-101)

mAb (rat) – Mag Beads (capture)

Abbott 510K, K170652

Influence of the Antibody – pAbs vs. mAbs

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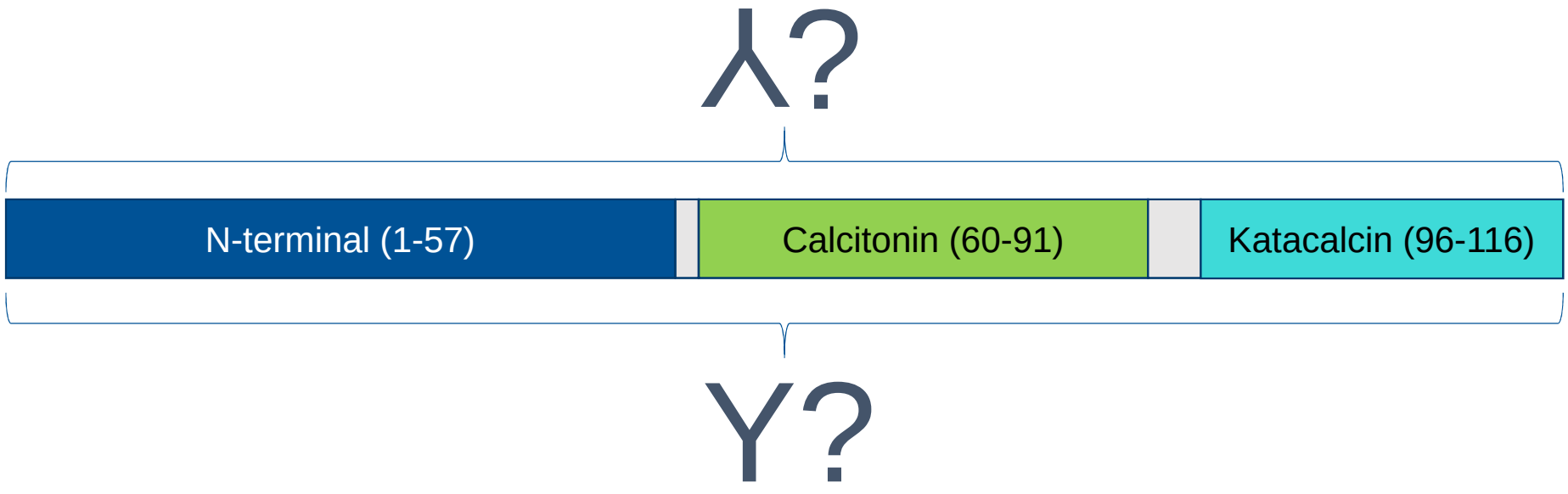
Diagnostic Systems

Elecsys BRAHMS PCT (Roche Diagnostics)

mAb (mouse) – Ruthenium (tracer)



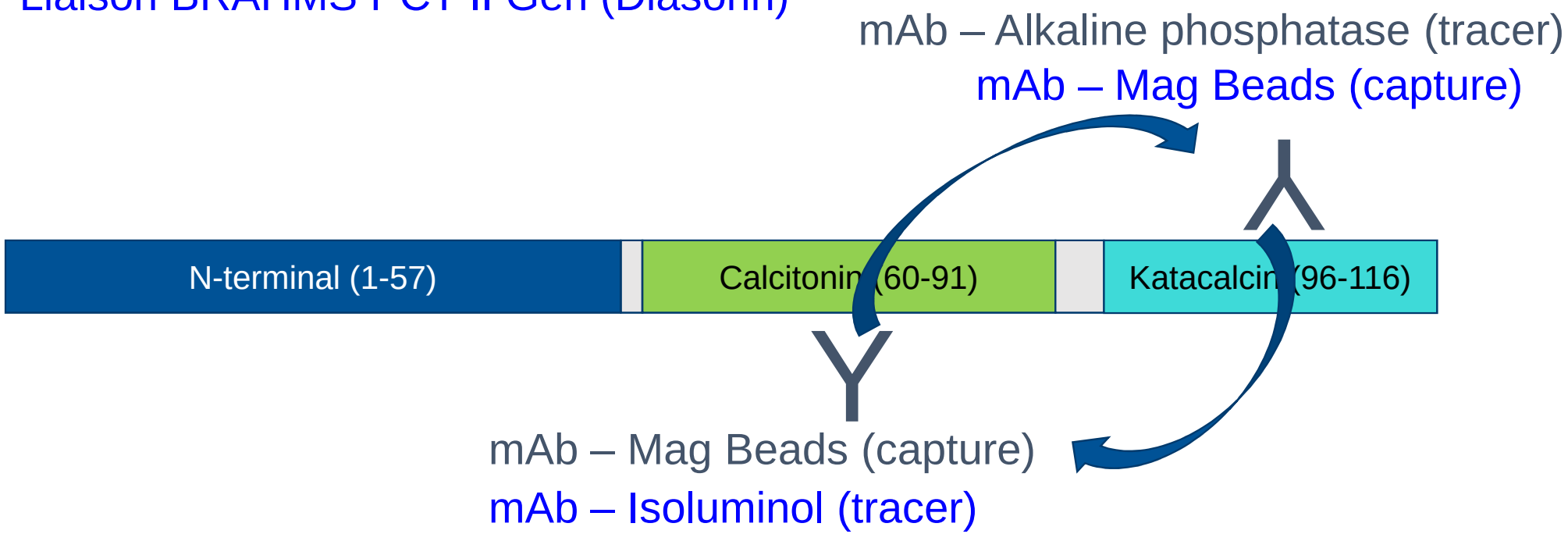
mAb (mouse) – Mag Beads (capture)

Huynh H. et al., 2021
https://doi.org/*10.1016/j.cca.2021.01.004*

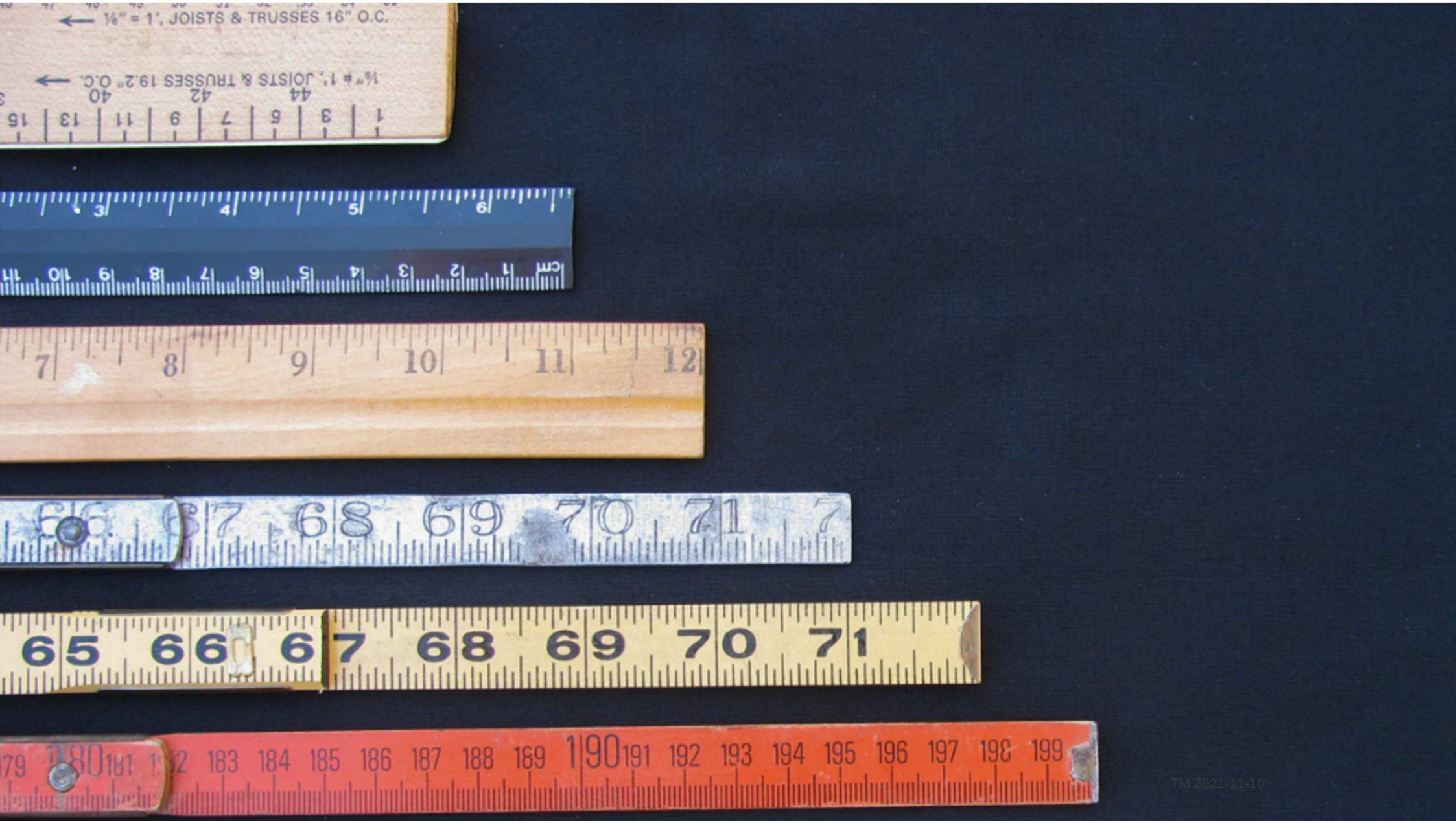
Influence of the Antibody – pAbs vs. mAbs

Lumipulse G BRAHMS PCT (Fujirebio)

Liaison BRAHMS PCT II Gen (Diasorin)



Huynh H. et al., 2021
https://doi.org/*10.1016/j.cca.2021.01.004*


⟵ 1/8" = 1', JOISTS & TRUSSES 16" O.C.
⟵ 1/4" = 1', JOISTS & TRUSSES 19.2" O.C.
1 3 5 7 9 11 13 15
40 42 44
3 4 5 6
cm 1 2 3 4 5 6 7 8 9 10 11
7 8 9 10 11 12
66 67 68 69 70 71
65 66 67 68 69 70 71
179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199

Calibrator standardization on an already existing immunoassay (used as reference)

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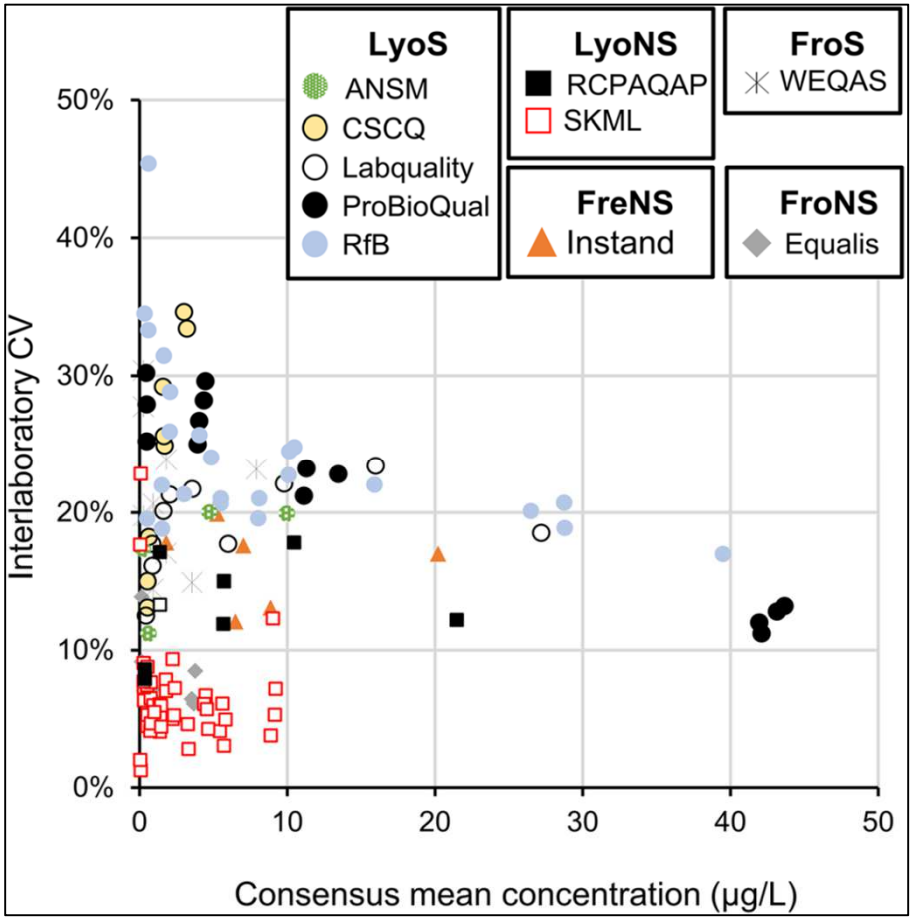
	Gravimetric value [ng/mL]	CLIA A [ng/mL]	CLIA B [ng/mL]
Level 5	53.52	26.01	27.10
Level 4	26.58	15.41	11.92
Level 3	12.65	7.74	5.60
Level 2	5.08	2.27	2.21
Level 1	1.15	0.46	0.54
Level 0	0.00	0.00	0.03

Uncertainty unknown

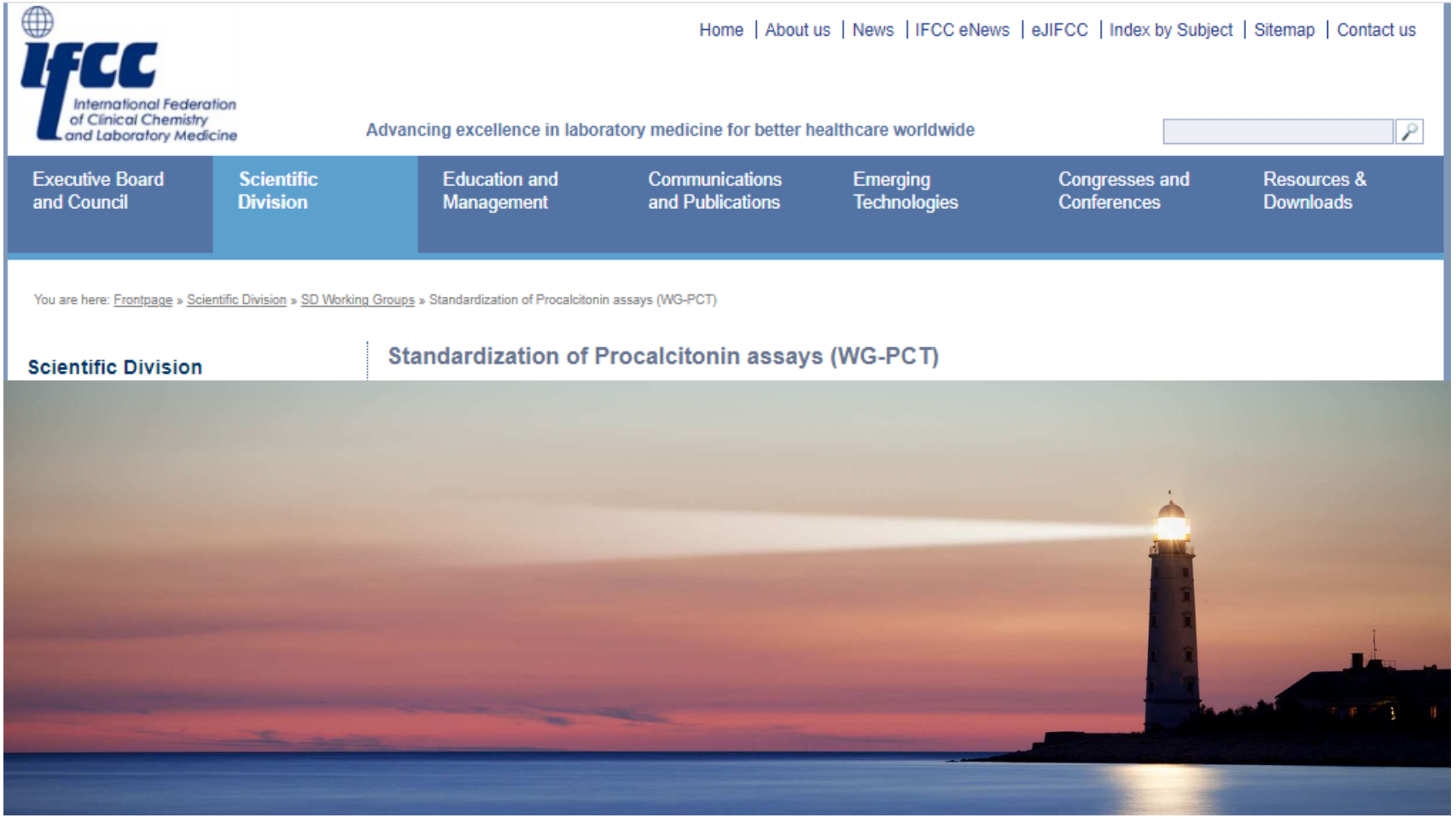
Harmonization through EQAs



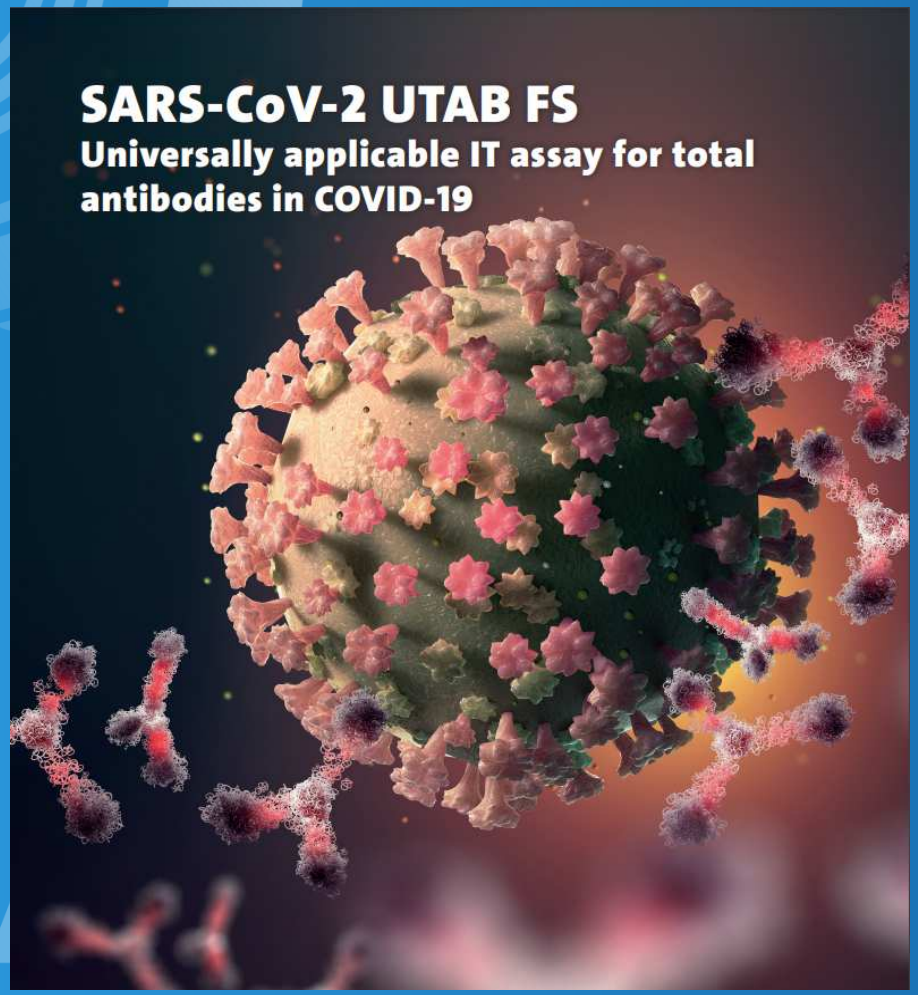
1. Interlaboratory CVs are highly heterogeneous across the different surveys
2. Materials with varying concentrations of PCT and various types of matrices (fresh, frozen, lyophilized, spiked with recombinant PCT or not) were used
3. Are these materials commutable?

Huynh H. et al., 2021
https://doi.org/10.1515/cclm-2021-0566

IFCC PCT Standardisation Working Group



SARS-CoV-2


SARS-CoV-2 UTAB FS
Universally applicable IT assay for total antibodies in COVID-19

Medicines & Healthcare products Regulatory Agency

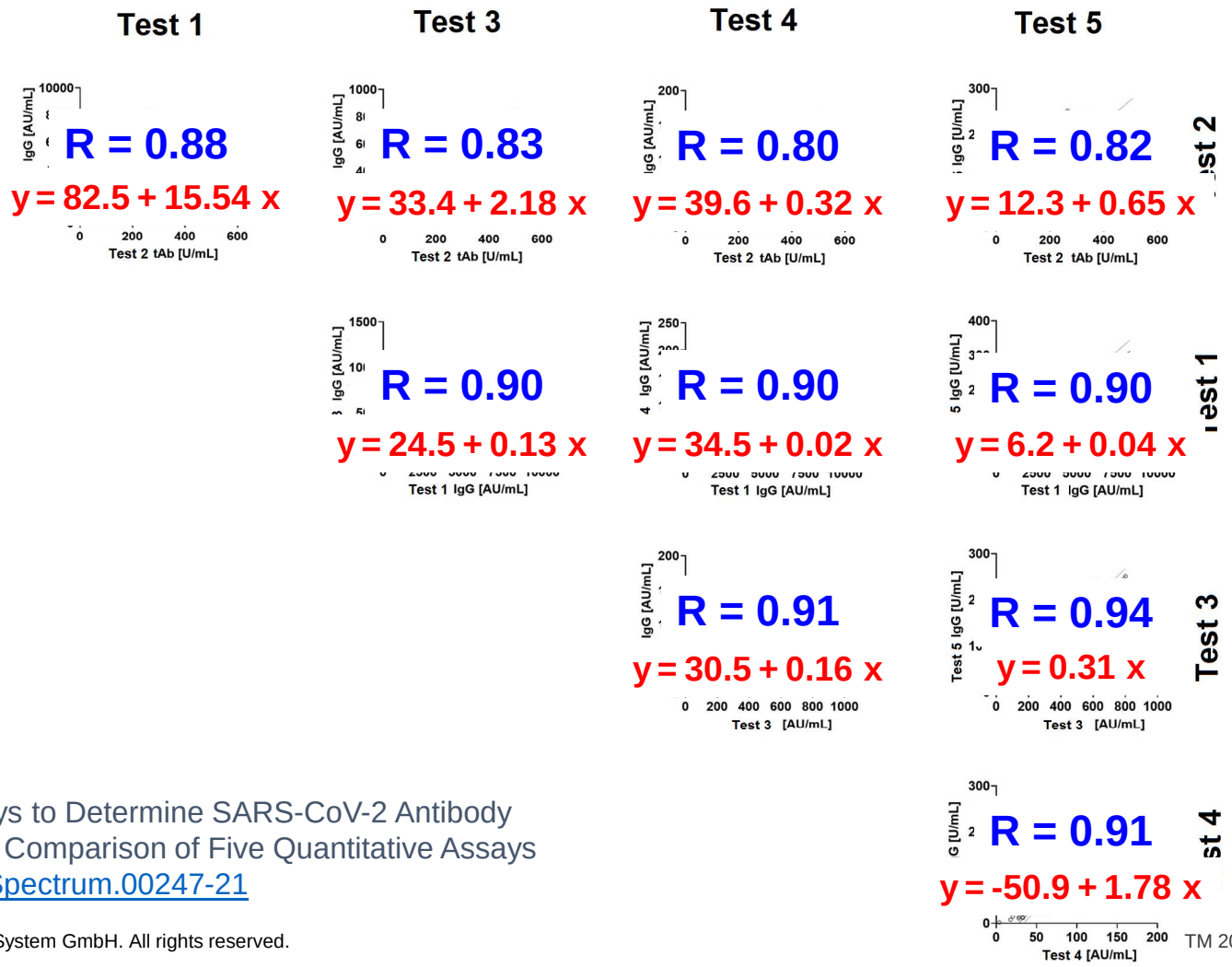
WHO International Standard
First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human)
NIBSC code: 20/136
Instructions for use
(Version 2.0, Dated 17/12/2020)

3. UNITAGE

The assigned potency of the WHO International Standard for SARS-CoV-2 is 250 IU/ampoule for neutralising antibody activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/mL.

For binding antibody assays, an arbitrary unitage of 1000 binding antibody units (BAU)/mL can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (e.g. anti-RBD IgG, anti-N IgM, etc.)

Differences between WHO-standarised tests



Perkmann et al., 2021
Anti-Spike Protein Assays to Determine SARS-CoV-2 Antibody Levels: a Head-to-Head Comparison of Five Quantitative Assays
https://doi.org/10.1128/Spectrum.00247-21

Enzyme-linked immunosorbent assay (ELISA)

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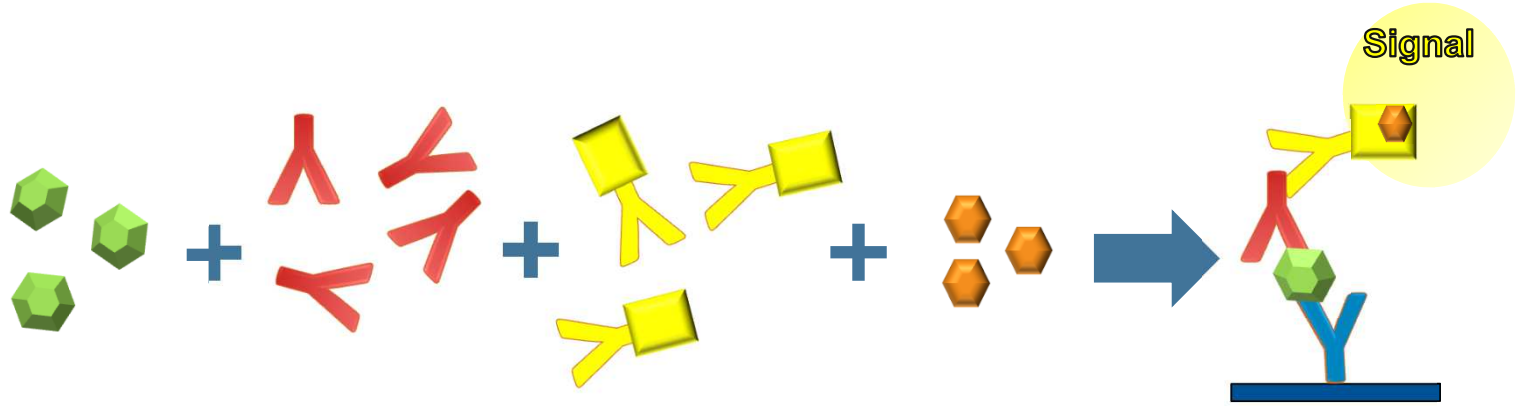

- Analyte (anti SARS-CoV-2 antibody)
- Plate immobilised antibody
- Antigen (SARS-CoV-2 protein)
- Detection antibody, bound to POD
- Substrate (TMB)

Courtesy of Leoni Wey

Additional efforts shall also be made for improving harmonization, standardization and comparability of anti-SARS-CoV-2 serology.

Plebani et al., 2021
SARS-CoV-2 antibody assay after vaccination: one size does not fit all
https://doi.org/10.1515/cclm-2021-0703

Calprotectin

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Non-homogeneity of measurement procedures

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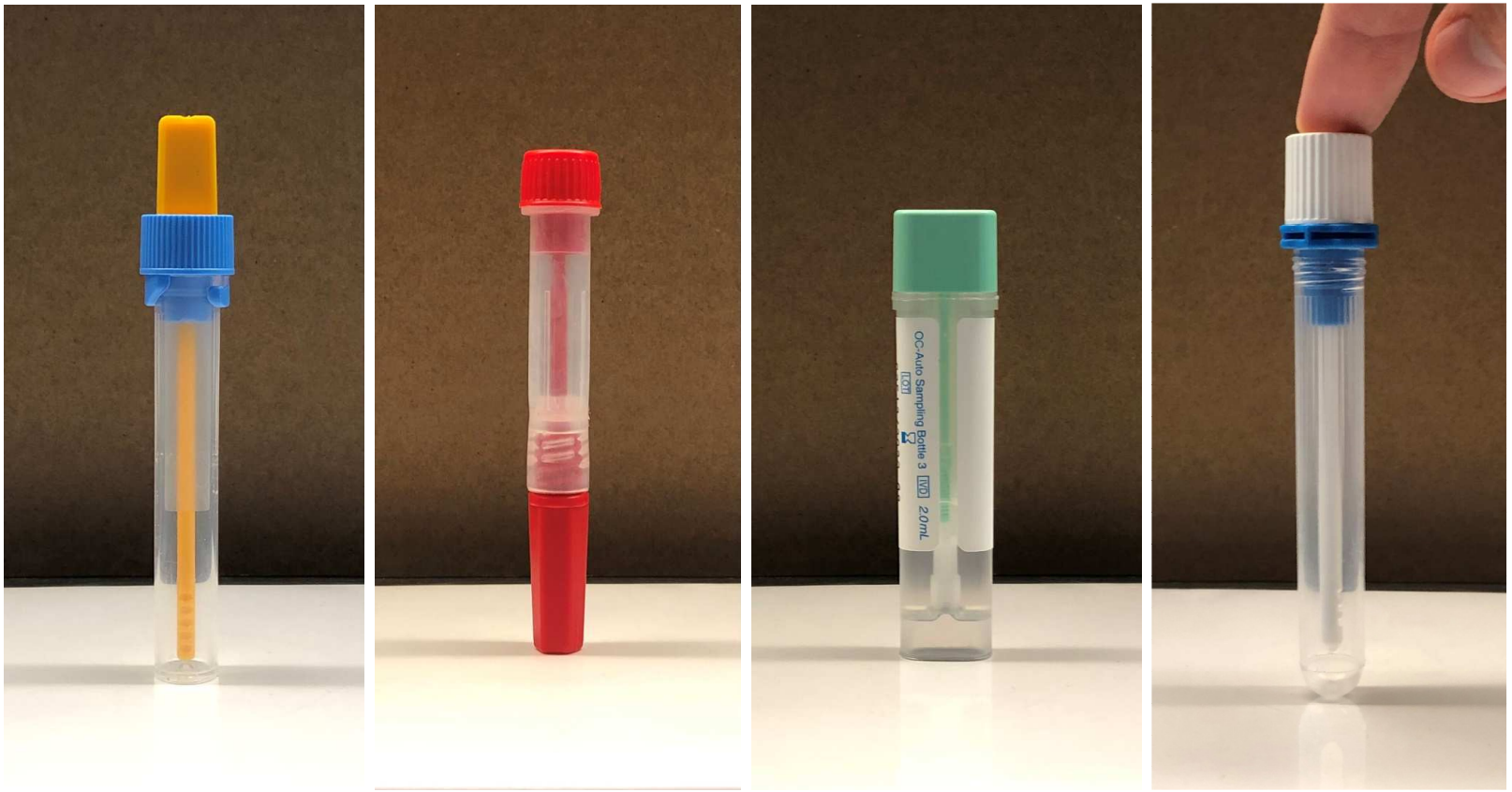
Greatest problem is the matrix itself: stool

- Stool is highly inhomogene in comparison to serum / plasma
- Interferences by host proteins, salts / ions, bacterial proteins, lipids etc.
- Process of dissolution in specific matrix tedious and not standardized

Stool sample tubes for clinical analyzers

- Differences by manufactures in sizes, forms, amount of stool and collecting systems
- Amount of stool collected reproducible?
- Handling of stool samples varies with tube (e.g. vortex), preparation and dilution factor

Influence of the stool tubes



Courtesy of Tobias Becker

IFCC Fecal Immunochemical Testing (WG-FIT)

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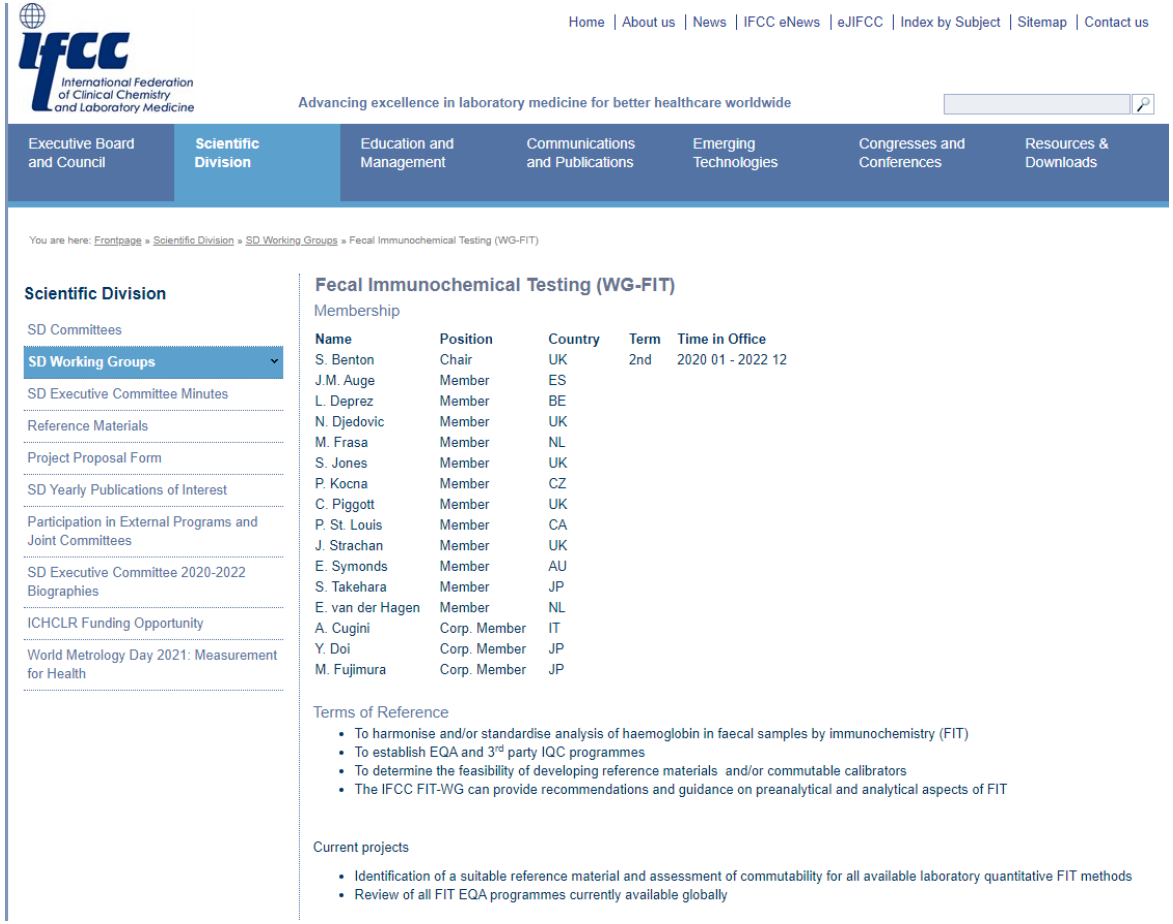

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Scientific Division

- SD Committees
- SD Working Groups
- SD Executive Committee Minutes
- Reference Materials
- Project Proposal Form
- SD Yearly Publications of Interest
- Participation in External Programs and Joint Committees
- SD Executive Committee 2020-2022 Biographies
- ICHCLR Funding Opportunity
- World Metrology Day 2021: Measurement for Health

Fecal Immunochemical Testing (WG-FIT)

Membership

Name	Position	Country	Term	Time in Office
S. Benton	Chair	UK	2nd	2020 01 - 2022 12
J.M. Auge	Member	ES		
L. Deprez	Member	BE		
N. Djedovic	Member	UK		
M. Frasa	Member	NL		
S. Jones	Member	UK		
P. Kocna	Member	CZ		
C. Piggott	Member	UK		
P. St. Louis	Member	CA		
J. Strachan	Member	UK		
E. Symonds	Member	AU		
S. Takehara	Member	JP		
E. van der Hagen	Member	NL		
A. Cugini	Corp. Member	IT		
Y. Doi	Corp. Member	JP		
M. Fujimura	Corp. Member	JP		

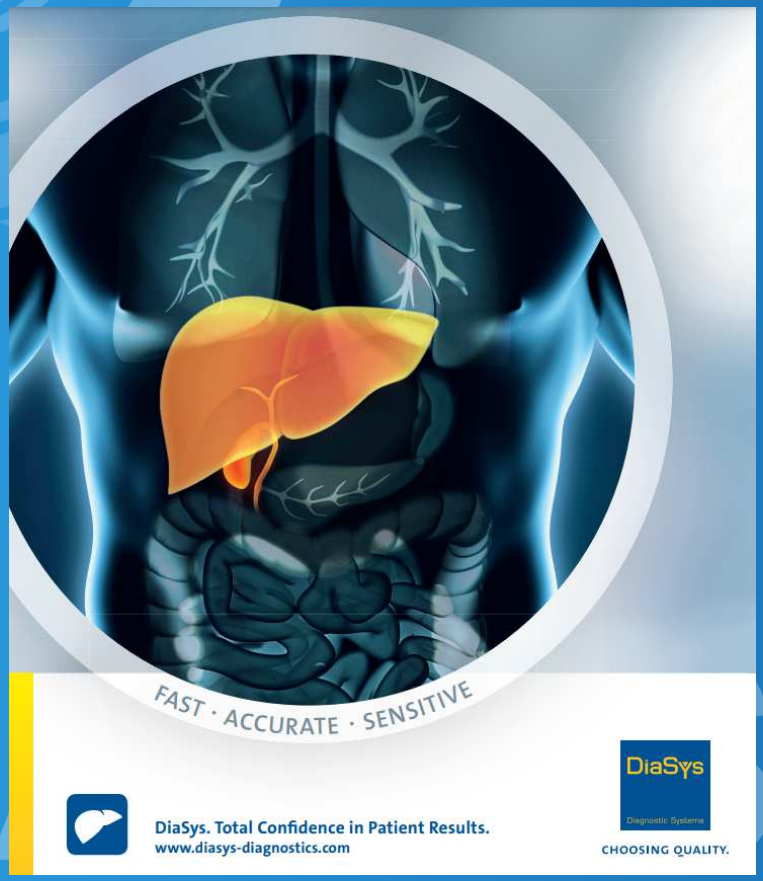
Terms of Reference

- To harmonise and/or standardise analysis of haemoglobin in faecal samples by immunochemistry (FIT)
- To establish EQA and 3rd party IQC programmes
- To determine the feasibility of developing reference materials and/or commutable calibrators
- The IFCC FIT-WG can provide recommendations and guidance on preanalytical and analytical aspects of FIT

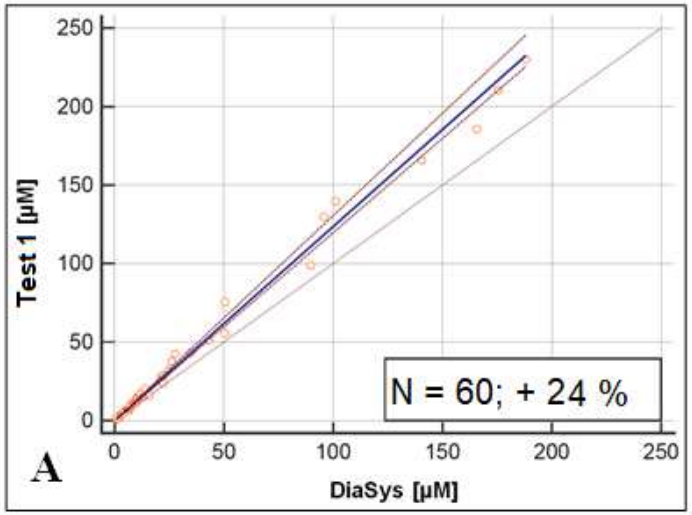
Current projects

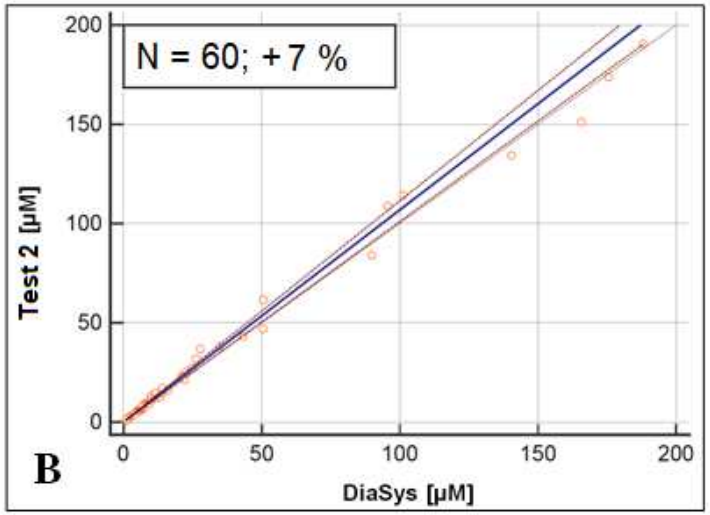
- Identification of a suitable reference material and assessment of commutability for all available laboratory quantitative FIT methods
- Review of all FIT EQA programmes currently available globally

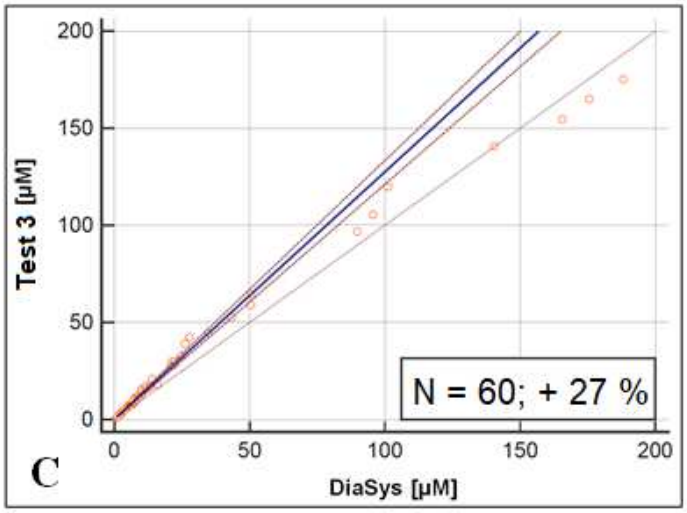
Total Bile Acids 21 FS

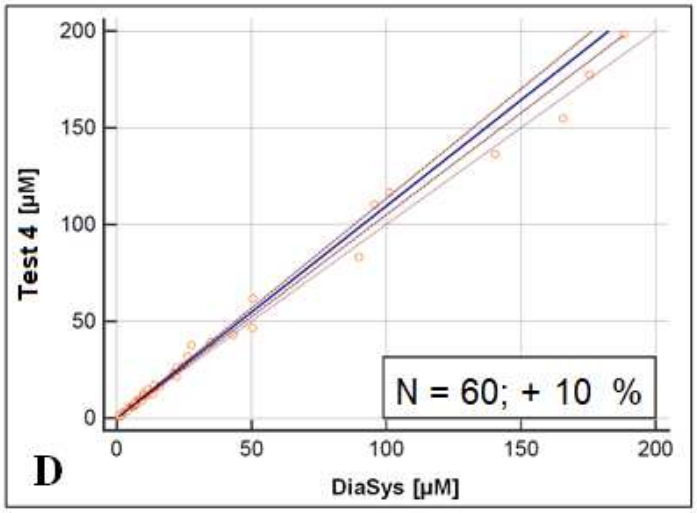


DiaSys vs. commercial tests P&B regression

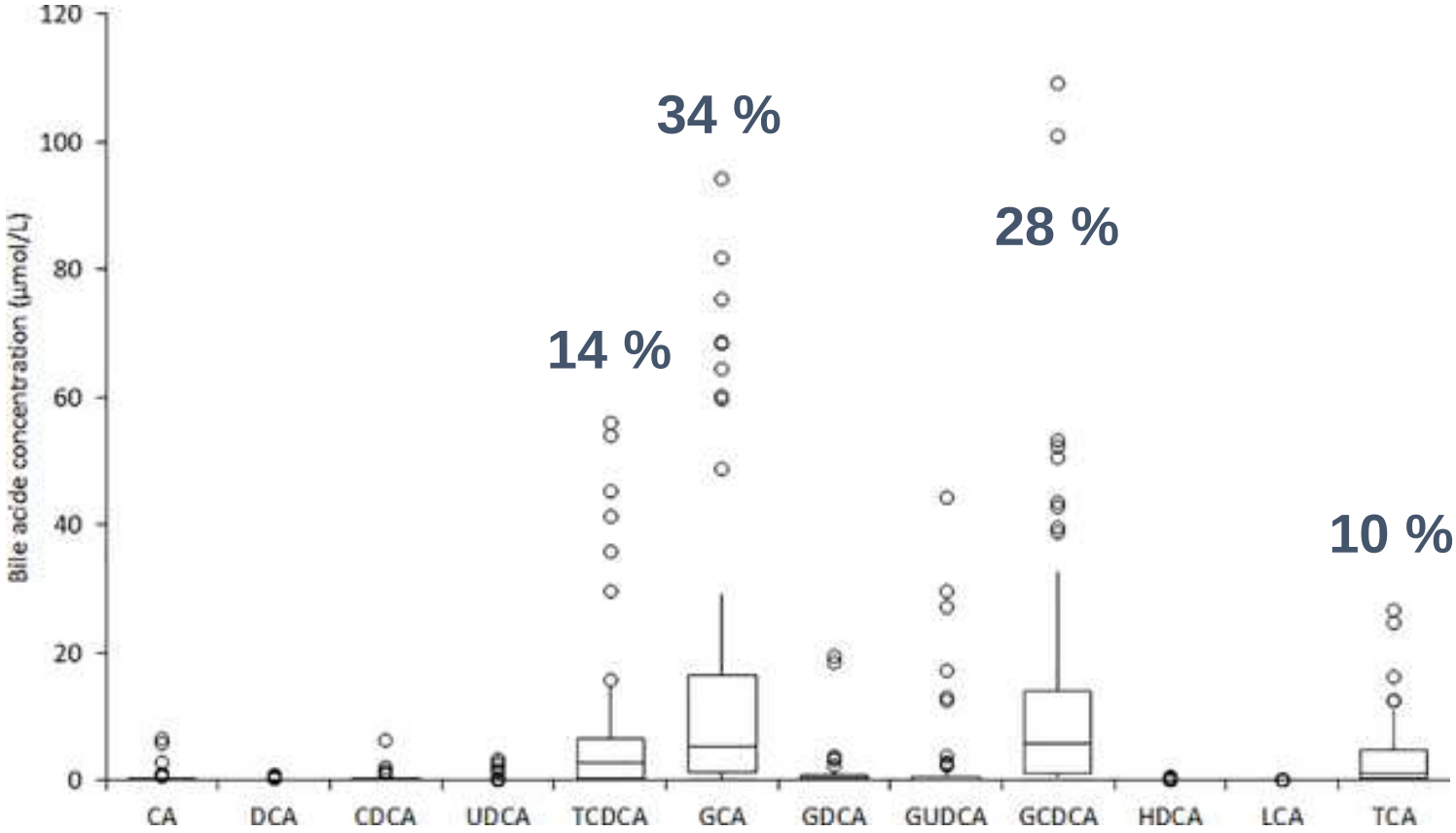






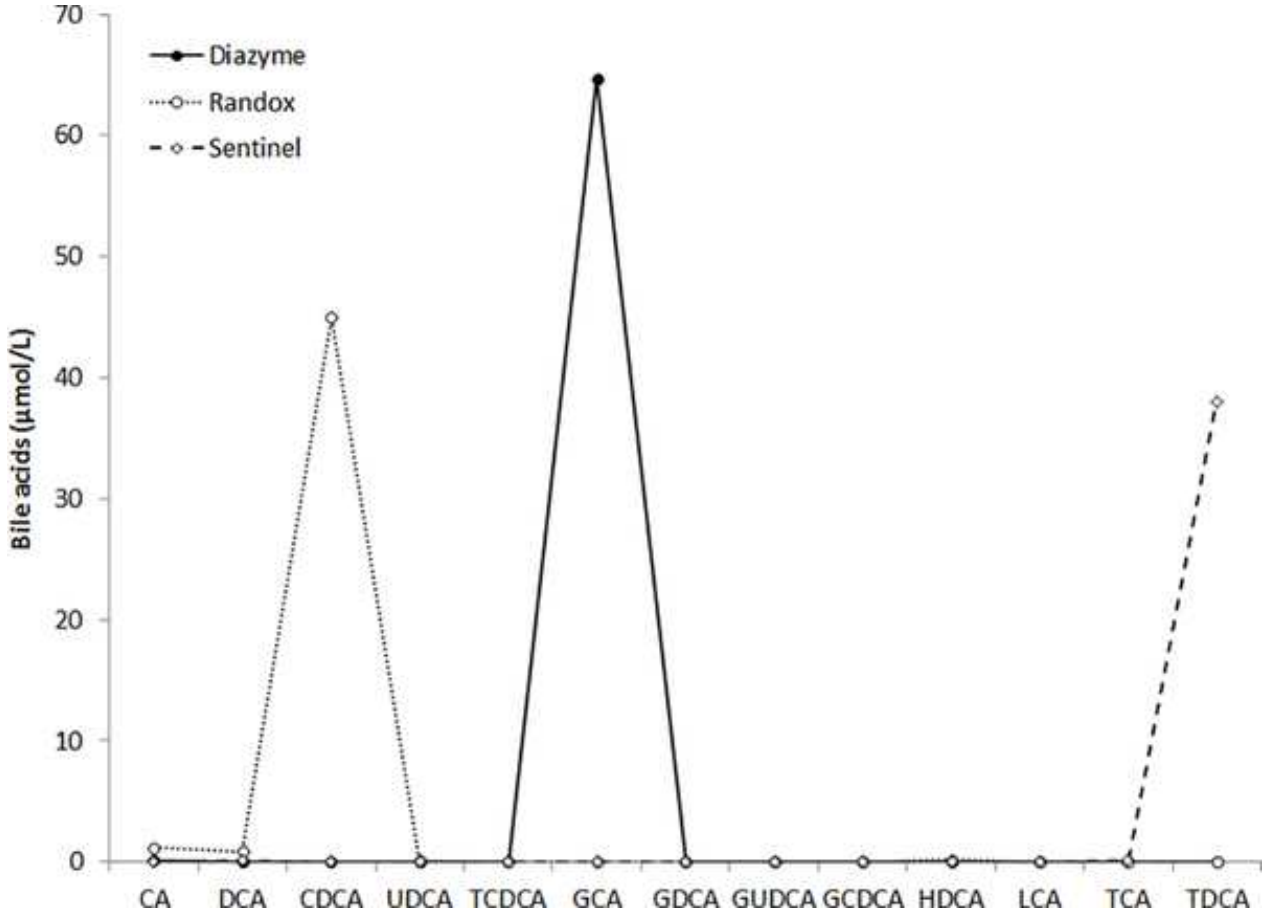


Bile acids composition of plasma samples



Danese et al., 2017
Analytical evaluation of three enzymatic assays for measuring total bile acids in plasma using a fully-automated clinical chemistry platform
https://doi.org/10.1371/journal.pone.0179200

Bile acids composition of calibrators of three commercial assays



Futher parameters

The long and winding road…

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Parameter	Standardisation / measurement issues
β-Hydroxybutyrate	No reference material/method; different Enzymes used by manufacturer + D/L impurity of calibration material
LpPLA2	No reference material; Enzyme activity vs. mass
ACE	No reference material, method; different matrices; enzyme vs. mass
Cys-C	No commutable reference material on diverse matrices (serum / urine)
Presepsin	No reference material, method
D-Dimer	No reference material, method; update
APOs	Update of reference material, method

Conclusions

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- Regulations and guidelines, e.g. the new IVDR 2017/746 (EU), strengthen the traceability aspects “through suitable reference measurement procedures and/or suitable reference materials of a higher metrological order”

- Standardisation (recovery) issues do affect assay parameters (e.g. LOQ) and development process (e.g. reaction enhancer, beads concentrations, etc.)

- Different conditions (mAb-pAntibodies, epitopes, labelling, detection systems, techniques) or enzymes can lead to different quantitations → importance of the specificity

- Absence of reference material (CRM) implies harmonization / standardisation of results through different methods (EQA, etc.)

- Defining the sample matrix (blood, stool, liquor, urine) and the commutability

Thank you for your attention

DIASYS