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Clinical applications of procalcitonin (PCT) and challenges in measurement standardization

Agenda

- Welcome and introductions – *Li Han*
- Speeches
  - Standardization of Procalcitonin: the modern Troy? – *Thomas Masetto*
  - Challenges in PCT standardization and where next – *Matthias Grimmler*
- Q&A – *Speakers and panelist*

One of the most important raw material suppliers for the IVD industry

We develop and produce monoclonal antibodies and antigens that are mainly used as key components of laboratory tests.

Established in 1994

- **Headquarters in Finland**, operations in **China, Russia** and **North America**
- Sales to over **50 countries**
- Active participation in **IFCC** and **AACC/ADLM** standardization committee work
- Operations compliant with ISO 9001:2015

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Comprehensive product line

PRODUCT CATEGORIES

Monoclonal antibodies
Polyclonal antibodies
Antigens
Plasma and serum

CORE PRODUCT AREAS


Cardiac Markers


Infectious Diseases


Blood Coagulation and Anemia


Inflammation

OTHER PRODUCT AREAS


Veterinary


Kidney Diseases

Fertility and Pregnancy

Hormones


Tumor Markers


Neuroscience


Miscellaneous


Immunology and Serology

Gangliosides


Microbial and Plant Toxins


Thyroid Diseases

Metabolic Syndrome

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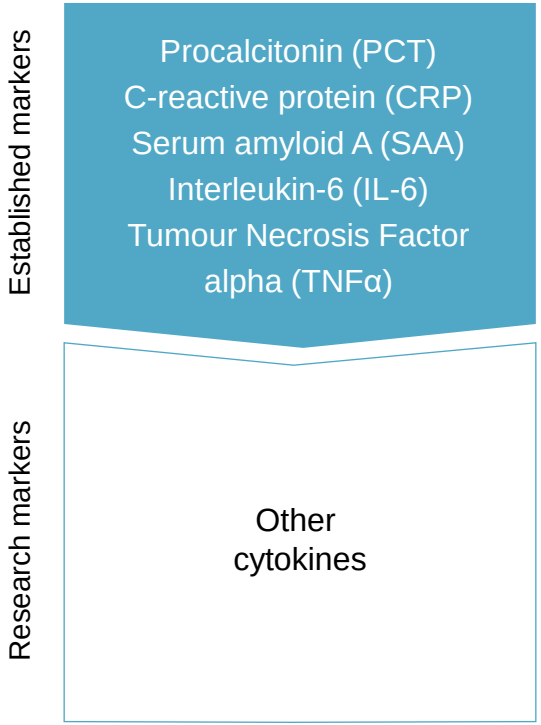
Inflammation product line



Speaker: Prof. Dr. Matthias Grimmler

Member of IFCC WG-PCT, WG-APO MS, Task Force on COVID-19 and Association of the German Diagnostics Industry

- Dr. Grimmler works as the Head of Scientific & Medical Affairs at DiaSys Diagnostic Systems. He also holds two other management positions at DiaServe Laboratories, as the Head of New Products/Technology and Head of Customer Business Care.
- He is a professor of Immunology, Institute for Biomolecular Research at University of Applied Sciences Fresenius.
- Dr. Grimmler has extensive research experience. His research interests involve the biochemical and cytological characterization of multi-protein complexes, with a particular focus on phospho-proteomics in the regulation of cell cycle control and tumorigenesis.
- Dr. Grimmler holds a PhD from Max Planck Institute of Biochemistry.

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Speaker: Thomas Masetto

Member of IFCC WG-PCT, EFLM, AACC and Italian Society of Clinical Biochemistry and Clinical Molecular Biology

- Mr. Masetto works as the Head of R&D Immunoturbidimetry at DiaSys Diagnostic Systems. Prior to his current role, he has worked in various positions in the biotech and clinical industry for more than 15 years.
- Mr. Masetto is passionate about medical and clinical research. His research interests involve immunoassay development and validation, biostatistics, metrology and assay standardization, protein and antibody production, purification and characterization.
- Besides the outstanding working experience, he is also a doctoral student at the University of Düsseldorf.

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Panelist: Dr. Vladimir Filatov

- Dr. Filatov works as a Group Leader of the Research and Development department of HyTest. He has led multiple challenging development projects during his 25-year experience with HyTest. These projects resulted in excellent products, e.g. PCT, IL-6, RBP4, D-dimer, troponins, adiponectin and etc.
- Dr. Filatov is an acknowledged scientists. He has published more than 15 articles in various well-known international journals, such as Clinical Chemistry and Clinical Biochemistry.
- Dr. Filatov's research interests involve antibody and prototype immunoassay development and production, immunoassay standardization, purification and characterization of protein and antibodies.

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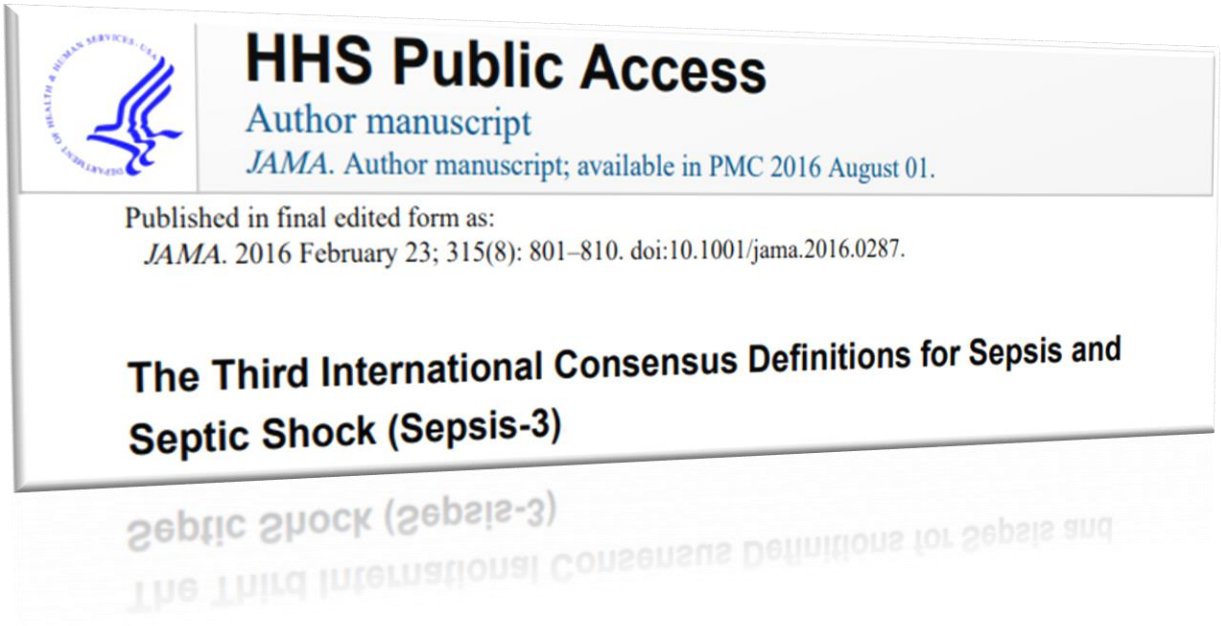
Standardisation of Procalcitonin: the modern Troy?

Thomas Masetto

20th September 2023

Sepsis: a new definition (Sepsis-3)



HHS Public Access

Author manuscript

JAMA. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

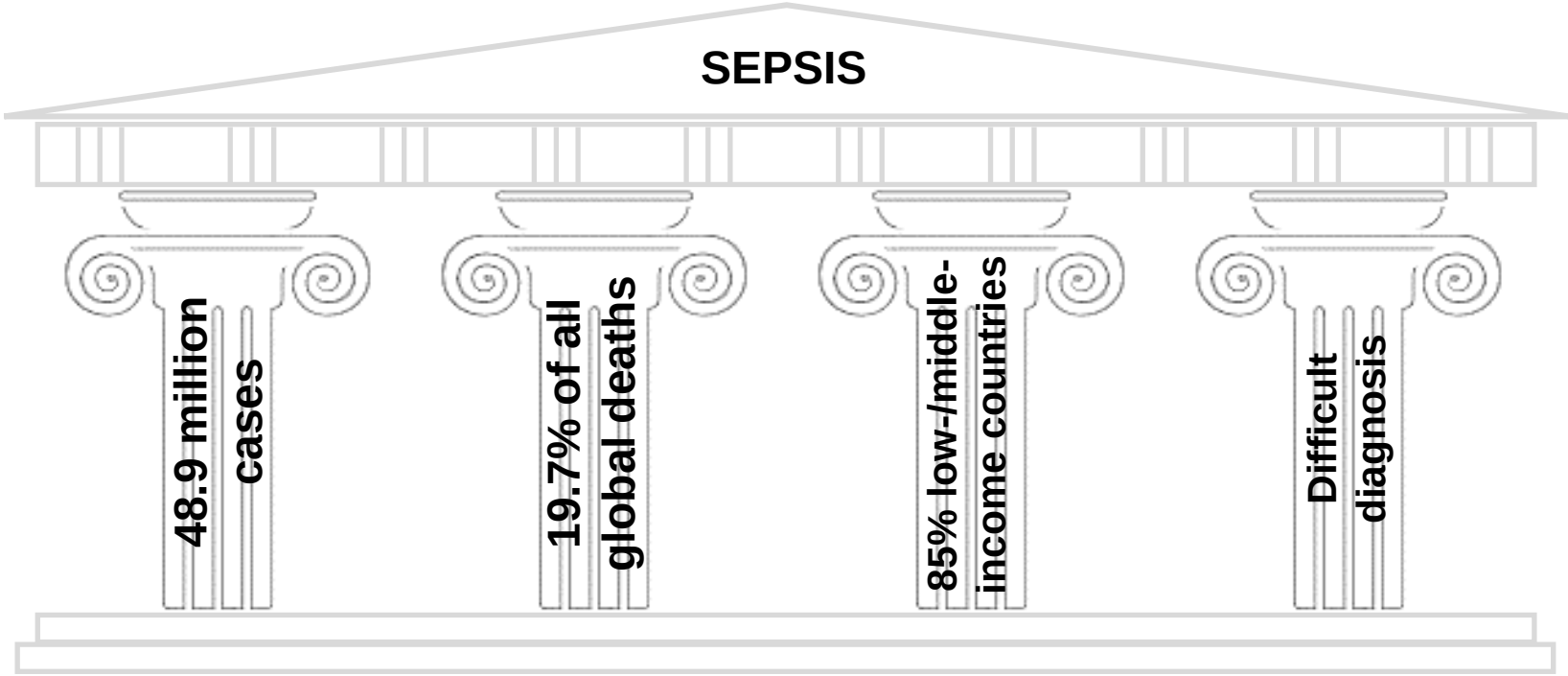
JAMA. 2016 February 23; 315(8): 801–810. doi:10.1001/jama.2016.0287.

The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)

"The life-threatening organ dysfunction caused by a dysregulated host response to infection"

Singer et al., 2016, 10.1001/jama.2016.0287

Sepsis: a worldwide threat



WHO, 2020, https://www.who.int/news-room/fact-sheets/detail/sepsis
Rudd et al., 2017, 10.1016/S0140-6736(19)32989-7

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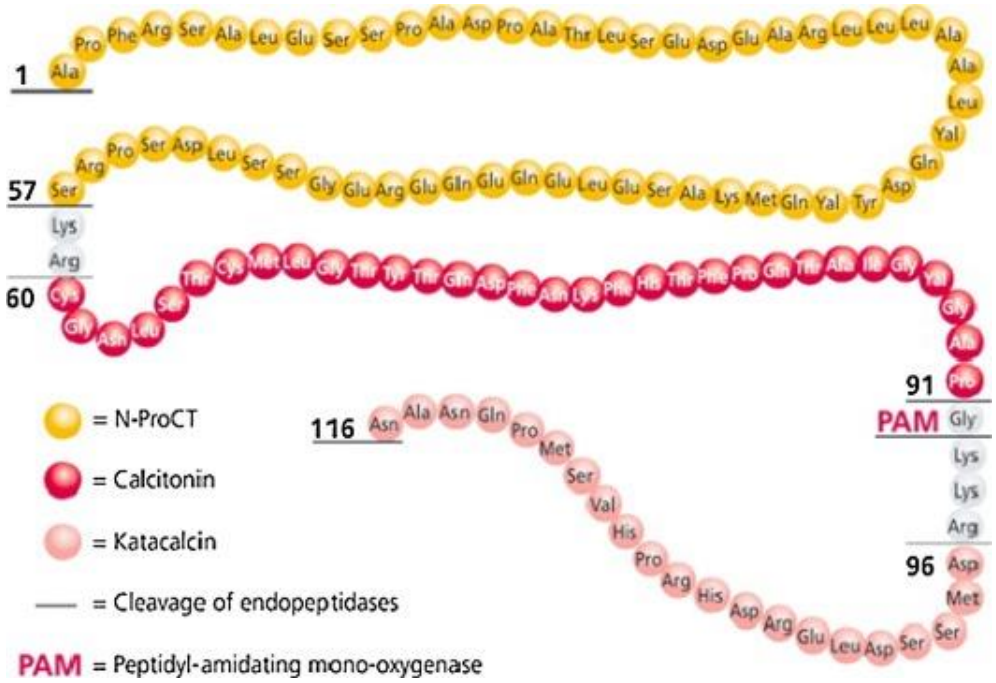
Procalcitonin: the molecule

- 116 aa, MW approx. 13 kDa
- Precursor hormone Calcitonin (only thyroidal C-cells)
- Normal physiological conditions PCT < 0.05 ng/mL



M. Meisner, 2000, ISBN 978-3-8374-1241-3

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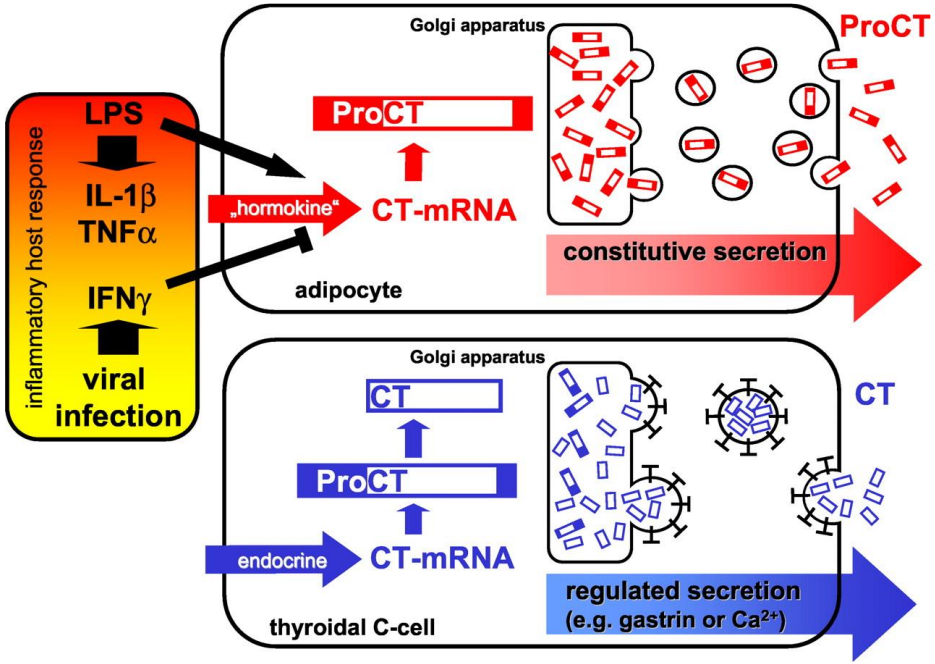
Procalcitonin: pathophysiology

- Assicot et al. (1993)
  PCT ↔ infection
- Bacterial endotoxins (LPS) and/or cytokines
  PCT ↑ up to 1000x
- Viral infections (IFNγ)
  PCT ↓
- Extra-thyroidal synthesis



Assicot et al., 1993, 10.1016/0140-6736(93)90277-n
P. Linscheid et al., 2003, 10.1210/en.2003-0854

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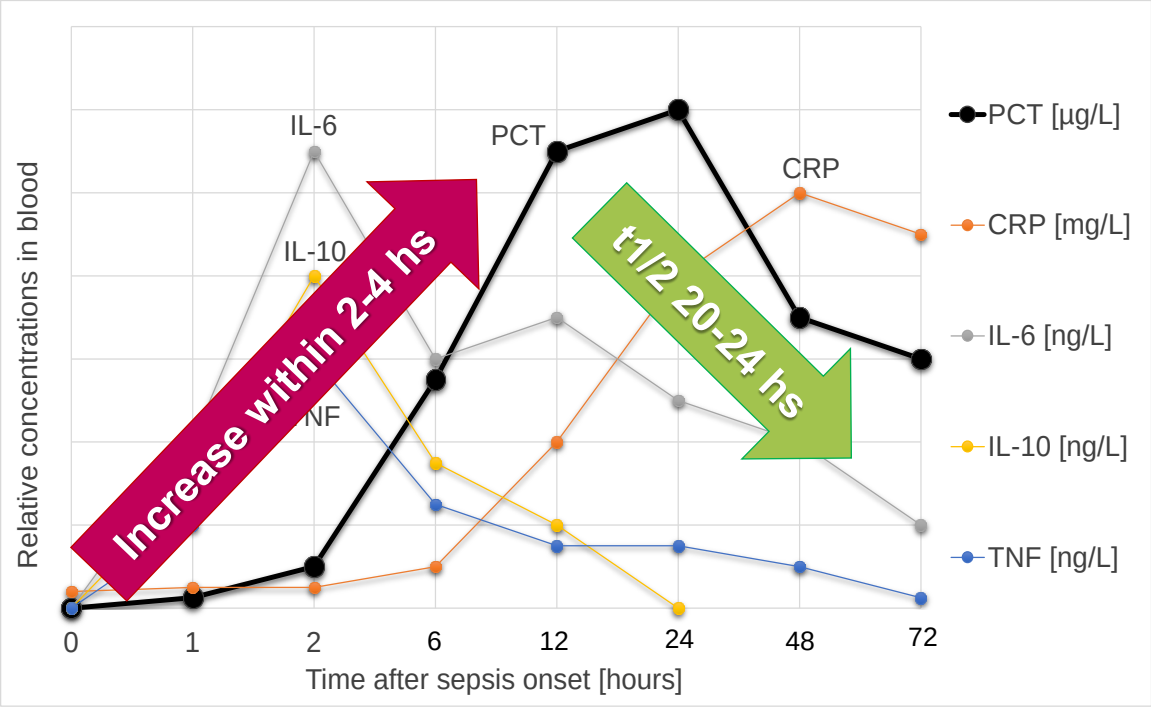
Procalcitonin and bacterial infection: kinetics



Adapted from M. Meisner, 1999, 10.1515/labm.1999.23.5.263

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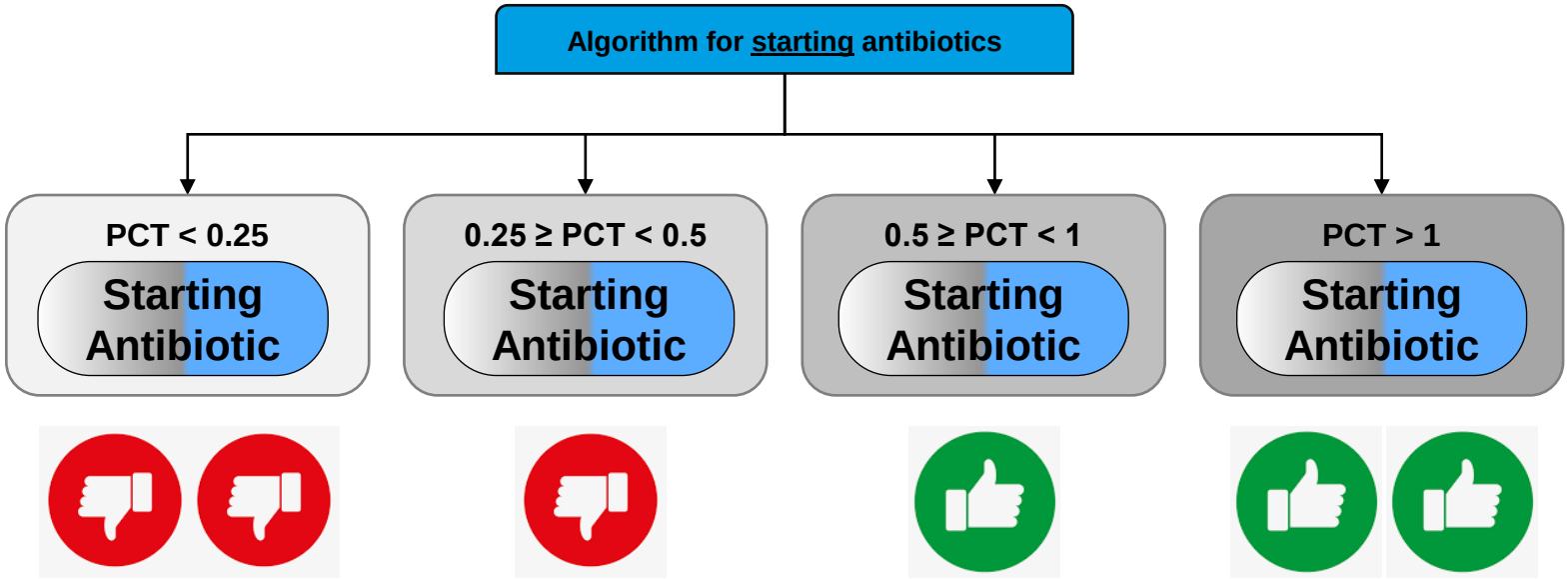
Proposed uses of PCT: sepsis diagnosis

Biomarker	Meta-analyses (n)	Range AUC	Range sensitivity	Range specificity
IL-6	2	0.79-0.80	0.68-0.72	0.73-0.73
LBP	2	0.68-0.71	0.62-0.70	0.56-0.70
nCD64	2	0.95-0.96	0.76-0.87	0.85-0.93
PCT	10	0.78-1.00	0.71-1.00	0.61-0.88
CRP	3	0.71-0.77	0.75-0.91	0.36-0.67
Presepsin	6	0.86-0.89	0.77-0.85	0.73-0.88
sTREM-1	2	0.85-0.87	0.78-0.83	0.68-0.78
suPAR	1	0.82	0.80	0.80

Lippi et al., 2017, 10.4081/ecj.2017.6877

Proposed uses of PCT: PRORATA trial

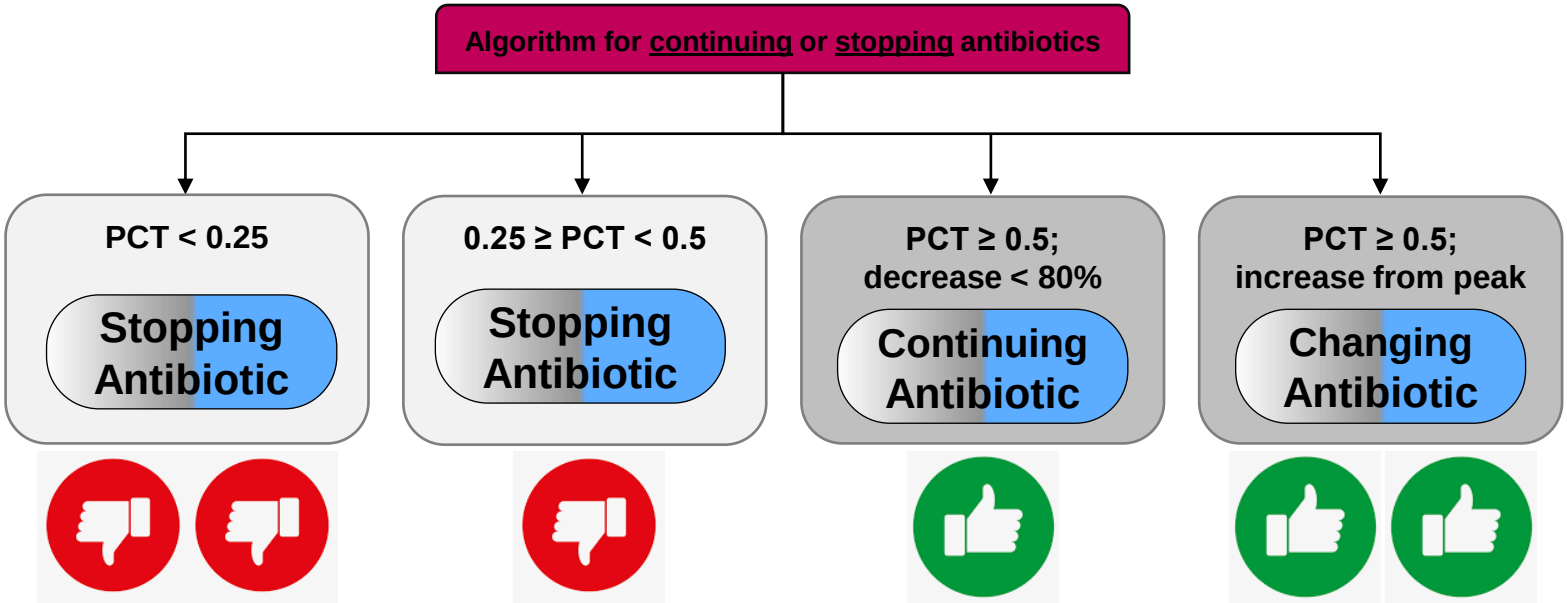
PROcalcitonin to Reduce Antibiotic Treatments in Acutely ill patients



Bouadma et al., 2010, 10.1016/S0140-6736(09)61879-1

Proposed uses of PCT: PRORATA trial

PROcalcitonin to Reduce Antibiotic Treatments in Acutely ill patients



Bouadma et al., 2010, 10.1016/S0140-6736(09)61879-1

Proposed uses of PCT: antibiotic stewardship

Authors	Biomarkers	Studies and population	Setting	Heterogeneity	Results
Kopterides *et al.*, 2010[44]	PCT	7 studies, 1131 patients	Intensive care	Significant	Reduction of 4.2 days (95% CI, 3.4-5.0) duration of antibiotic therapy; reduction of 18% antibiotic therapy expenditure
Heyland *et al.*, 2011[45]	PCT	5 studies, 947 patients	Intensive care	Non-significant	Reduction of 2.1 days (95% CI, 1.8-2.5) duration of antibiotic therapy; likely economic benefit
Schuetz *et al.*, 2011[46]	PCT	14 studies, 4467 patients	Primary care, emergency department, intensive care	Non-significant	Reduction of 29% (95% CI, 15-37%) duration of antibiotic therapy (34%; 95% CI, 15-53 for emergency department)
Soni *et al.*, 2013[47]	PCT	18 studies, number of patients unavailable	Intensive care	Non-significant	Reduction of 2.0 days (95% CI, 1.5-2.6) duration of antibiotic therapy
Prkno *et al.*, 2013[48]	PCT	7 studies, 1075 patients	Intensive care	Non-significant	Reduction of 27% (95% CI, 5-53%) duration of antibiotic therapy
Andriolo *et al.*, 2017[49]	PCT	10 studies, 1215 patients	Unselected population	Significant	Reduction of 1.3 days (95% CI, 0.6-2.0) duration of antibiotic therapy

Duration of antibiotic therapy significantly reduced

Lippi et al., 2017, 10.4081/ecj.2017.6877

Proposed uses of PCT: SAPS trial

Stop Antibiotics on guidance of Procalcitonin Study (SAPS)

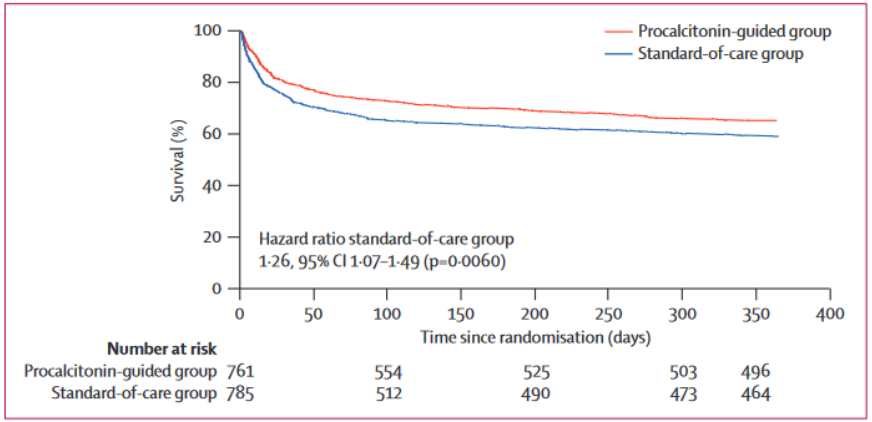


Figure 2: **Kaplan-Meier plot for probability of survival from random assignment to day 365, in the modified intention-to-treat population**

	Procalcitonin-guided group (n=761)	Standard-of-care group (n=785)	Between-group absolute difference in means (95% CI)	p value
Antibiotic consumption (days)				
Daily defined doses in first 28 days	7·5 (4·0 to 12·8)	9·3 (5·0 to 16·5)	2·69 (1·26 to 4·12)	<0·0001
Duration of treatment	5·0 (3·0 to 9·0)	7·0 (4·0 to 11·0)	1·22 (0·65 to 1·78)	<0·0001
Antibiotic-free days in first 28 days	7·0 (0·0 to 14·5)	5·0 (0 to 13·0)	1·31 (0·52 to 2·09)	0·0016
Mortality (%)				
28-day mortality	149 (19·6%)	196 (25·0%)	5·4% (1·2 to 9·5)	0·0122
1-year mortality	265 (34·8%)	321 (40·9%)	6·1% (1·2 to 10·9)	0·0158
Adverse events				
Reinfection	38 (5·0)	23 (2·9)	−2·1% (−4·1 to −0·1)	0·0492
Repeated course of antibiotics	175 (23·0)	173 (22·0)	−1·0% (−5·1 to 3·2)	0·67
Time (days) between stop and reinstitution of antibiotics	4·0 (2·0 to 8·0)	4·0 (2·0 to 8·0)	−0·22 (−1·31 to 0·88)	0·96
Costs				
Total cumulative costs of antibiotics	€150 082	€181 263	NA	NA
Median cumulative costs antibiotics per patient	€107 (51 to 229)	€129 (66 to 273)	€33·6 (2·5 to 64·8)	0·0006
Length of stay (days)				
On the intensive care unit	8·5 (5·0 to 17·0)	9·0 (4·0 to 17·0)	−0·21 (−0·92 to 1·60)	0·56
In hospital	22·0 (13·0 to 39·3)	22·0 (12·0 to 40·0)	0·39 (−2·69 to 3·46)	0·77

Data are median (IQR), n (%), or mean (95% CI). Between-group absolute differences were calculated using the mean values, percentage differences, and 95% CIs. NA=not applicable.

Table 2: **Primary and secondary outcome measures**

de Jong et al., 2016, 10.1186/1471-2334-13-178

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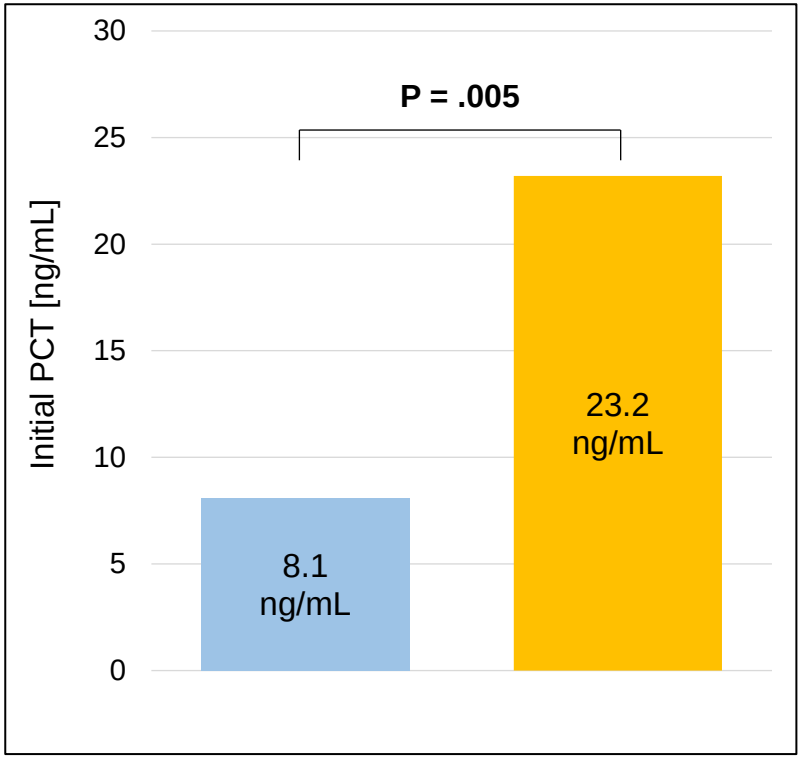
Proposed uses of PCT: prognosis of sepsis

Non-survivor	*P* value	Odd ratio	95% CI
Age	.022	1.055	1.008-1.105
PCT (log scale)	.005	2.004	1.240-3.238
SOFA score	<.001	1.303	1.142-1.486



Jekarl et al., 2019, 10.1002/jcla.22996

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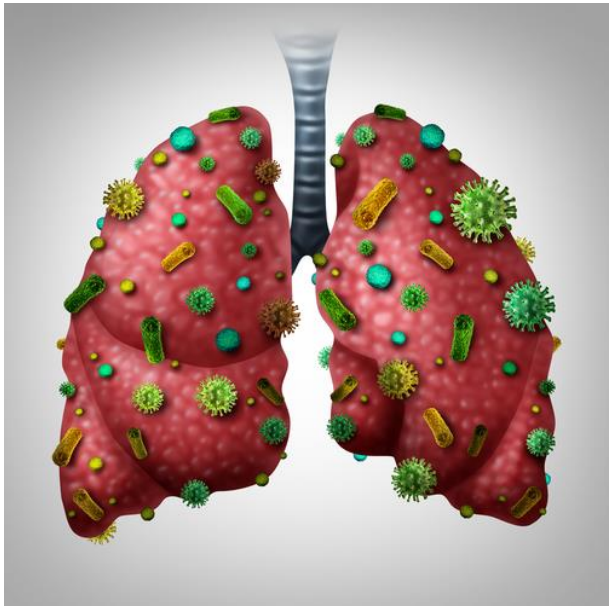
Proposed uses of PCT: lower respiratory tract infections, *ProHOSP trial*



- Multicenter (86 sites, N = 1359)
- No significant difference in deaths, ICU admissions, or disease-specific complications
- Significant decrease in antibiotic days → 5.7 (PCT) vs. 8.7 (control)
- Significant decrease in antibiotic associated adverse events → 19.8% (PCT) vs. 28.1% (control)

Schuetz et al., 2009, 10.1001/jama.2009.1297

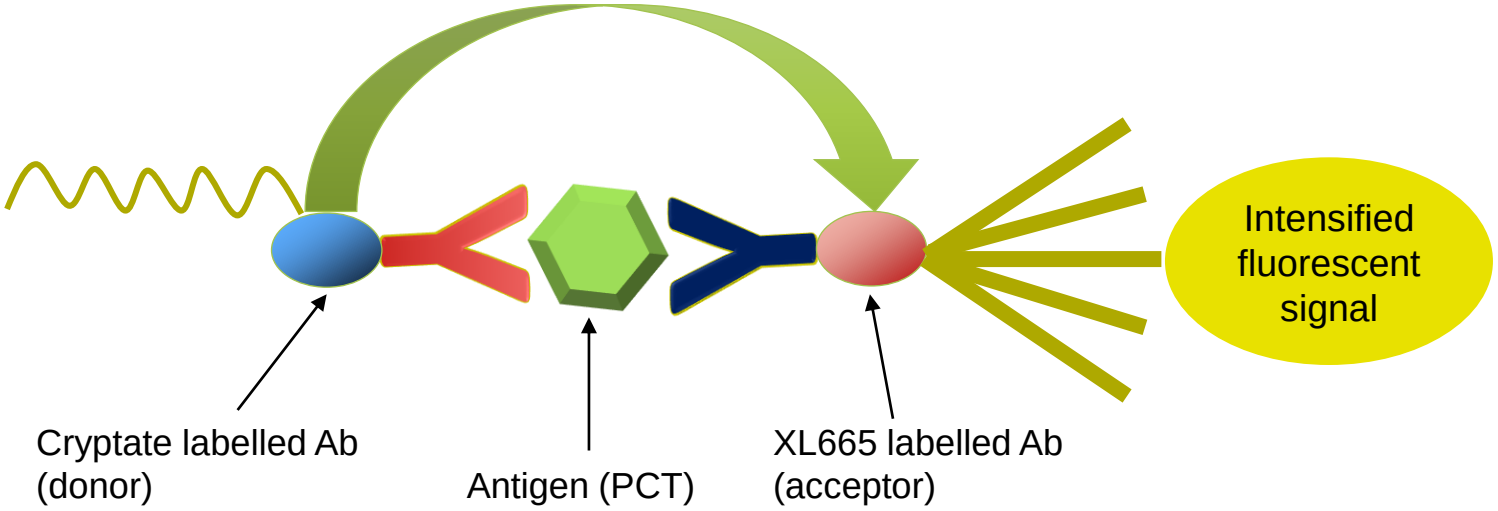
Proposed uses of PCT: further uses

Clinical scenario	PCT cut-off [ng/mL]	Clinical scenario	PCT cut-off [ng/mL]
Arthritis	0.1 to 0.25 ng/mL	Pneumonia	0.1 to 0.5 ng/mL
Bacteremic Infections	0.25 ng/mL	Postoperative Fever	0.1 to 0.5 ng/mL
Blood Stream Infection (primary)	0.1 ng/mL	Postoperative Infections	0.5 to 1.0 ng/mL
Acute Bronchitis/Chronic Obstructive Pulmonary Disease (COPD) Exacerbations	0.1 to 0.5 ng/mL	Severe Sepsis With or Without Shock	0.2 to 0.5 ng/mL
Infective Endocarditis	2.3 ng/mL	Upper Respiratory Tract Infections	0.1 to 0.25 ng/mL
Meningitis	0.5 ng/mL	Urinary Tract Infections	0.25 ng/mL
Neutropenia	0.1 to 0.5 ng/mL	Ventilator-associated Pneumonia	0.1 to 0.25 ng/mL

Cleland & Eranki, 2023, NCBI Bookshelf ID: NBK539794, PMID: 30969616

Immunoassay technologies to measure PCT

Time Resolved Amplified Cryptate Emission (TRACE)



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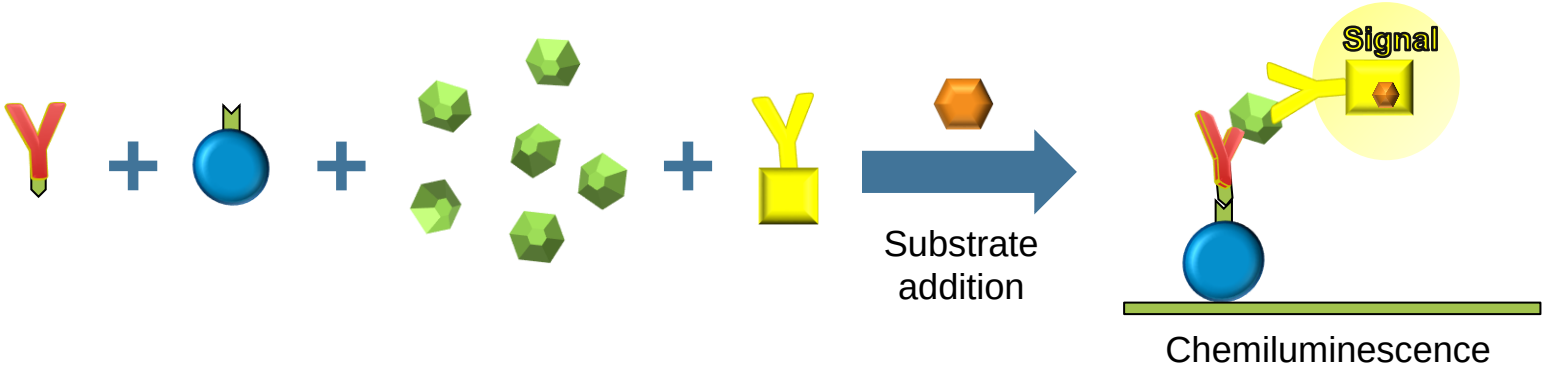
Immunoassay technologies to measure PCT

ChemiLuminescence Immuno Assay (CLIA)



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

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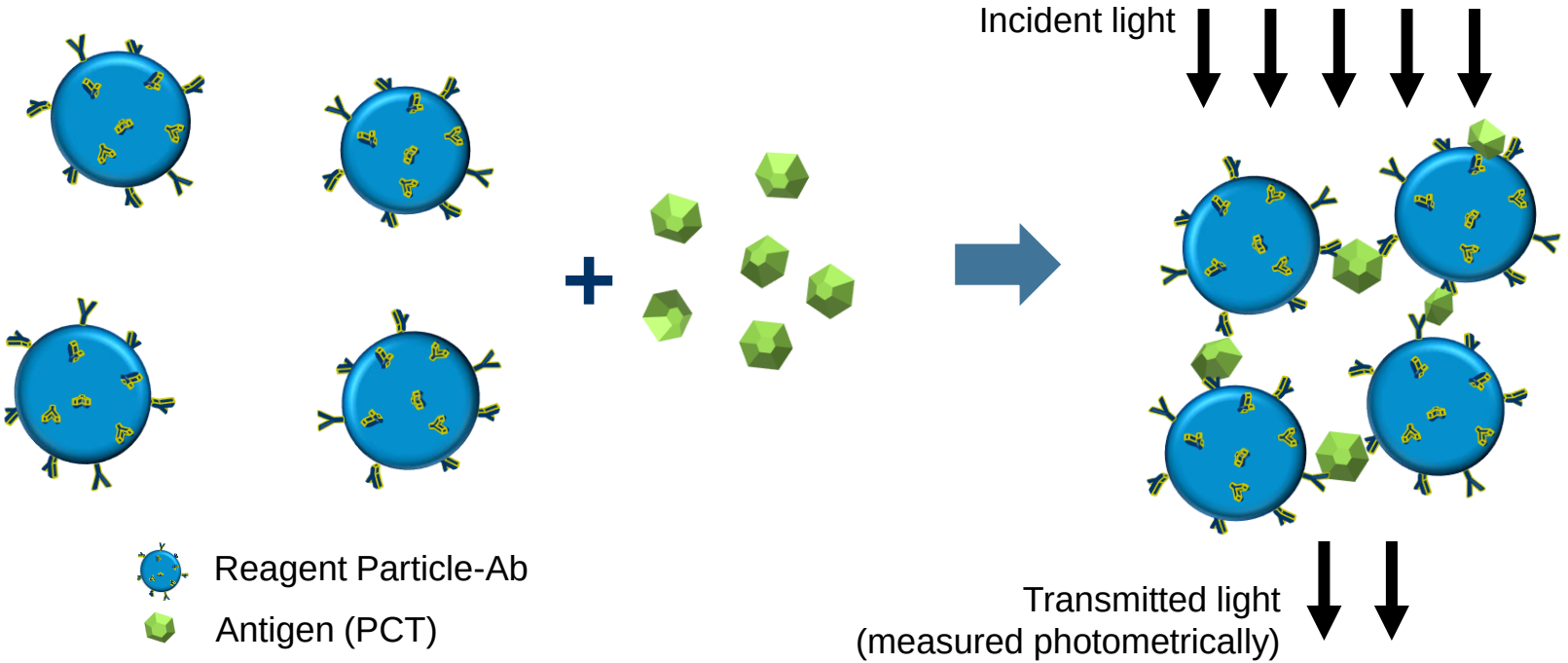
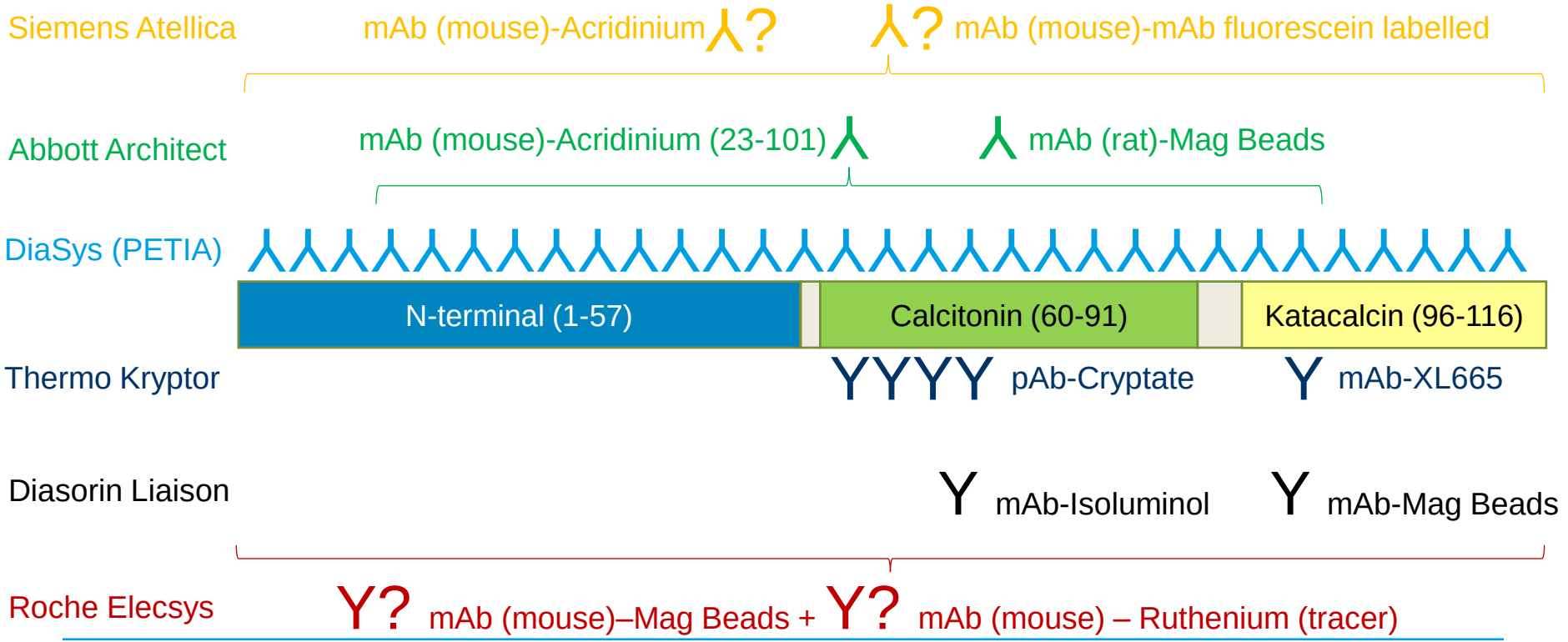
Immunoassay technologies to measure PCT

Particle Enhanced Turbidimetric Immunoassay (PETIA)



Anti-PCT antibodies employed by different IAs

All for one and one for all



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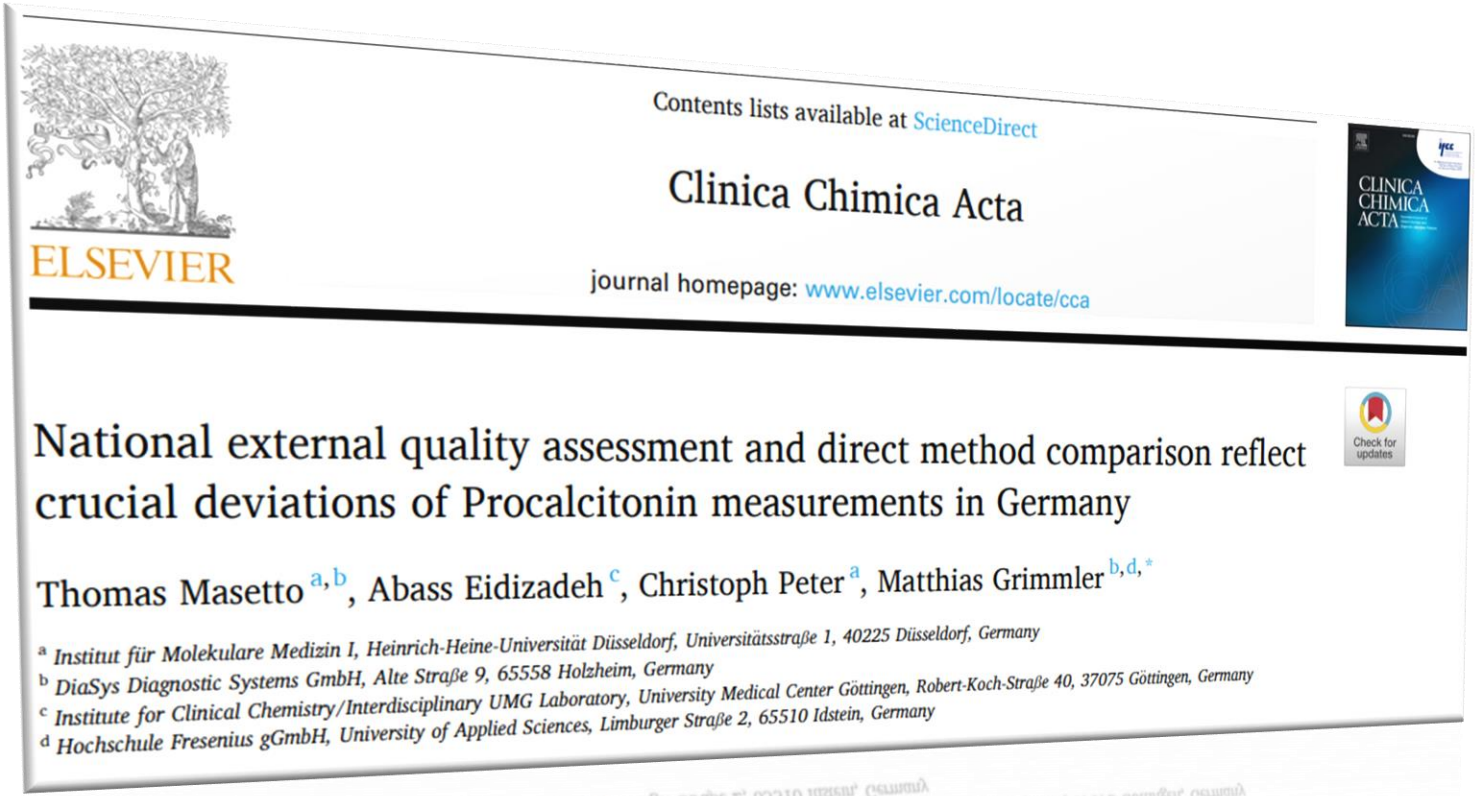

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

National external quality assessment and direct method comparison reflect crucial deviations of Procalcitonin measurements in Germany

Thomas Masetto [a,b], Abass Eidizadeh [c], Christoph Peter [a], Matthias Grimmler [b,d,*]

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[b] DiaSys Diagnostic Systems GmbH, Alte Straße 9, 65558 Holzheim, Germany
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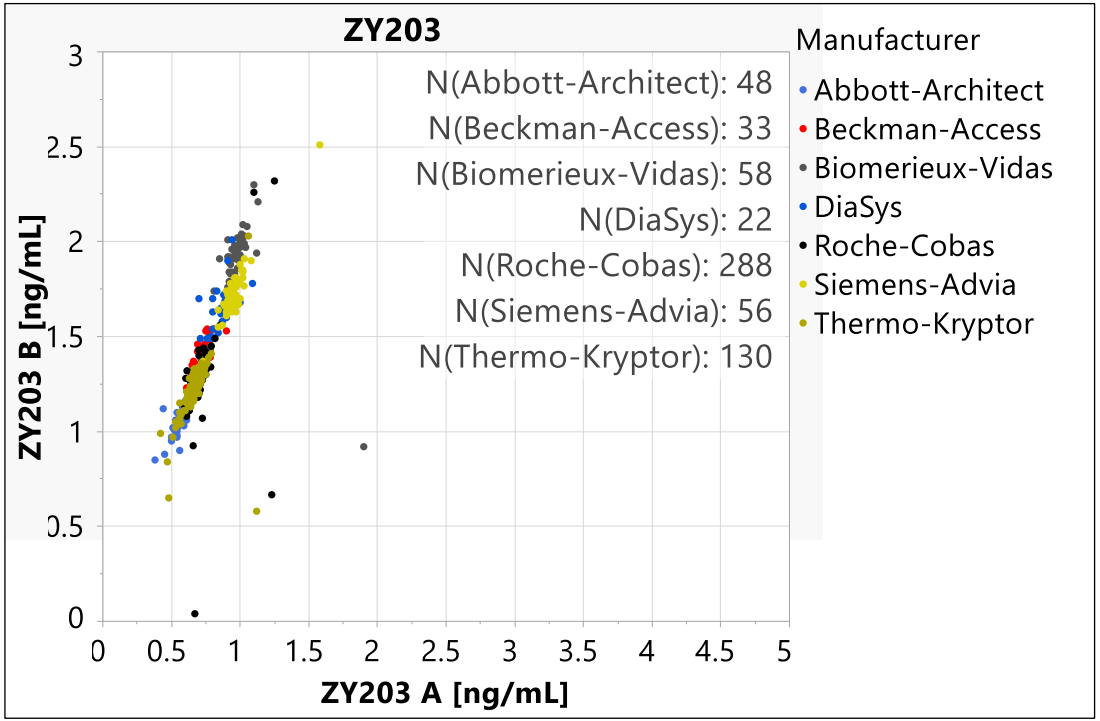
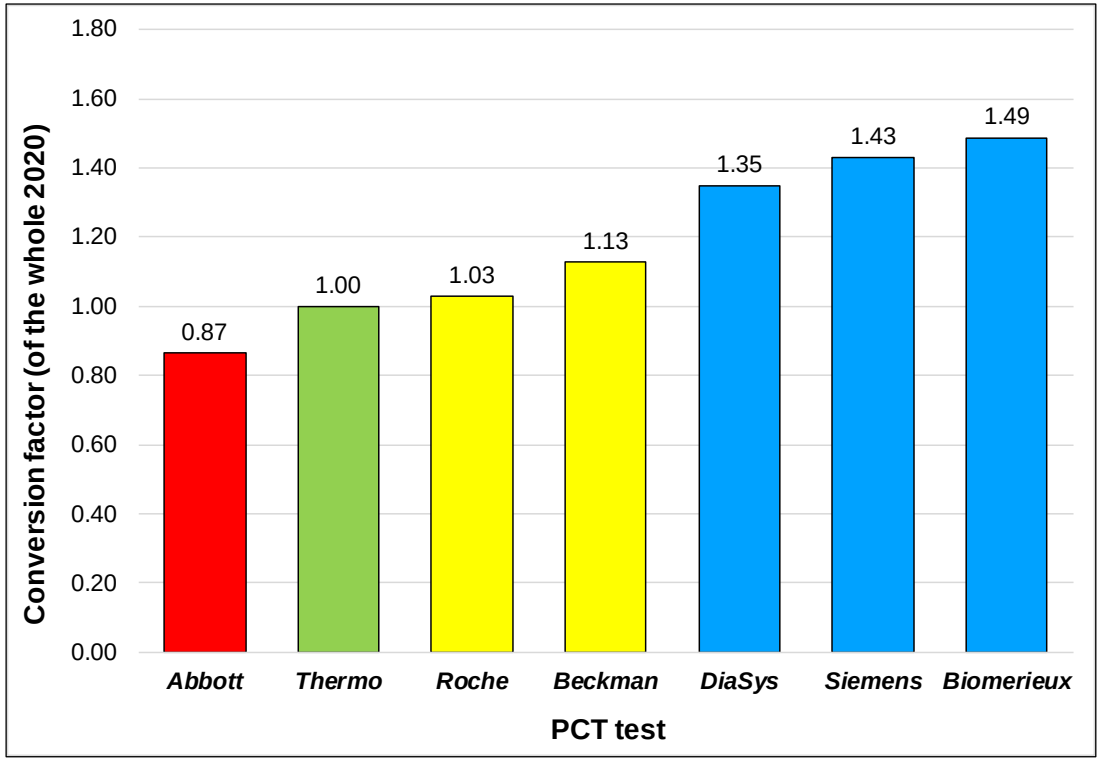
[d] Hochschule Fresenius gGmbH, University of Applied Sciences, Limburger Straße 2, 65510 Idstein, Germany

RfB EQA scheme 2020/3 (Germany) – The players



Masetto et al., 2022, 10.1016/j.cca.2022.02.007

RfB EQA scheme 2020/3 (Germany) – “Relation” factors



Masetto et al., 2022, 10.1016/j.cca.2022.02.007

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Take-home message

- Procalcitonin is currently the best commercially available biomarker for sepsis diagnosis… even though not perfect (Sensitivity approx. 0.85, Specificity approx. 0.75)
- Procalcitonin can be used also to guide antibiotic therapy, for prognosis of sepsis, in low respiratory tract infections, etc.
- Different immunoassay technologies and antibodies lead to a complex measurement situation (very different recoveries even though the cut-offs remain the same)
- EQA schemes also depict this very heterogenenous situation
- The standardisation or harmonisation of the different tests appears highly necessary

Thank you!

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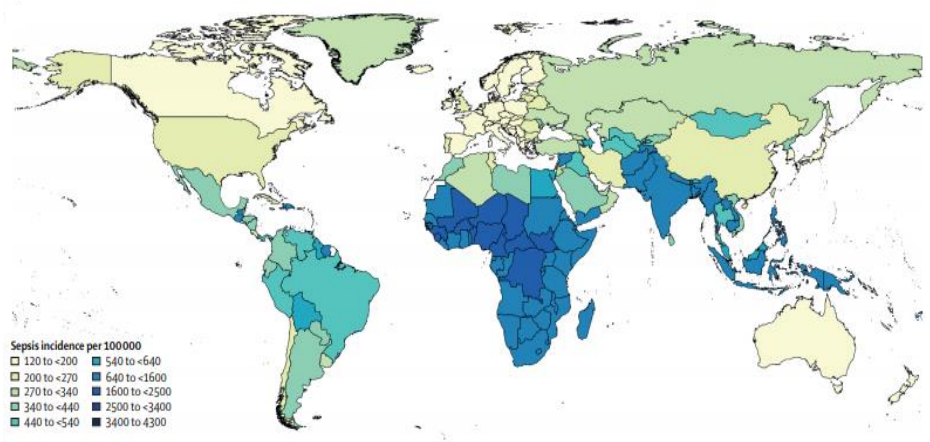
Challenges in PCT standardization and where next

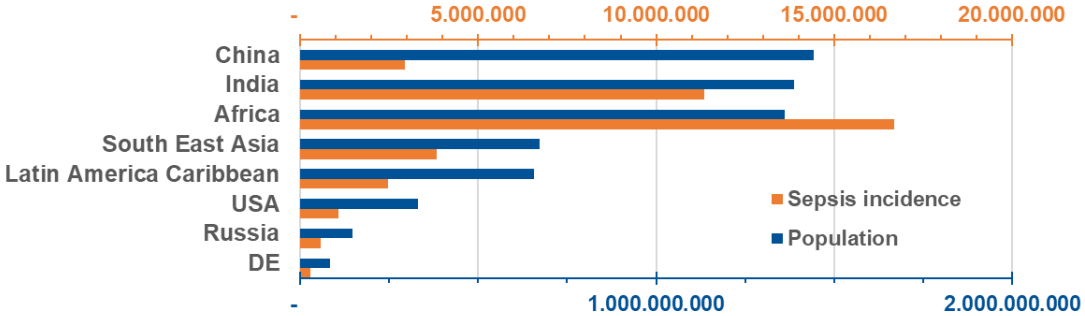
Prof. Dr. Matthias Grimmler

20th September 2023

Sepsis: A global health concern



- Sepsis is a tremendous challenge in developing countries
- 60-80% of deaths are due to sepsis in developing countries
- Affordable sepsis diagnosis significantly improves patient management and decreases mortality



K. E. Rudd, S. C. Johnson, K. M. Agesa, et al., The Lancet, 2020, 395, 200–211
Lee et al. ´Procalcitonin (PCT)-guided antibiotic stewardship in Asia-Pacific countries: adaptation based on an expert consensus meeting´ CCLM 2020, 58 (12), 1983

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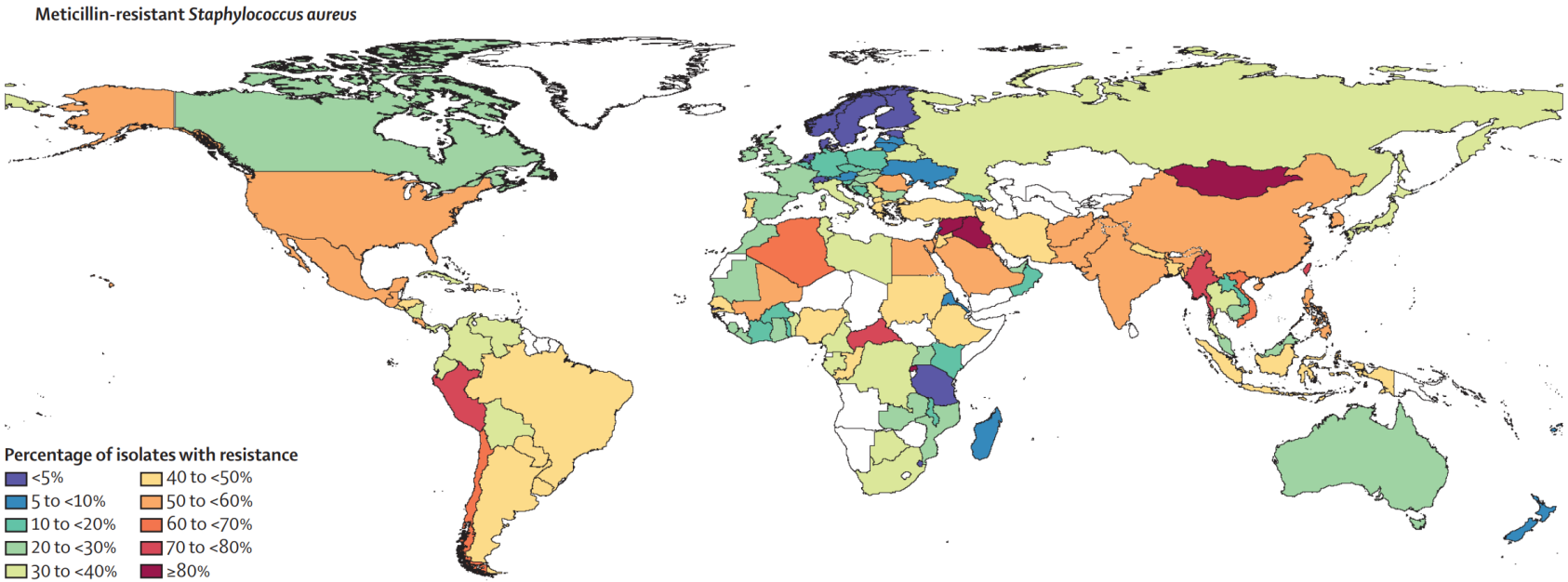
Sepsis: A global health concern



Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022 Feb 12;399(10325):629-655.

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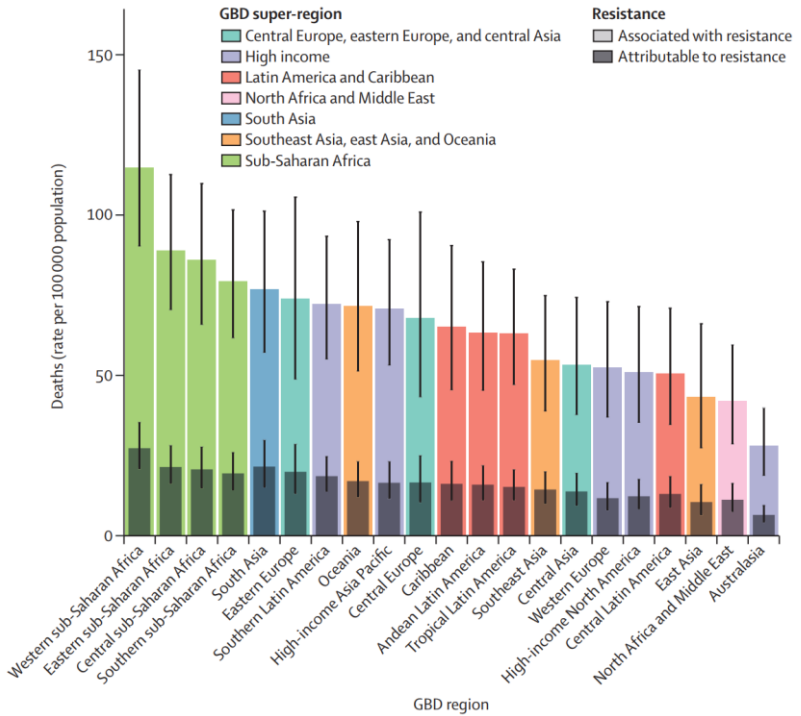
Sepsis: A global health concern

All-age rate of deaths attributable to and associated with bacterial antimicrobial resistance by GBD region, 2019



4.95 million deaths associated to antimicrobial resistance in 2019 - a globally growing cohort due to antimicrobial resistance / antibiotic crisis.

Are today's assays ready for this?

Lee et al. K. E. Rudd, S. C. Johnson, K. M. Agesa, et al., The Lancet, 2020, 395, 200–211

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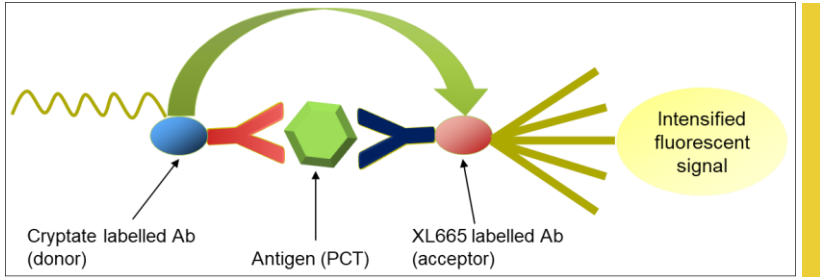
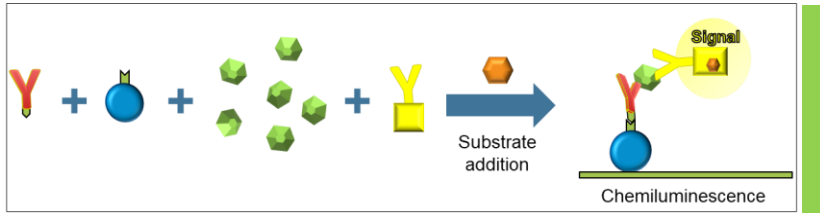
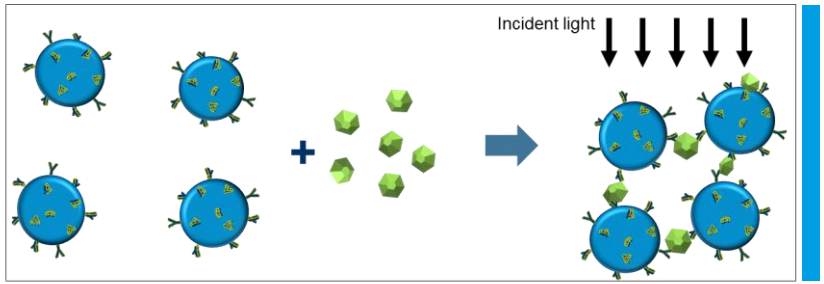
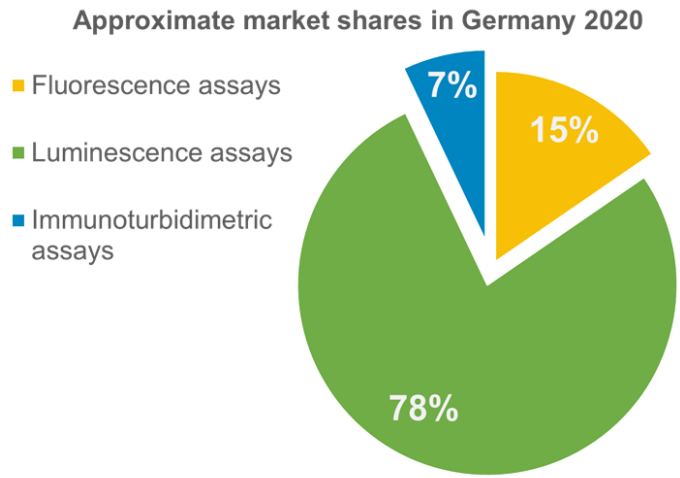
Procalcitonin: Major technologies/principles







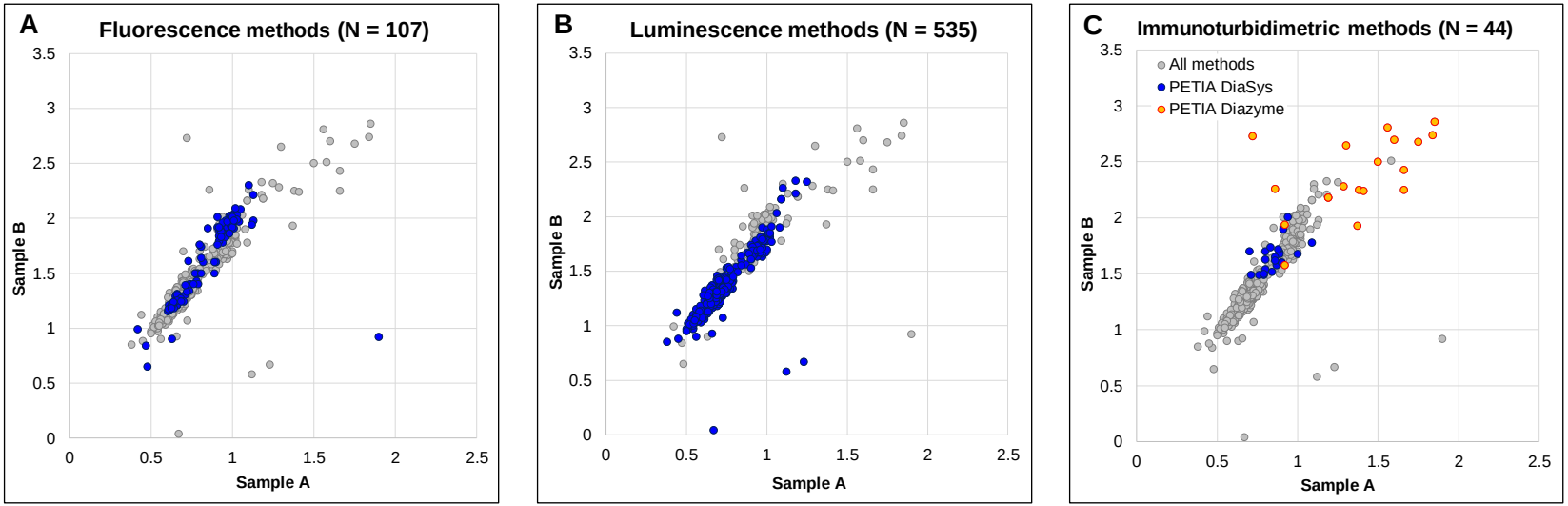


Procalcitonin

- Sensitive to bacterial origin
- Leading parameter in sepsis diagnostics
- Use of PCT significantly improves patient management

HyTest

PCT: EQA results on quantification in Germany

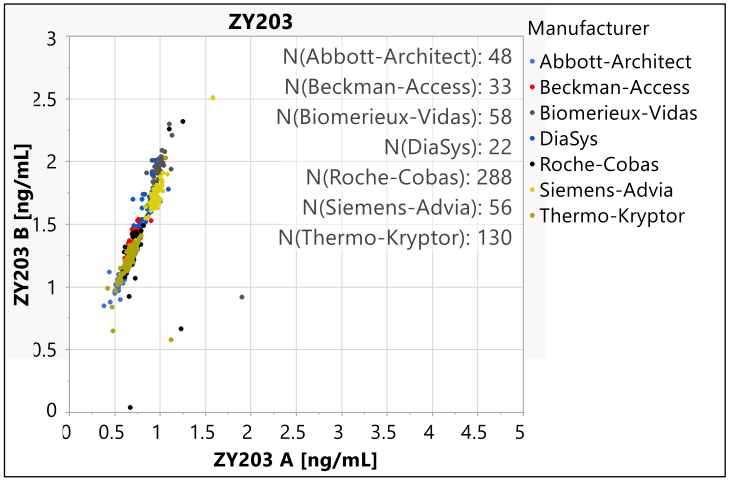


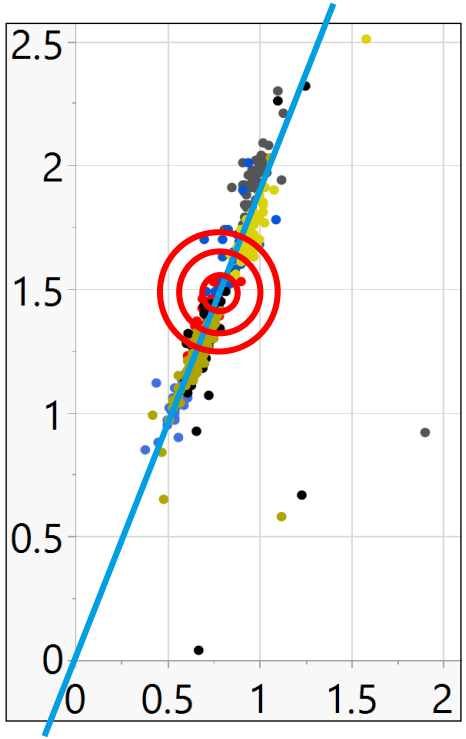
Results of PCT measurements within German laboratories for the RfB EQA scheme ZY2037”020 – overall and linked to technologies and tests.

Masetto T, Eidizadeh A, Peter C, Grimmler M. National External Quality Assessment and direct method comparison reflect crucial deviations of procalcitonin measurements in Germany. Clin Chim Acta., 2022 Apr;529:67–75.

HyTest

PCT: EQA results on quantification in Germany





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HyTest

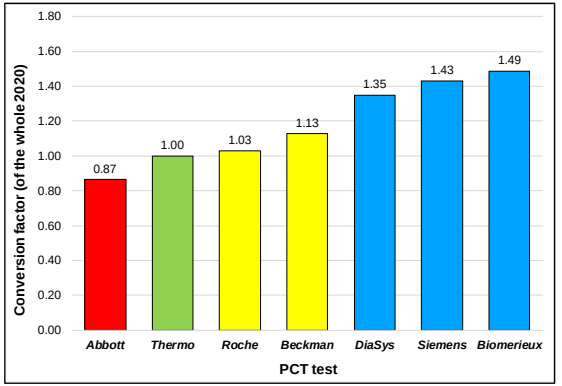
PCT: EQA results on quantification in Germany

Mean value A + B [ng/mL]	0.8215	1.0632	1.4311	1.2527	0.9802	1.3494	0.9494
Reference	**Measured**						
	Abbott	**Beckman**	**Biomerieux**	**DiaSys**	**Roche**	**Siemens**	**Thermo**
Abbott	1.00	1.29	1.74	1.52	1.19	1.64	1.16
Beckman	0.77	1.00	1.35	1.18	0.92	1.27	0.89
Biomerieux	0.57	0.74	1.00	0.88	0.68	0.94	0.66
DiaSys	0.66	0.85	1.14	1.00	0.78	1.08	0.76
Roche	0.84	1.08	1.46	1.28	1.00	1.38	0.97
Siemens	0.61	0.79	1.06	0.93	0.73	1.00	0.70
Thermo	0.87	1.12	1.51	1.32	1.03	1.42	1.00

Are today's assays ready for this?

Considering cut off / decision sepsis yes/no at 0.5 ng/mL, the answer clearly is:

No, they are not!



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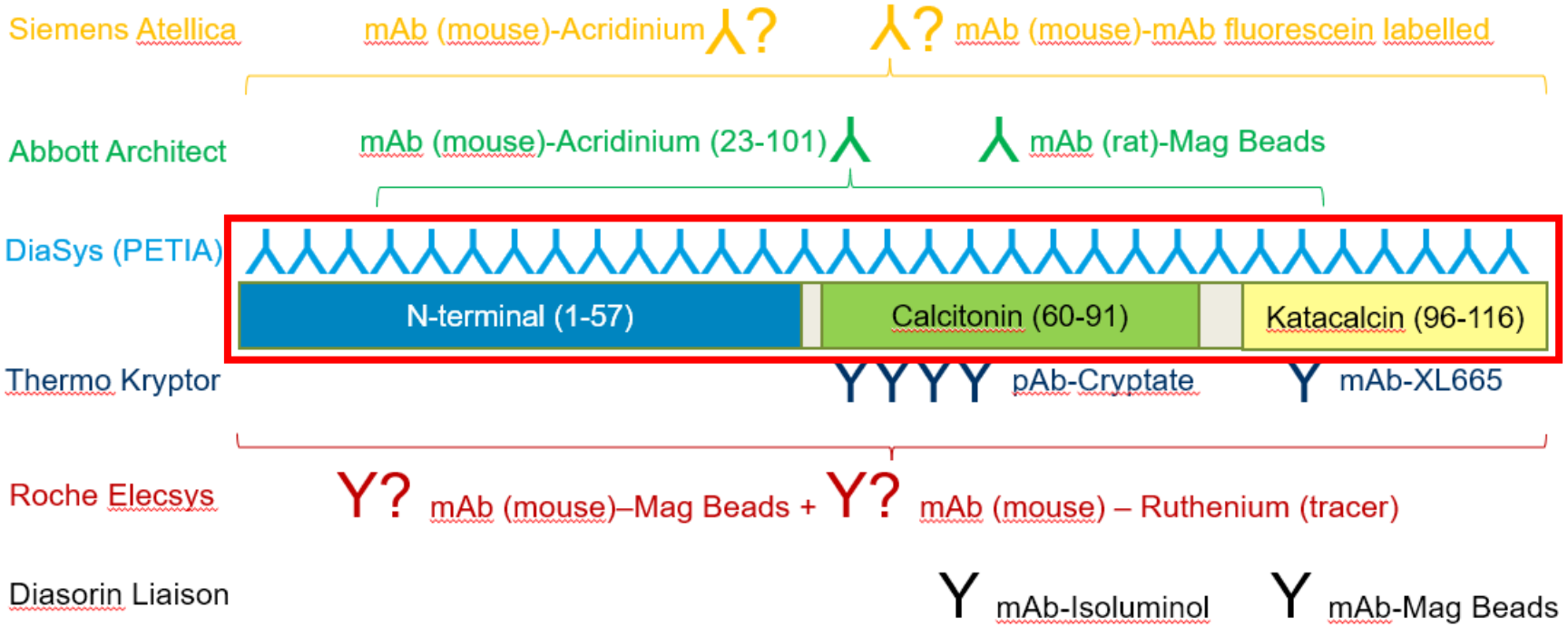
PCT: EQA results on quantification in Germany



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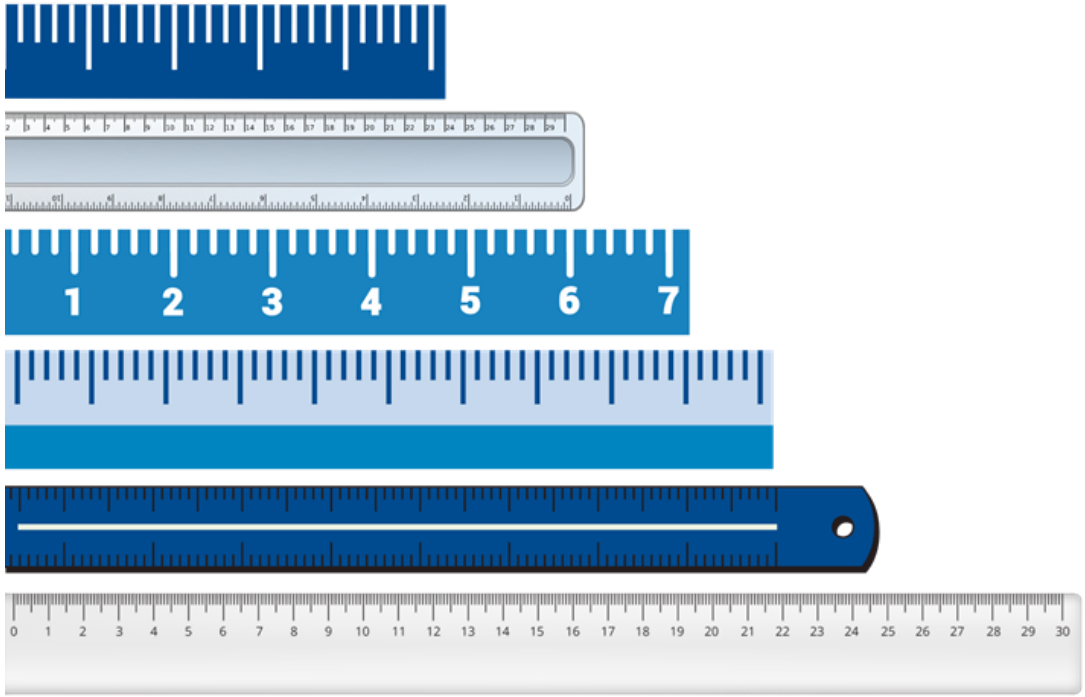
PCT: EQA results on quantification in Germany



What to do?

STANDARDIZATION

Huynh HH, Bœuf A, Vinh J, Delatour V; IFCC Working Group on Standardization of Procalcitonin assays (WG-PCT). Evaluation of the necessity and the feasibility of the standardization of procalcitonin measurements: Activities of IFCC WG-PCT with involvement of all stakeholders. Clin Chim Acta. 2021 Apr;515:111-121.

HyTest

IFCC Working Group on Standardization of PCT Assays

https://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-pct/

- Three laboratories, **LNE** (Laboratoire national de métrologie et d'essais), **Hochschule Fresenius** and **DiaSys** are the three core members to develop the reference method on PCT quantification. 30 members in total; 16 scientists and 14 company representatives
- **Goal:** Develop and validate a reference measurement procedure for absolute PCT quantification by Stable Isotope Dilution Mass Spectrometry; provide respective reference material (primary calibrators) and commutable EQA materials to ensure comparability of commercially available PCT assays

Selection of Related Publications:

1. Evaluation of the necessity and the feasibility of the standardization of procalcitonin measurements: Activities of IFCC WG-PCT with involvement of all stakeholders. DOI: 10.1016/j.cca.2021.01.004
2. Harmonization status of procalcitonin measurements: what do comparison studies and EQA schemes tell us? DOI: 10.1515/cclm-2021-0566
3. National external quality assessment and direct method comparison reflect crucial deviations of Procalcitonin measurements in Germany. DOI: 10.1016/j.cca.2022.02.007
4. Candidate High-Resolution Mass Spectrometry-Based Reference Method for the Quantification of Procalcitonin in Human Serum Using a Characterized Recombinant Protein as a Primary Calibrator. DOI: 10.1021/acs.analchem.1c03061
5. Comprehensive Comparison of the Capacity of Functionalized Sepharose, Magnetic Core, and Polystyrene Nanoparticles to Immuno-Precipitate Procalcitonin from Human Material for the Subsequent Quantification by LC-MS/MS. DOI: 10.3390/ijms241310963
6. Immunoaffinity LC−MS/MS Quantification of the Sepsis Biomarker Procalcitonin Using Magnetic- and Polystyrene-Bead Immobilized Polyclonal Antibodies. DOI: 10.1021/acs.jproteome.3c00082

IFCC Working Group on Standardization of PCT Assays

How to standardize PCT?

Easy, if you have a simple and abundant analyte.
PCT is complex by ways of proteolytic processing (talk by Thomas), it is low abundant and may be post translationally modified.

For this: You need to know your analyte – and you need to enrich!

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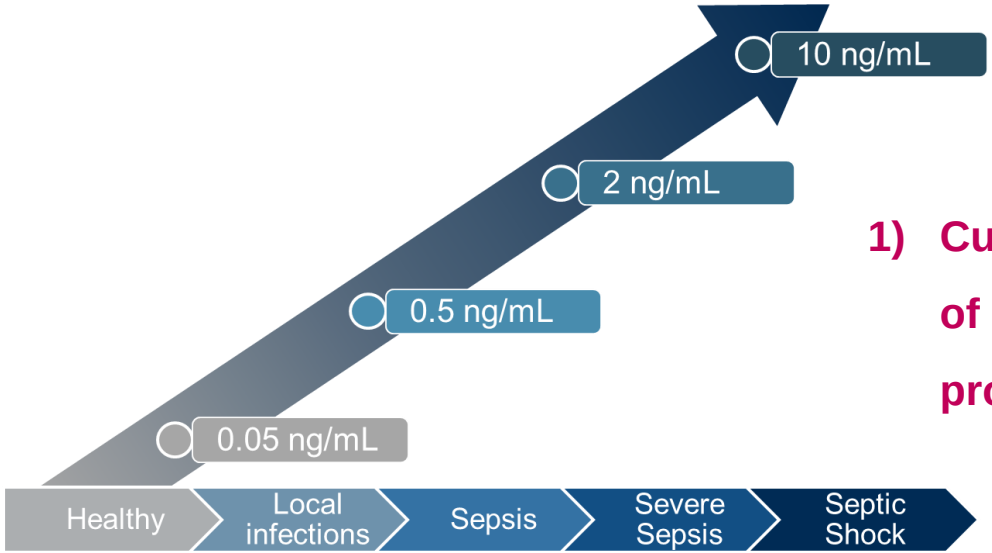

1) Cut off levels PCT are at the limit of detection of LC-MS/MS on protein analytes!

S. Harbarth, K. Holeckova, C. Froidevaux, D. Pittet, B. Ricou, G. E. Grau, L. Vadas, J. Pugin and Geneva Sepsis Network, Am. J. Respir. Crit. Care Med., 2001, 164, 396–402.
M. Meisner, Procalcitonin: ein neuer, innovativer Infektionsparameter ; biochemische und klinische Aspekte, Georg Thieme Verlag, Stuttgart New York, 3. Edition, 2000.
M. Meisner, Procalcitonin - biochemistry and clinical diagnosis, UNI-MED Verl, Bremen, 1st ed., 2010.
K. L. Becker, R. Snider and E. S. Nylen, British Journal of Pharmacology, 2010, 159, 253–264.
K. L. Becker, E. S. Nylén, J. C. White, B. Müller and R. H. Snider, The Journal of Clinical Endocrinology & Metabolism, 2004, 89, 1512–1525.
N. G. Morgenthaler, J. Struck, C. Fischer-Schulz, E. Seidel-Mueller, W. Beier and A. Bergmann, Clin. Lab., 2002, 48, 263–270.

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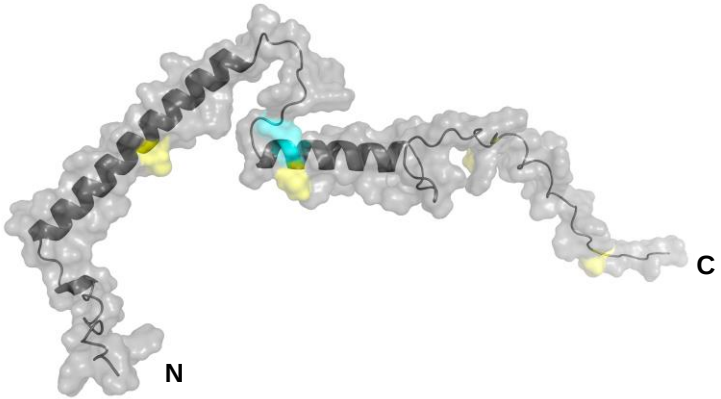
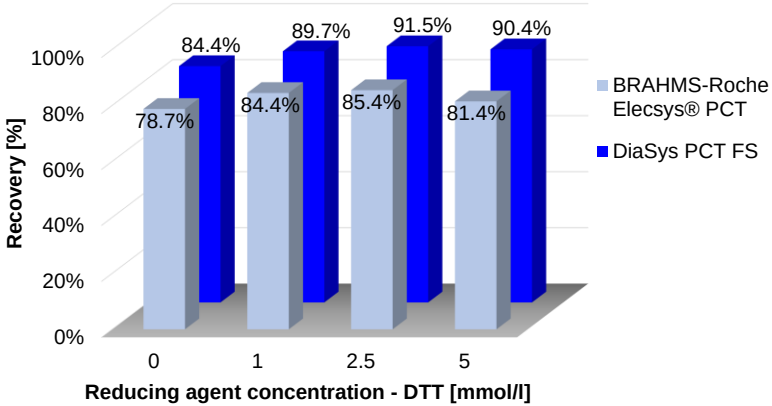


Predicted structure of human PCT and impact of oxidation



Recovery of rhPCT after 10 days of incubation at 37 °C with increasing amounts of reducing agents (DTT) by different immunoassays:

2) For quantitative enrichment, you should get ALL forms/modifications!

Masetto T, Matzenbach K, Reuschel T, Tölke SA, Schneider K, Esser LM, Reinhart M, Bindila L, Peter C, Grimmler M. Comprehensive Comparison of the Capacity of Functionalized Sepharose, Magnetic Core, and Polystyrene Nanoparticles to Immuno-Precipitate Procalcitonin from Human Material for the Subsequent Quantification by LC-MS/MS. Int J Mol Sci. 2023 Jun 30;24(13):10963.

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Two strategies to „concentrate“ a protein from a patient sample:

1) **Chemical enrichment/precipitation**
2) **Immuno enrichment/precipitation**

LNE was doing the first strategy

Fresenius University/DiaSys the second

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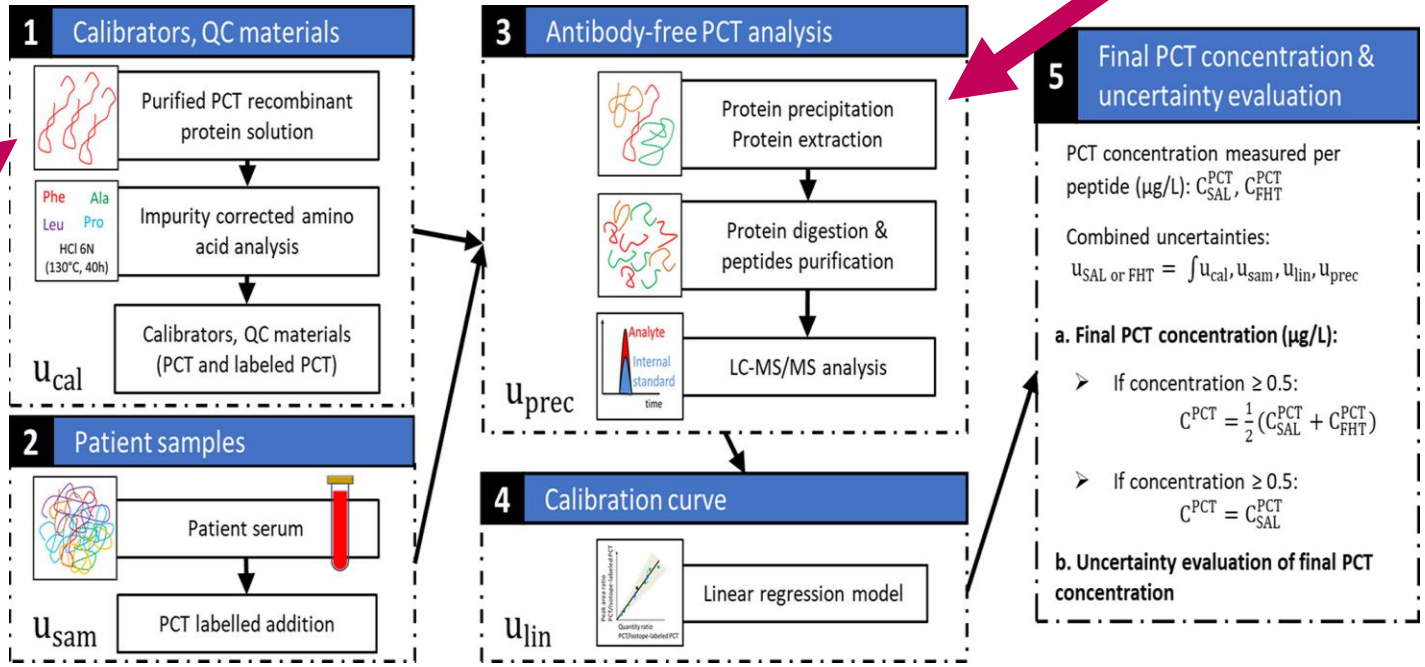
Workflow for SI-traceable quantification of PCT in human serum and its uncertainty



Huynh HH, Delatour V, Derbez-Morin M, Liu Q, Boeuf A, Vinh J. Candidate High-Resolution Mass Spectrometry-Based Reference Method for the Quantification of Procalcitonin in Human Serum Using a Characterized Recombinant Protein as a Primary Calibrator. Anal Chem. 2022 Mar 15;94(10):4146-4154.

Huynh HH, Bœuf A, Derbez-Morin M, Dupuy AM, Lalere B, Delatour V, Vinh J. Development of an antibody-free ID-LC MS method for the quantification of procalcitonin in human serum at sub-microgram per liter level using a peptide-based calibration. Anal Bioanal Chem. 2021 Aug;413(19):4707-4725

IFCC Working Group on Standardization of PCT Assays

Summary:

- Validated robust LC-MS/MS method, based on denaturing protein precipitation (SDC detergent / acetonitrile)
  Instrument: Dionex Ultimate 3000 ultraperformance liquid chromatography system coupled to a
  Q Exactive Focus hybrid QuadrupoleOrbitrap mass spectrometer (Thermo Scientific)
- LLOQ: 0.25 ng/mL
- Dynamic range 0.25 to 13.74 ng/mL, possible to extend by sample dilution
- Sample consumption: 0.5 mL Serum/Plasma
- Time to result (complete workflow): up to 2 days

IFCC Working Group on Standardization of PCT Assays

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UNIVERSITY OF APPLIED SCIENCES

DiaSys
Diagnostic Systems

Immuno-enrichment

- **which antibody to use to best ensure quantitative enrichment of all forms/modifications of PCT?**

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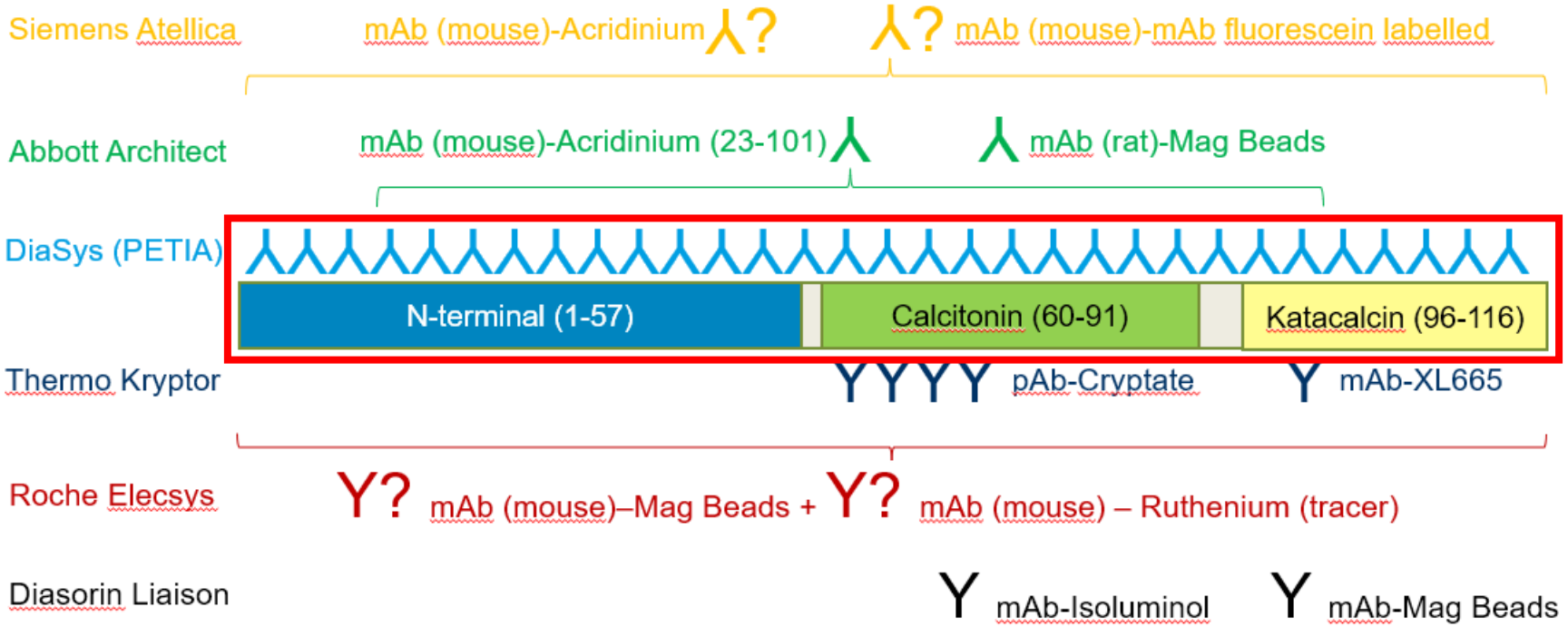

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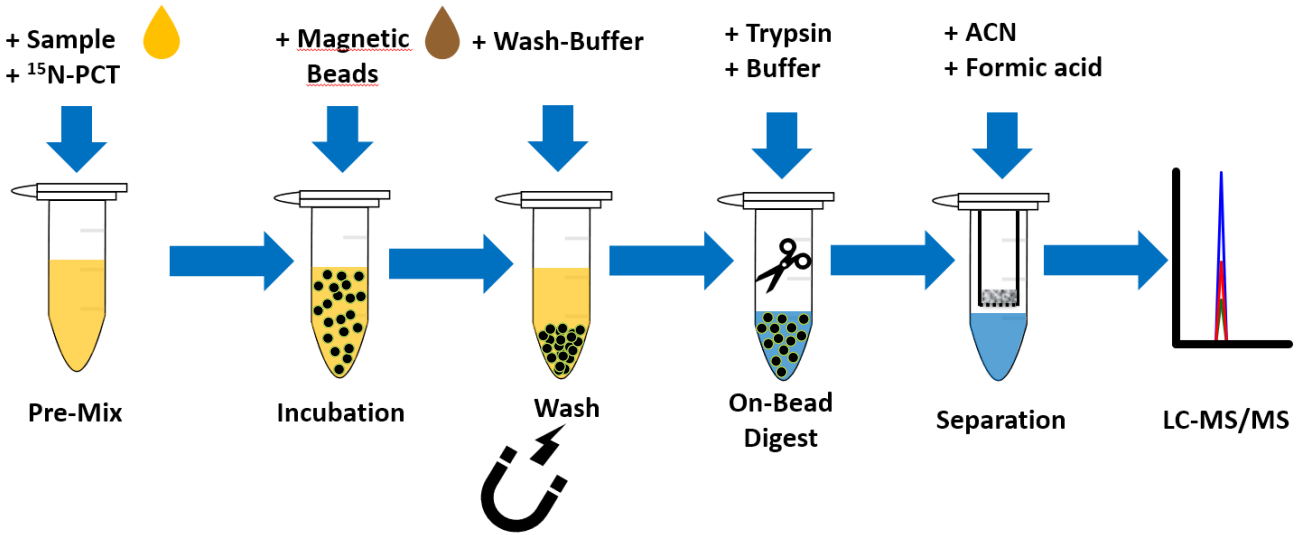

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Tölke SA, Masetto T, Reuschel T, Grimmler M, Bindila L, Schneider K. Immunoaffinity LC–MS/MS Quantification of the Sepsis Biomarker Procalcitonin Using Magnetic- and Polystyrene-Bead Immobilized Polyclonal Antibodies. J. Proteome Res. 2023, DOI: 10.1021/acs.jproteome.3c00082

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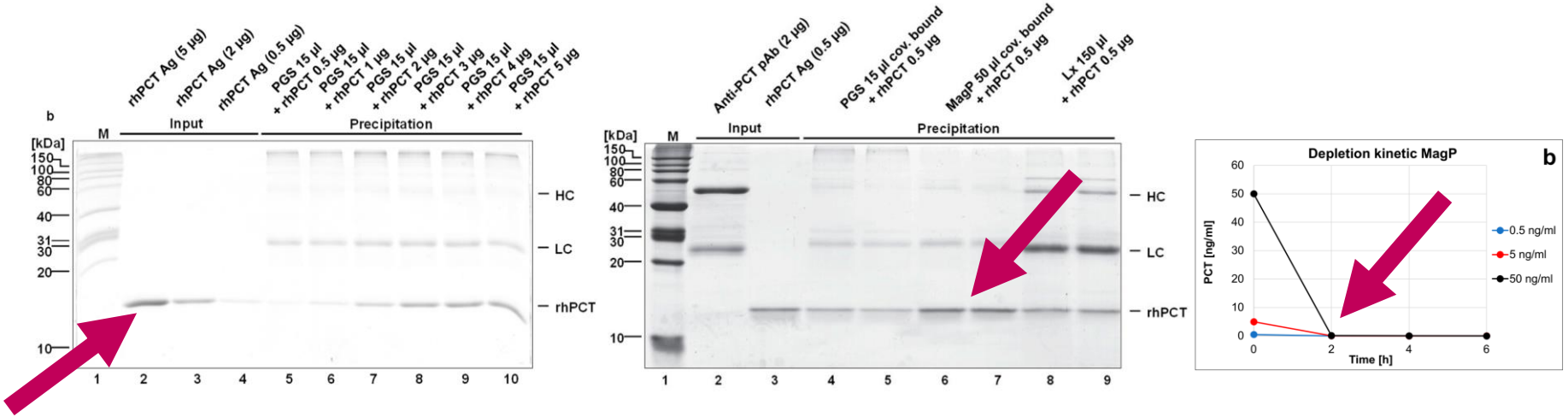

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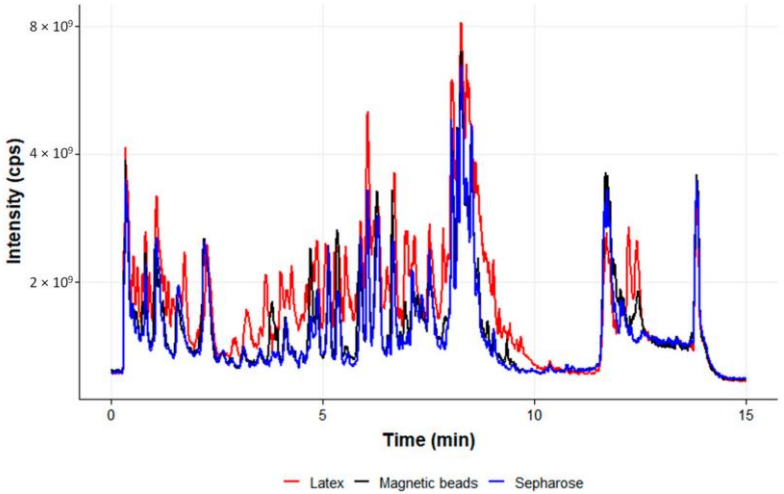

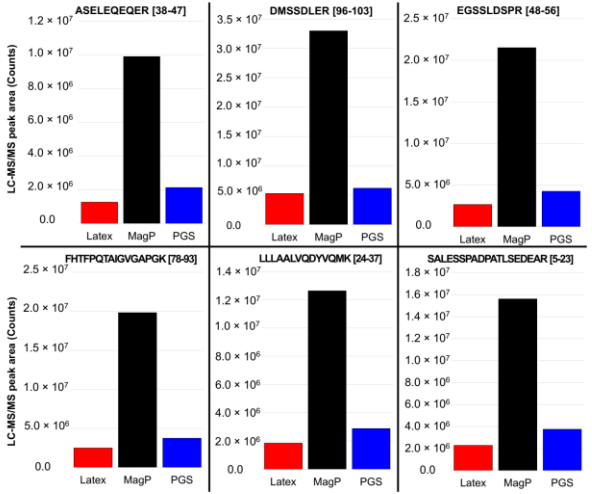

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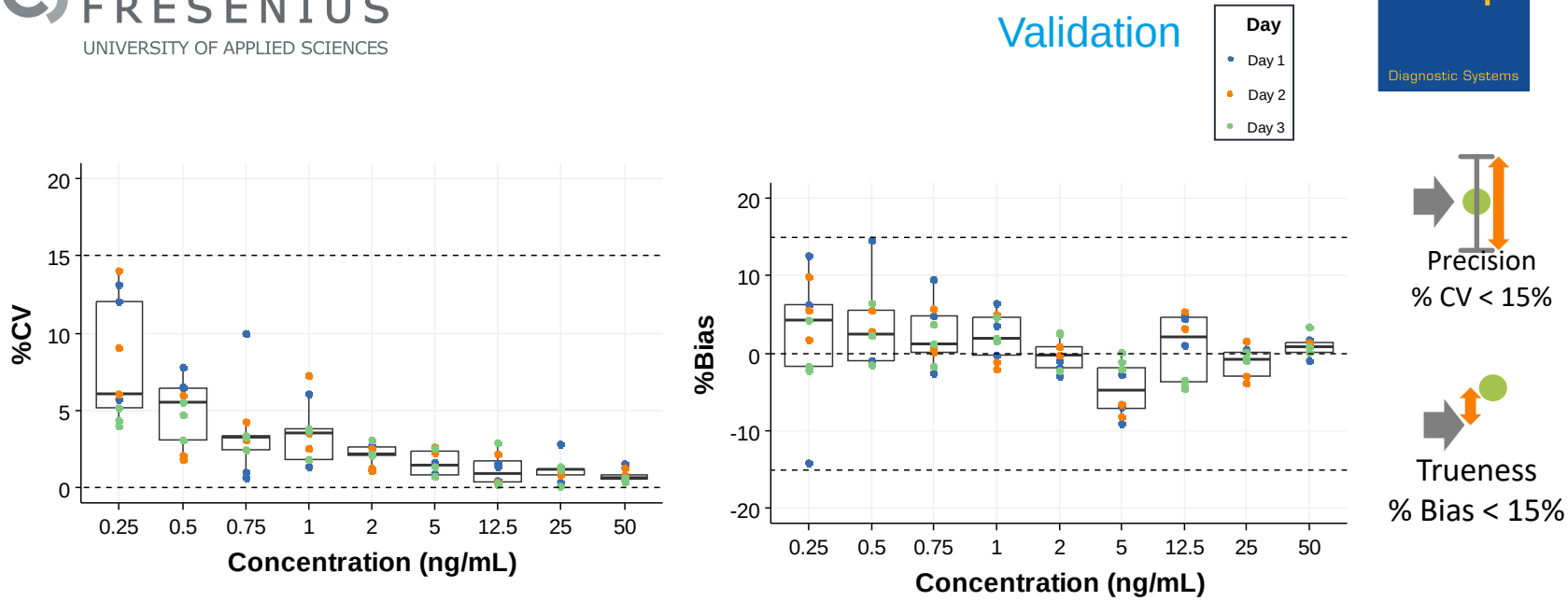
DiaSys Diagnostic Systems

Validation



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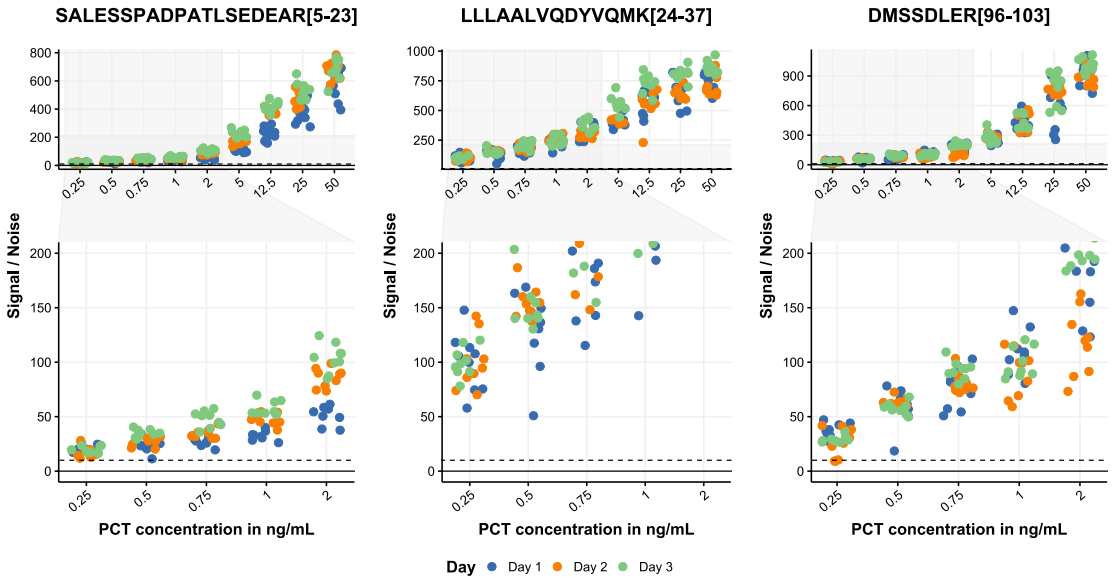

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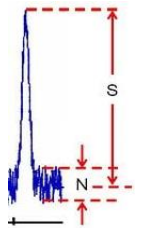

Signal to Noise
S/N > 10

Tölke SA, Masetto T, Reuschel T, Grimmler M, Bindila L, Schneider K. Immunoaffinity LC–MS/MS Quantification of the Sepsis Biomarker Procalcitonin Using Magnetic- and Polystyrene-Bead Immobilized Polyclonal Antibodies. J. Proteome Res. 2023, DOI: 10.1021/acs.jproteome.3c00082

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IFCC Working Group on Standardization of PCT Assays

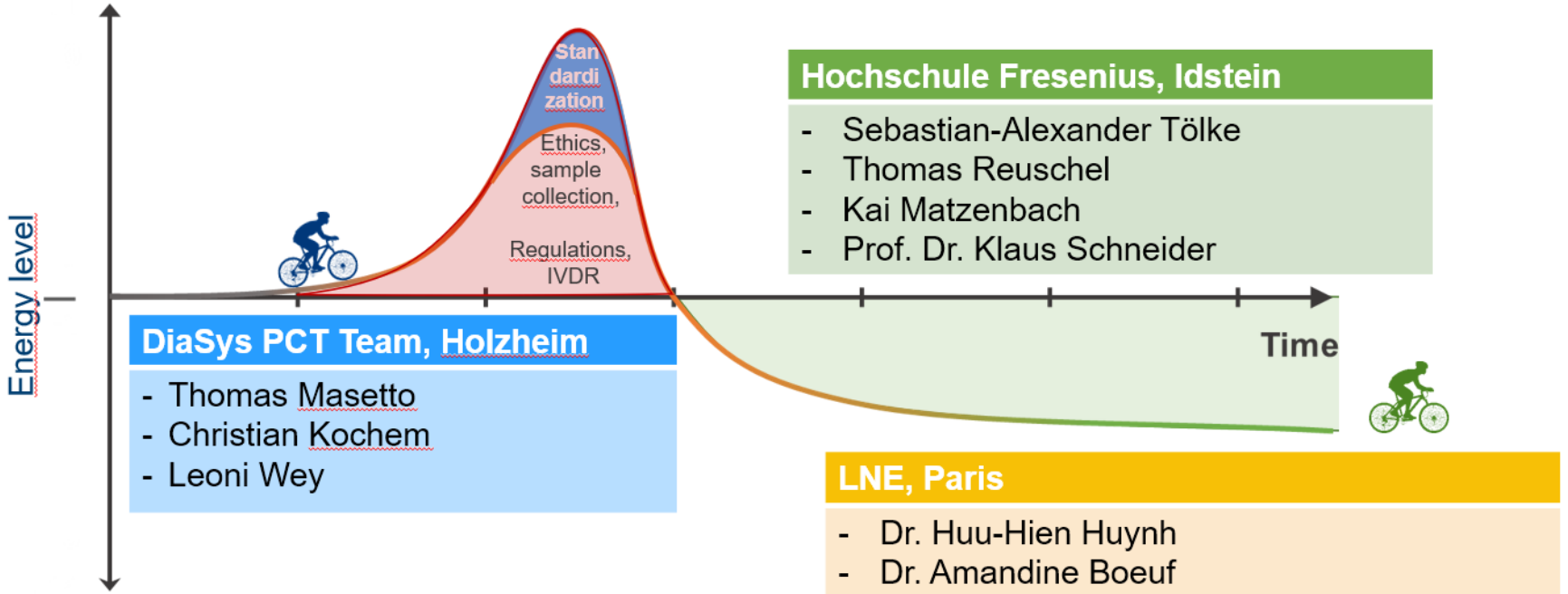
Summary:

- Validated robust immuno-LC-MS/MS method, based on pAbs
  ^{15}N-Labeled PCT as ISTD. Instrument: ESI-QqQ (Sciex 5500 QTRAP)
- LLOQ: 0.25 ng/mL
- Dynamic range 0.25 ng/mL – 50 ng/mL
- Sample consumption: 1 mL Serum/Plasma
- Time to result (complete workflow): up to 2 days

Take-home message

- Sepsis is a global burden, especially attributed to developing countries and antimicrobial resistance. PCT is a crucial diagnostic tool.
- A broad variability of methods and used antibodies on PCT quantification in the market causes high variation among different PCT assay/manufacturers.
- *IFCC WG on Standardization of PCT assays* is evaluating this variation and is working on respective reference methods and material to reduce this heterogenous situation.
- Stable Isotope Dilution Mass Spectrometry-based reference methods are developed by chemical precipitation/denaturation or by immuno precipitation.
- Good success in both methods, but by 0.25 ng/mL so far achieved, sensitivity still needs to be improved further.

IFCC Working Group on Standardization of PCT Assays



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DiaSys PCT Team, Holzheim

- Thomas Masetto
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LNE, Paris

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HyTest

Thank you!

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University of Applied Science/HOCHSCHULEN FRESENIUS gGmbH
Idstein, Germany

HyTest

Q & A

Q&A

Q: What is the overall status with the PCT Standardization?

A: The standardization of PCT was initiated in 2018 by the IFCC WG on PCT. Currently, IFCC has intensivley analyzed the current situation in different countries and assay systems and did observe/confirm quite some variety of results coming along with different assay types and manufacturers.

In the last years two methods have been developed, based on Stable Isotope Dilution Mass Spectrometry. One is based on SDC detergent / acetonitrile (LNE) one is based on immunoenrichment by polyclonal Abs (Fresenius/DiaSys). Both methods robustly quantify PCT down to a LLOQ of 0.25 ng/mL. We need to further reduce sensitivity down to 0.1 ng/mL or less. This is ongoing work. On the other hand, IFCC is initiating a global EQA study, based on patient samples to directly compare the most relevant PCT immunomethods around the globe as well as LC-MS/MS methods. Based on the outcome (probably in 2024) defining suitable and commutable first order reference material will be initiated. Also defining more centers to act as PCT reference labs will be necessary. For sure, still quite a long lasting and complex work ahead!

Q&A

Q: How to develop a good antibody for diagnosis application? What's the critical technique or methods?

A: It is really not that easy to develop good diagnostic antibody. Good antibody by definition have to react with native antigen, particular form of it, which exist in biological fluid to be tested. Good antibody has to be resistant to any influence which may affect its interaction with such analyte. Therefore one needs to know antigen, its biochemical and immunochemical properties.

Next thing to mention is that development of antibody with particular characteristics (for instance recognizing only specific form of an analyte) is a quite tricky thing in comparison to development of “just an antibody” when you are not aiming at some properties in particular. Unfortunately, there is no critical technique which guarantees development of good antibody. After getting knowledge of an antigen, one usually employs several methods for development of antibodies hoping that broader the method portfolio more likely it is to select antibody with desired properties.

Q&A

Q: How will PCT continue to be relevant as a stand-alone test, as additional markers are becoming available?

A: As showed during the webinar, PCT has a sensitivity for sepsis diagnosis of approx. 85%. This means that PCT will miss to diagnose as positive 15 out of 100 septic patients. PCT remains the best commercially available biomarker for sepsis diagnostic that we currently have, but it is not perfect. Missing 15% of the positive diagnoses is quite much. Consequently, PCT should never be used alone to diagnose sepsis. In this direction Schütz et al. published an expert consensus (10.1515/cclm-2018-1181) indicating, in my opinion, the best way to diagnose sepsis: a combination of clinical patient assessment, probability of infection and biochemical markers (e.g. PCT). After a confirmed diagnosis of sepsis, however, PCT can be used also alone to follow up antibiotic therapy. Decreasing PCT blood concentrations indicate to discontinue antibiotic treatment, while increasing concentrations the other way round. The algorithm to guide antibiotic treatment is defined here 10.1016/S0140-6736(09)61879-1. However, a combined evaluation with the whole clinical situation of the patient is always preferable, also for antibiotic stewardship.

Q&A

Q: Is there an FDA approved lateral flow test for PCT? If not, what are the limiting factors?

A: Currently, there is no FDA-approved lateral flow assay for PCT. To the best of our knowledge there is also no LFA at all for PCT. First, LFA are mainly meant for qualitative purposes. Recently, some quantitative LFA application has been proposed (e.g. 10.3390/s22197398) but their real performances are still questionable.

In the case of PCT, the measurement accuracy is very important, consequently the system must be completely quantitative and validated. Moreover, the very low blood PCT concentrations and make it difficult for a LFA to reach the desired sensitivity, needed to differentiate among positive and negative patients.

Q&A

Q: What factors should be considered in designing a PCT kit using chemiluminescence immunoassay, and what are the potential pitfall?

A: Antibodies used are the key factor for designing an immunoassay. They need to represent the in vivo situation of an antigen/PCT in the on- and off-set of sepsis. Also, they need to consider post-translational processing, isoforms, modification of the antigen. As PCT unfortunately is not well biochemically characterized in this regard (eg. by age, gender, ethnies, reaction to medical treatment), this for sure is a difficult task. On the other hand, high purity, integrity of the protein, long time stability and reactivity/specificity is needed also coming along with low lot-to-lot variability to ensure best/homogenous conditions when linking the Ab to a carrier surface. Additionally, an assay needs to come along with calibrators + controls. For this, the antigen needs to fulfill similar specifications: representing the setting in sepsis in patients/normal samples as well as a high, robust immunoreactivity and stability.

Q&A

Q: Performance evaluation of existing nephelometric and turbidimetric PCT assays compared to "BRAHMS" PCT based on sandwich format

A: To the best of our knowledge, there is no nephelometric assay for PCT available on the market at the moment. There are however two turbidimetric assays, the first by the company Diazyme, the second by the company DiaSys. Many publications in the last years have shown that the Diazyme´s test has got questionable clinical performances (Ceriotti 2017 10.1515/cclm-2017-0159; Lippi 2015 10.1016/j.plabm.2015.07.001; Dupuy 2020 10.3390/diagnostics10070461, Jensch 2021 10.1515/cclm-2020-1541, Masetto 2022 10.1016/j.cca.2022.02.007), while the DiaSys´ one appears to have good clinical performances and comparability with sandwich assays by the company BRAHMS (Dupuy, 2020, 10.3390/diagnostics10070461; Eidizadeh, 2022, 10.1016/j.plabm.2022.e00274; Masetto, 2022, 10.1016/j.cca.2022.02.007; Di Deo-Vantaggiato, 2023, 10.1515/cclm-2023-0776). The comparison however should be divided at least in precision and recovery. While the DiaSys´ assay (but not the Diazyme´s) precision is comparable with BRAHMS licensed assays for concentrations ≥ 0.5 ng/mL (sepsis appplication), at lower concentrations (necessary for different clinical applications, e.g. LRTI) the turbidimetric technology can hardly compete with other more precise technologies (CLIA, ELFA, etc.). On the other hand, the recovery (trueness) is heavily affected by the missing standardization (Masetto, 2022, 10.1016/j.cca.2022.02.007) and each laboratory should establish its own reference limits and cut-offs, until a reference material will be made available by the IFCC working group on PCT standardization. This heterogenous measurement situation however affects all tests on the market and is not an exclusive problem of the turbidimetric-based ones.

Q&A

Q: How PCT can be used to prevent septic shock?

A: PCT is currently the best biomarker for sepsis diagnostic available on the market, even though not perfect (sensitivity 85%, specificity 75% approx.). As soon as the clinician, based on the probability of infection and the clinical situation, hypothesises that the patient could have a bacterial infection / sepsis, he should measure PCT and start antibiotic therapy (PCT ≥ 0.5 ng/mL) or not start antibiotic therapy (PCT < 0.5 ng/mL). Furthermore, the success of this antibiotic therapy should be monitored through PCT measurement (at least once every 24 h). Decreasing PCT blood concentrations suggest a successful therapy, while increasing ones suggest to continue the treatment or to change antibiotic, as the patient is not responding. For the detailed algorithms, please refer to Bouadma, 2010, 10.1016/S0140-6736(09)61879-1.

Moreover, one further possible useful marker of septic shock could be lactate, as reported by the task force which elaborated the present Sepsis-3 definition (Singer, 2016, 10.1001/jama.2016.0287, lactate > 18 mg/dL). Also Lee, 2016, 10.21037/jtd.2016.05.55 reports lactate´s utility as a prognostic toll of mortality / septic shock.

Q&A

Q: Why go choose PCT over CRP?

A: PCT is slightly more sensitive and overall much more specific for (bacterial) sepsis diagnosis than CRP. This means that if the goal is to monitor / diagnose a bacterial sepsis, PCT does have a big advantage over CRP, what could be elevated in many other different clinical conditions, independently from sepsis. Refer to Lippi, 2017, 10.4081/ecj.2017.6877 and slide 7 of the first presentation from the webinar.

Moreover, the rapid half-life in blood of PCT (20-24 hs) vs. CRP (approx. 7 days) makes its use for antibiotic stewardship also possible, as PCT blood concentrations would decrease rapidly if the antibiotic therapy is working. On the other hand, CRP is eliminated much slower, so even if the antibiotic therapy is working, anyway its blood concentrations would remain high for long time, impeding the recognition of the antibiotic threatment success.

Q&A

Q: In clinical chemistry analyzer, how accurate is the test of PCT compared to the CLIA, ELFA & eCLIA

A: We should first of all define about which turbidimetric test we are talking about. To the best of my knowledge, there are currently two turbidimetric assays on the market:, the first by the company Diazyme, the second by the company DiaSys. Many publications in the last years have shown that the Diazyme´s test has got questionable clinical performances (Ceriotti, 2017, 10.1515/cclm-2017-0159; Lippi, 2015, 10.1016/j.plabm.2015.07.001; Dupuy, 2020, 10.3390/diagnostics10070461; Jensch, 2021, 10.1515/cclm-2020-1541; Masetto, 2022, 10.1016/j.cca.2022.02.007), while the DiaSys´ one appears to have good clinical performances and comparability with sandwich assays by the company BRAHMS (Dupuy, 2020, 10.3390/diagnostics10070461; Eidizadeh, 2022, 10.1016/j.plabm.2022.e00274; Masetto, 2022, 10.1016/j.cca.2022.02.007; Di Deo-Vantaggiato, 2023, 10.1515/cclm-2023-0776). The accuracy comparison however should be divided in precision and recovery. While the DiaSys´ (but not the Diazyme´s) assay precision is comparable with BRAHMS-licensed assays for concentrations ≥ 0.5 ng/mL (sepsis appplication), at lower concentrations (necessary for different clinical applications, e.g. LRTI) the turbidimetric technology can hardly compete with other more precise technologies (CLIA, ELFA, etc.). On the other hand, the recovery (trueness) is heavily affected by the missing standardization (Masetto, 2022, 10.1016/j.cca.2022.02.007) and each laboratory should establish its own reference limits and cut-offs, until a reference material will be made available by the IFCC working group on PCT standardization. This heterogenous measurement situation however affects all tests on the market and is not an exclusive problem of the turbidimetric-based ones.

Q&A

Q: Importance of PCT in Emergency & Difference between PCT vs Lactate

A: If we talk about sepsis, PCT is currently the best biomarker for early diagnosis available on the market, even though not perfect (sensitivity 85%, specificity 75% approx.). As soon as the clinician, based on the probability of infection and the clinical situation, hypothesises that the patient could have a bacterial infection / sepsis, he should measure PCT and start antibiotic therapy (PCT ≥ 0.5 ng/mL) or not start antibiotic therapy (PCT < 0.5 ng/mL). Furthermore, the success of this antibiotic therapy should be monitored through PCT measurement (at least once every 24 h). Decreasing PCT blood concentrations suggest a successful therapy, while increasing ones suggest to continue the treatment or to change antibiotic, as the patient is not responding. There are many pieces of evidence demonstrating the utility of PCT in Emergency and ICU. However, please refer to Bouadma, 2010, 10.1016/S0140-6736(09)61879-1 and de Jong, 2016, 10.1186/1471-2334-13-178 as original clinical trials. In relation to other clinical conditions (e.g. LRTI) or cohorts (e.g. new borns) PCT can be a useful marker to rule out the source of infection (bacterial/viral) and to guide antibiotic therapy. However, the cut-offs could change and consequently sensitivity and specificity. In this case, you should refer to the literature but also define your own cut-offs according to the test you are using and to the application you need in your own population. As far as Lactate is concerned, we should once again differentiate among sepsis diagnosis and antibiotic stewardship. For the latter application lactate has no power and is usually not employed in this direction. Differently, lactate could indeed have some utility in sepsis diagnosis. The expert group which released the present Sepsis-3 definition (Singer, 2016, 10.1001/jama.2016.0287) actually excluded the use of lactate from the consensus, as it appeared to give no advantage over the qSOFA for the identification of patients at risk of sepsis. Nonetheless, the group reports that lactate could be a good marker of pre-shock (Mean Arterial Pressure ≥ 65 mmHg and lactate > 18 mg/dL). Also Lee, 2016, 10.21037/jtd.2016.05.55 reports lactate´s utility as a prognostic toll of mortality / septic shock. Furthermore, other authors report the lactate to be a sensitive, but unspecific, tool of cellular or metabolic stress (Kraut, 2014, 10.1056/NEJMra1309483). Consequently, the advantage of PCT over lactate as a biomarker of sepsis/bacterial infection is actually its higher specificity.

Q&A

Q: when PCT are demanded, dosage interferences?

A: For sure, PCT is requested to be measured in a very broad variety of settings in the hospitals/departments, associated to inflammation, traumata, burnings, follow up surgery, and so on. Therefore, PCT will also come along with a quite high and different presentation in a patient. Also, different medical circumstances of a patient will require huge differences in treatment/medication. All kinds of assays need to be aware of direct reactive substances, combinations or metabolized ones. Furthermore, as PCT only has a specificity of 80%, diagnostic labs have to consider other settings (inflammation caused by a virus, parazoan infections or specific tumors like thyroid turmors) that can significantly influence PCT concentration as well.

Q&A

Q: Stability of PCT after blood withdrawal?

A: According to Gruzdys, 2019, 10.1373/jalm.2018.028449, PCT is stable in plasma and serum 24 h at room temperature (approximately 23 °C); 5 days under refrigerated storage (4 °C); 14 days under frozen storage (−20 °C), and after 3 freeze–thaw cycles. We personally tested in DiaSys one freeze-thaw cycle without observing any change in PCT concentration (< 2%). However, we observed significant reduction of PCT concentrations (ca. 20-25%) upon storage of samples at 2-8 °C for > 2 weeks, what indeed seems to confirm Gruzdys´ data. Nonetheless, we did not measure this in a dedicated way, as of no interest for us, but just made an "eye-ball" observation. Moreover, we should also say that we don´t know if the observed decrease in concentration is due either to the proteolysis of PCT or to its denaturation or to its association with some other molecule in the blood.

Q&A

Q: Is there an advantage to using other proteins beyond PCT to discriminate viral from bacterial infections? If yes, which ones?

A: Differentiation of bacterial caused and viral caused sepsis is the primary diagnostic aim to initiate suitable medication! In fact, beside classical inflammatoric markers like CRP and lactate, there are only few parameters available that show suitable specificity for a bacterial caused sepsis (e.g. PCT or Presepsin/sdCD14). IL-6 is a fast but quite unspecific marker of inflammation. This cytokine often is used in the context of newborns to diagnose/monitor infections, also in the context of viral caused infections. SAA also is upregulated in various conditions (e.g. rheumatoid settings and many more, doi: 10.1016/bs.acc.2019.01.002.). In fact, this parameter seems to be more clearly related to viral caused sepsis in humans. Additionally, SAA is coming along with quite high genetic polymorphism, especially in Asian population that needs to be considered when quantifying this parameter in the context of a sepsis (doi:10.1016/j.gene.2016.02.044.).

Q&A

Q: Biomarker and AI, KPI's for Regulation of tests, metrics for AI testing Biomarker

A: This is an interesting question. AI is now disrupting every industry. Possible algorithms together with different parameters / clinical conditions, which could be introduced in the clinical practice, should be evaluated by MD on the field. Indeed, this is a very interesting application field, what could strongly help clinicians in early defining a sepsis through the concomitant and coordinated indications resulting from biomarker measurements, probability of infection, and clinical picture of each patient.

However, this goes beyond the scope of this webinar, which we highly recommend to follow industry trend for more information and insights related to this subject.

Thank you for your participation!

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