The Complete Blood Count (CBC): Reference Procedures and Traceability

- RiLiBÄK, Annex 1d:
  Requirements for internal / external Quality Assurance
- Flow Cytometric Blood Cell Counting

  $\mathbf{C_{ery}}$, $\mathbf{C_{leu}}$, $\mathbf{C_{thr}}$
- **PCV** / **Hkt** – Reference Values by Centrifugation
- Traceability
- New (Reference-) Procedures
- Summary

CBC: Requirements for Accuracy and Precision

RiliBÄK, i.e. Guidelines of the German Medical Association

Annex 1d - Measuring quantities in whole blood

1 Seq. No.	2 Analyte	3 Type of quantity	4 Target value[1]	5 Maximal admissible unprecision	6 Maximal admissible deviation from target value	7 Maximal admissible deviation of single measurement	8 Measuring range
5	Erythrocytes	Cell-concentration	RP	3 %	4 %	10 %	
8	Haematocrit	Ratio of volumes	SP	3 %	3 %	9 %	
9	Haemoglobin	Concentration of Mass	RP	2 %	2 %	6 %	
14	Leucocytes	Cell-concentration	SP	6 % 120 / µL	6 % 120 / µL	18 % 360 / µL	≥ 2000 / µL < 2000 / µL
17	Thrombocytes	Cell-concentration	SP	7% 2800 / µL	7% 2800 / µL	21% 8400 / µL	≥ 40000 / µL < 40000 / µL

[1]RP=value measured by a reference procedure, SP= nominal value for a selected procedure. The type of the target value listed in this table is mandatory for the execution of the external quality assurance

DIN

Reference Procedures:

DIN 58932-3

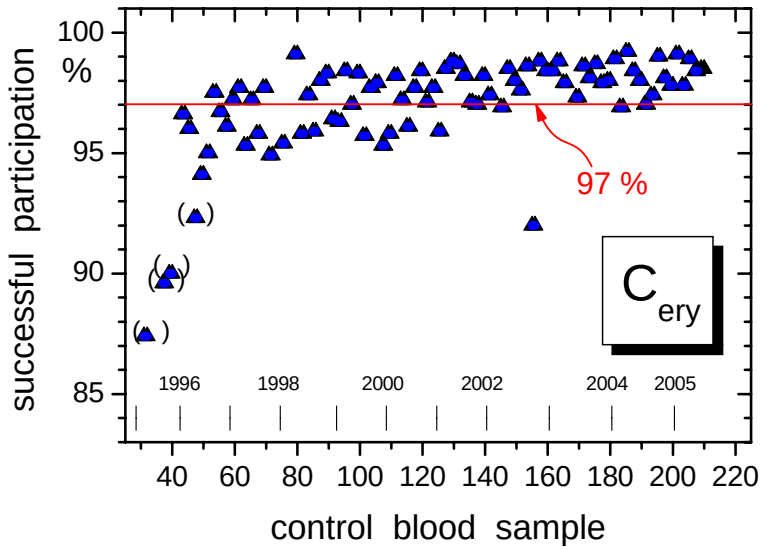
DIN 58933-1

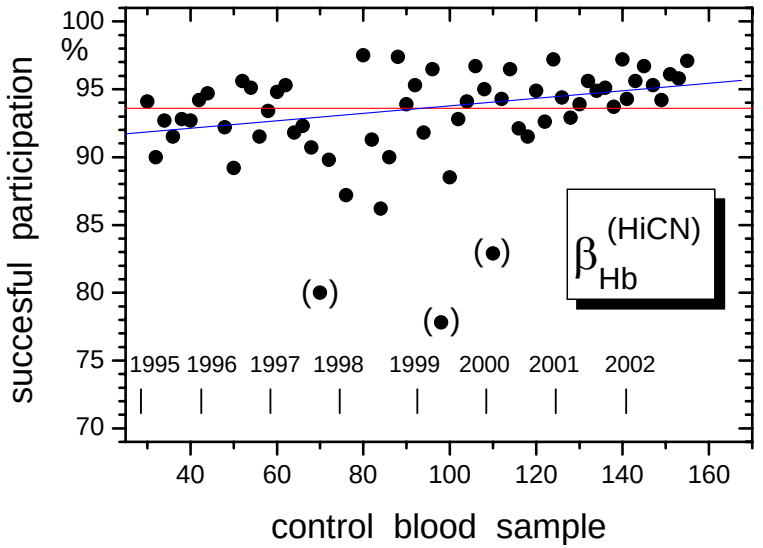
DIN 58931

DIN 58932-4

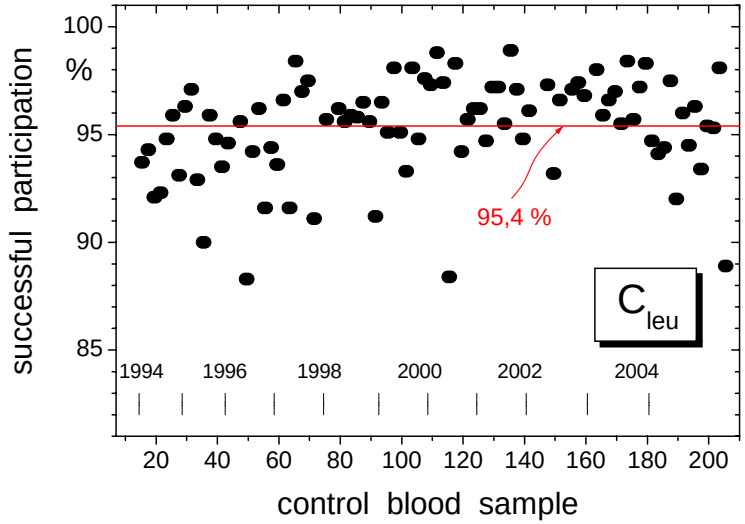
DIN 58932-4

External Quality Assurance: Evaluation of Round Robin Experiments



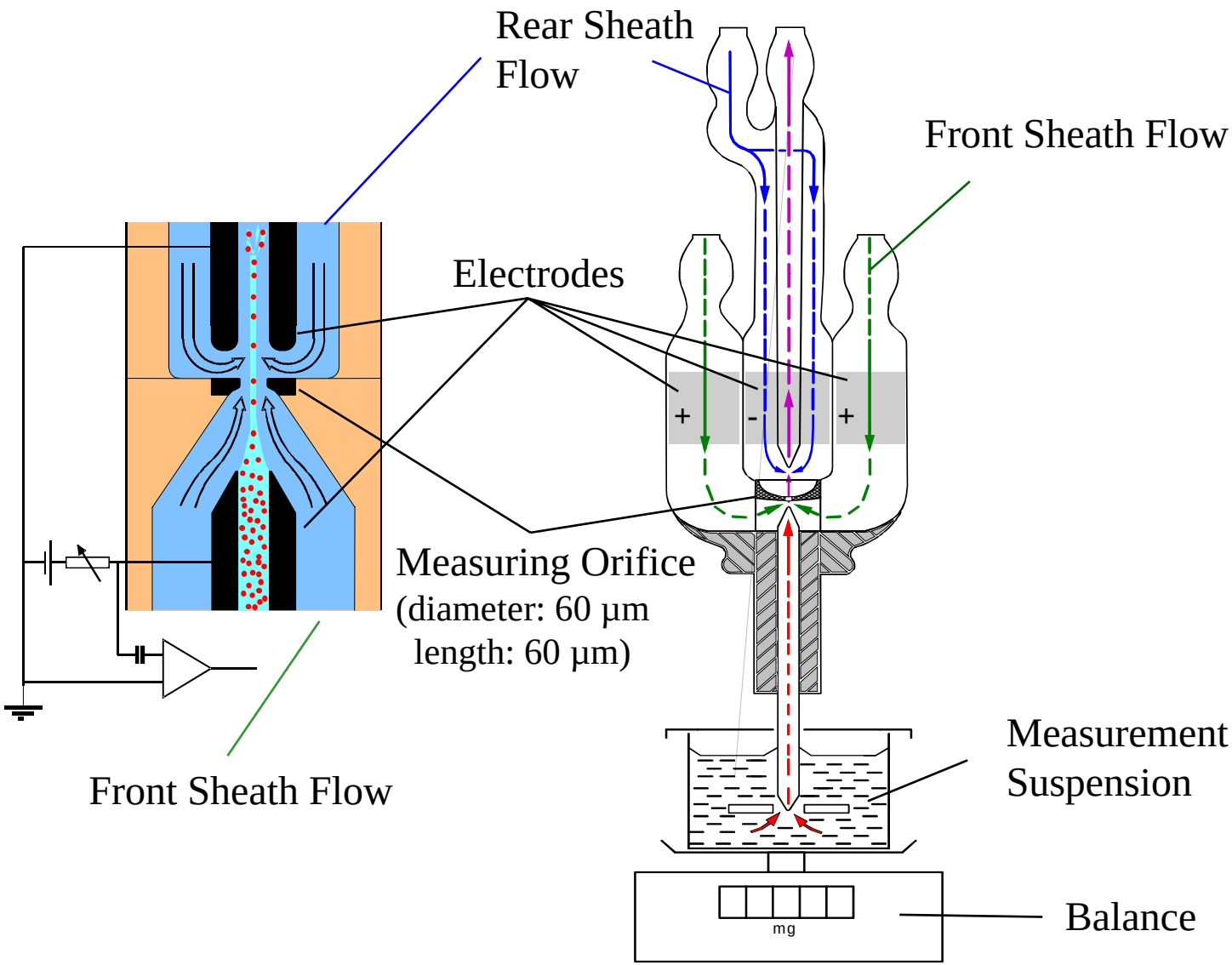


Control blood samples (DGKL, INSTAND)

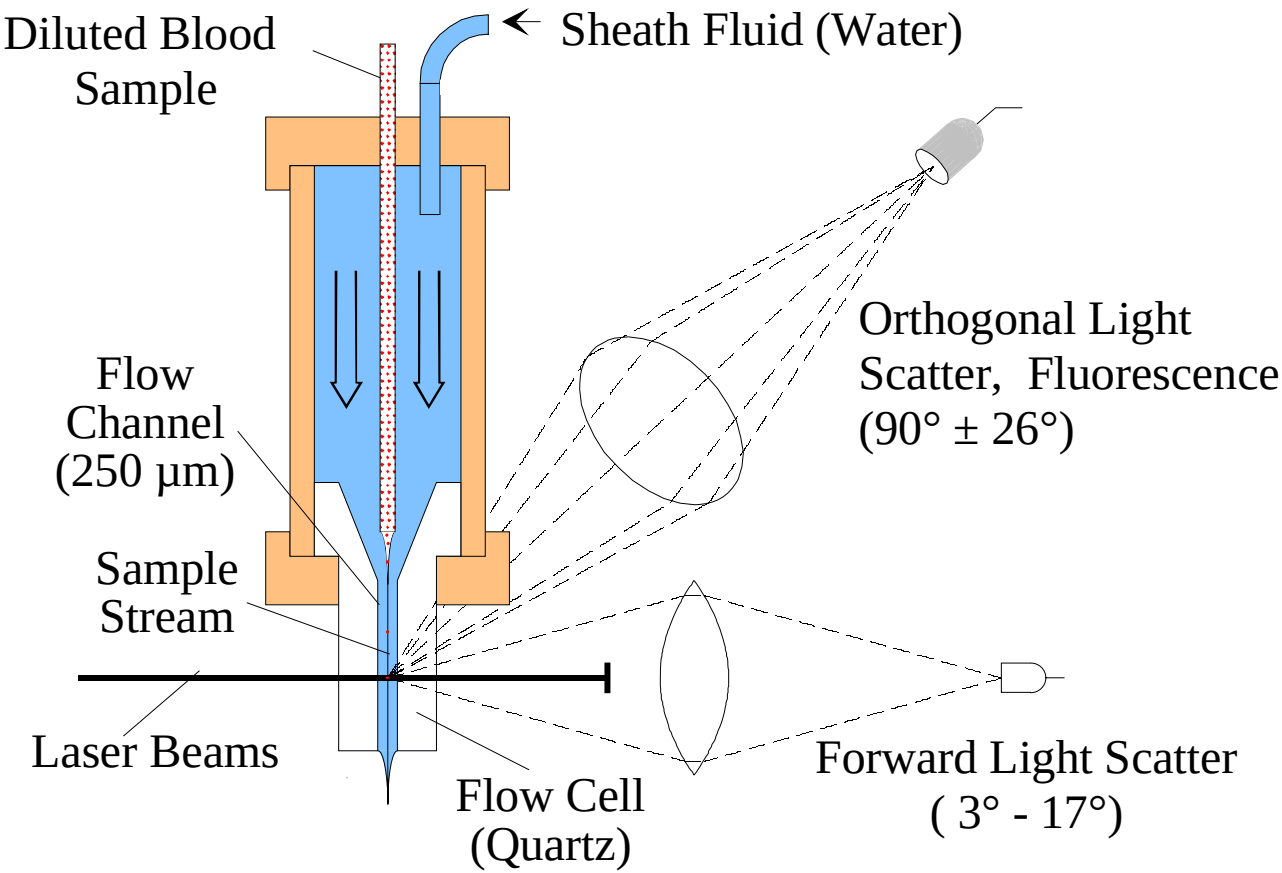


⇒ Reference procedures should be
⇒ introduced to evaluate leukocyte
⇒ concentrations in round robin tests

Flow Cytometric Blood Cell Counting by Impedance Measurements



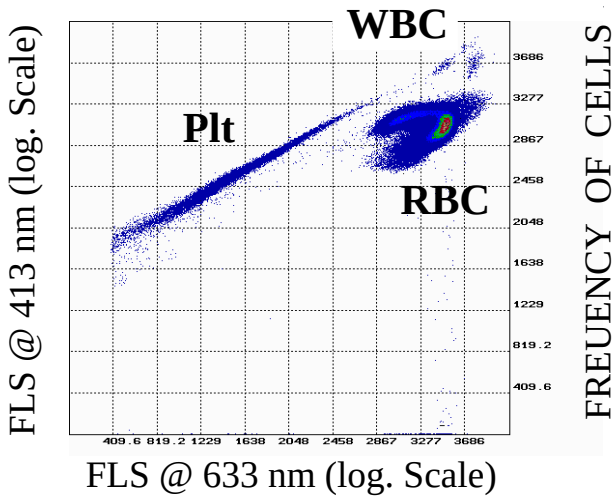
Blood Cell Counting by Laser Flow Cytometry

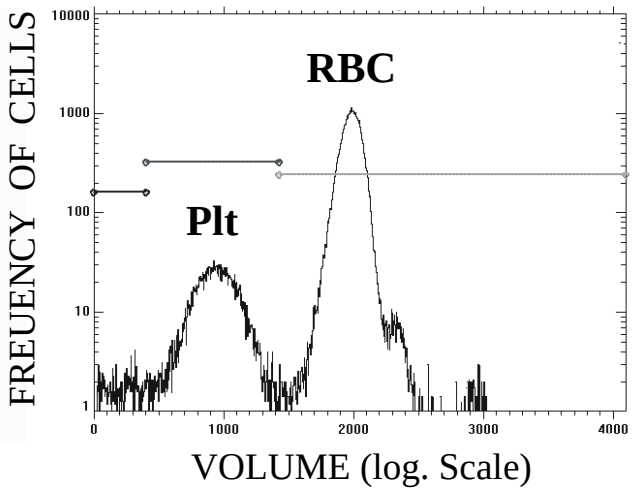


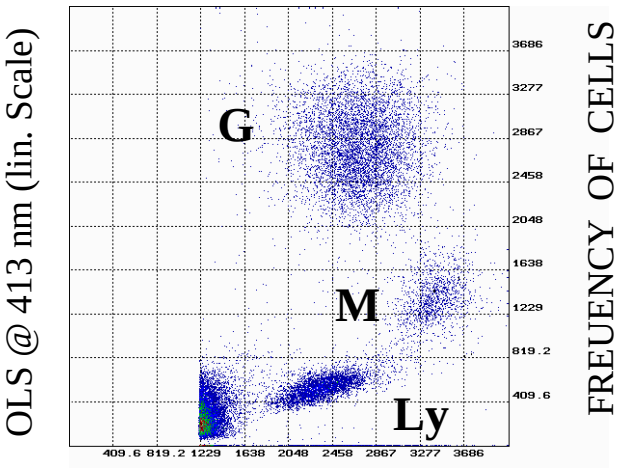
- Simultaneous detection of erythrocytes (RBC), leukocytes (WBC), thrombocytes (Plt)
- interaction time 2 µs
- analysis of typical 5000 cells / s
- sensitivity 500 fluorochromes

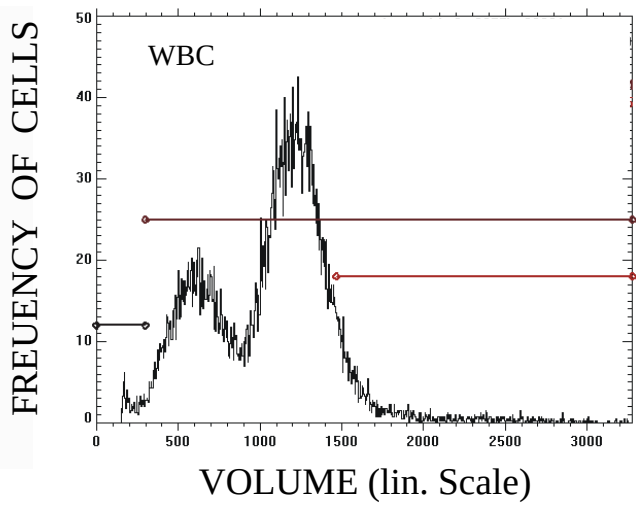
Flow Cytometric Differentiation of Blood Cells

Complete Blood Count (CBC)









RBC
Red Blood Cells

WBC
White Blood Cells
G: Granulocytes
Ly: Lymphocytes
M: Monoctes

Plt
Platelets

FLS
Forward Light Scatter

OLS
Orthogonal Light Scatter

Primary Reference Procedure Followed at the PTB

Concentration C of primary sample

$$C = \frac{N}{V}$$

Recorded concentration C of analytical solution

$$C_{\mathrm{ri,j}} = \frac{N_{\mathrm{ri,j}}}{V_{\mathrm{i,j}} \cdot \phi_{\mathrm{i}}}$$

Coincidence correction by dilution series ⇒ N

$$\ln \frac{\overline{N}_{\mathrm{ri}}}{\phi_{\mathrm{i}}} = \ln N - \phi_{\mathrm{i}} \cdot N \cdot \mathrm{p}$$

Definition of Symbols

N conventional true value of the number of particles
V volume of primary sample derived from $V_{i,j}$
$N_{ri,j}$ recorded number of events
$V_{i,j}$ volume of analytical solution i,repeat measurement j
ϕ_i volume fraction of primary sample in the analytical solution i
p coincidence parameter

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Determination of Volume and Density

V, ϕ_i gravimetrical measurement of volume V and volume fraction ϕ_i

ρ density measurement using the mechanical oscillator method

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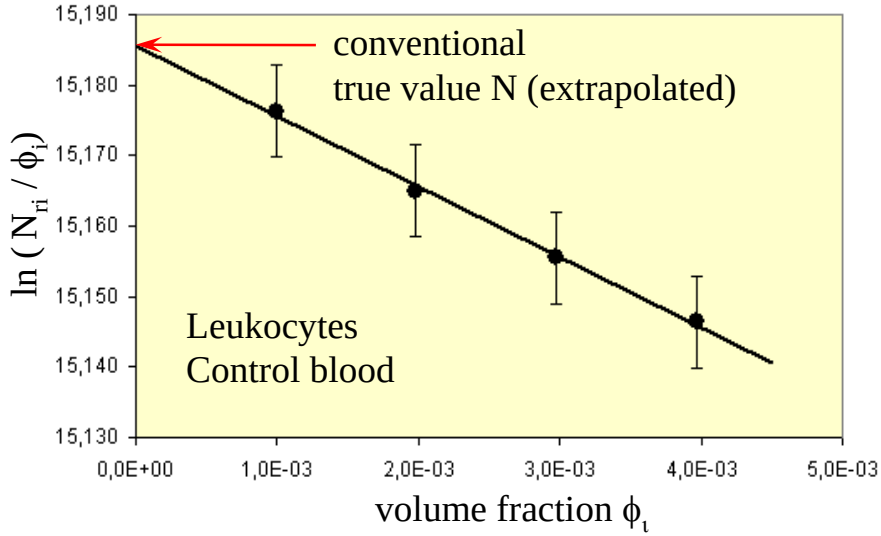

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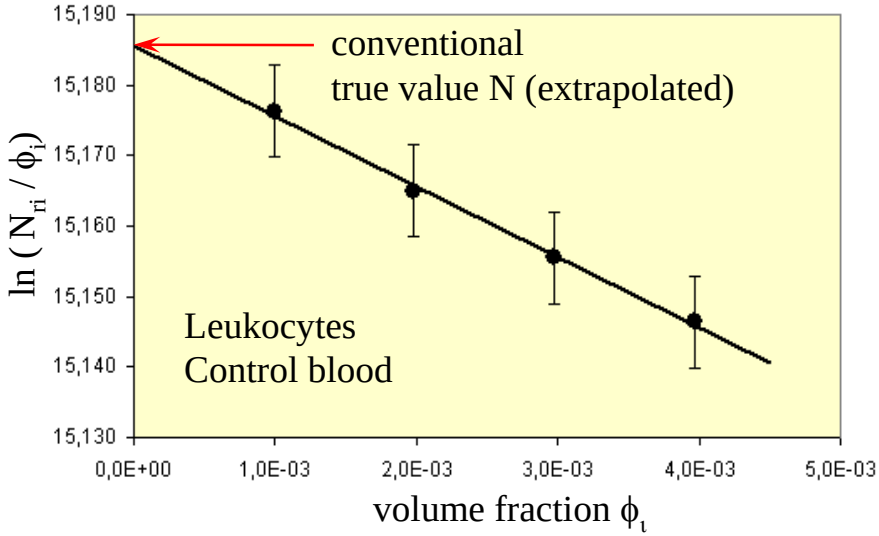

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Control of Influence Quantities

adhesion: determination of concentration immediately and 30 min after preparation

agglomeration: analysis of pulse height distributions, scatter plots, integrated dead time

sedimentation: stirring during measurement, time dependence of $N_{ri,j}$

carry over: background determination between different series of measurements

lysis: comparison experiments using various reagents

Definition of Symbols

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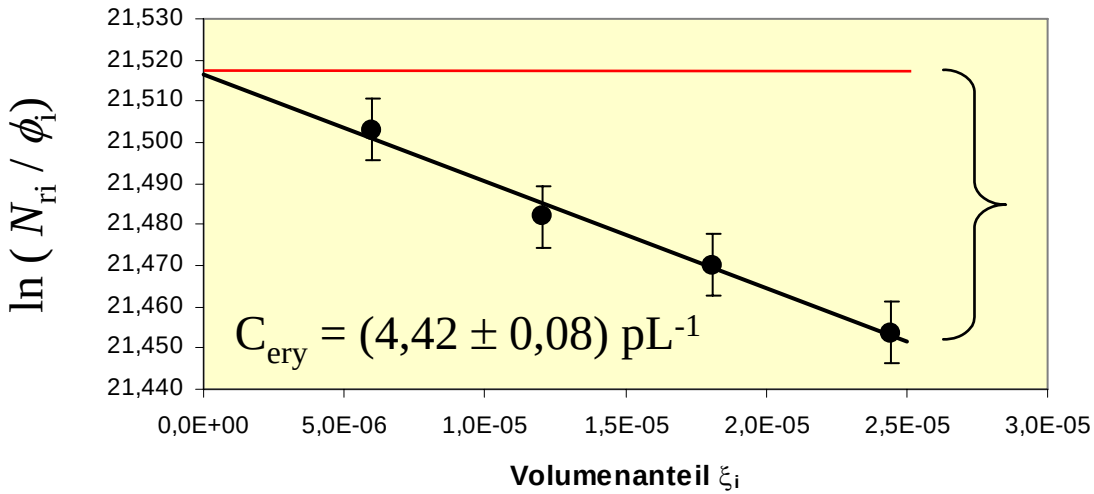
Determination of Cell / Counting Losses

$$C = \frac{N}{V} \quad \rightarrow \quad C_{ri,j} = \frac{N_{ri,j}}{V_{i,j} \cdot \phi_i}$$

i Measuring suspension, j Repeat measurements

ϕ_i Volume fraction

1. Dilution series* (Coulter Counter ZM)

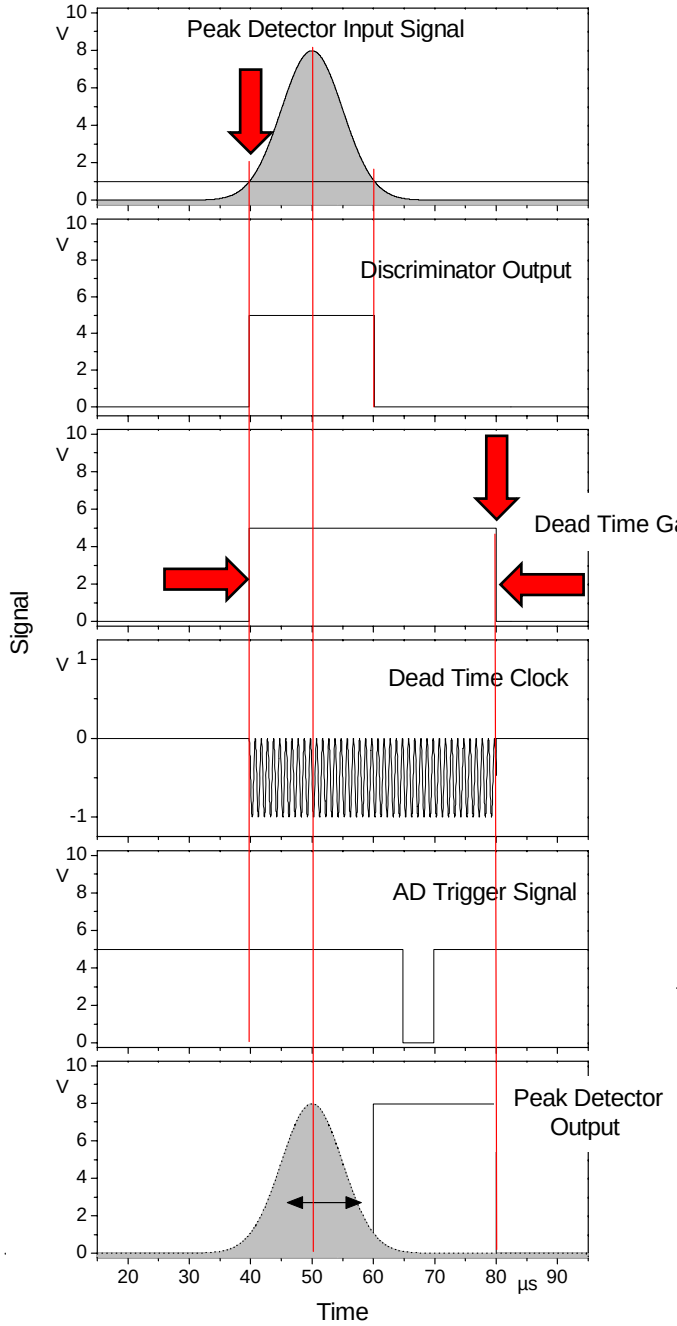


Coincidence losses, specific for instrument specific for sample

2. Determination of pulse width & electronic dead time

*see e.g. S.M. Lewis et al. 1989, *Phys. Med. Biol.* and Recommendations of the International Commitee of Standardization in Haematology (ICSH) 1988, *Clin. Lab. Haematol.*

Determination of Total Dead Time

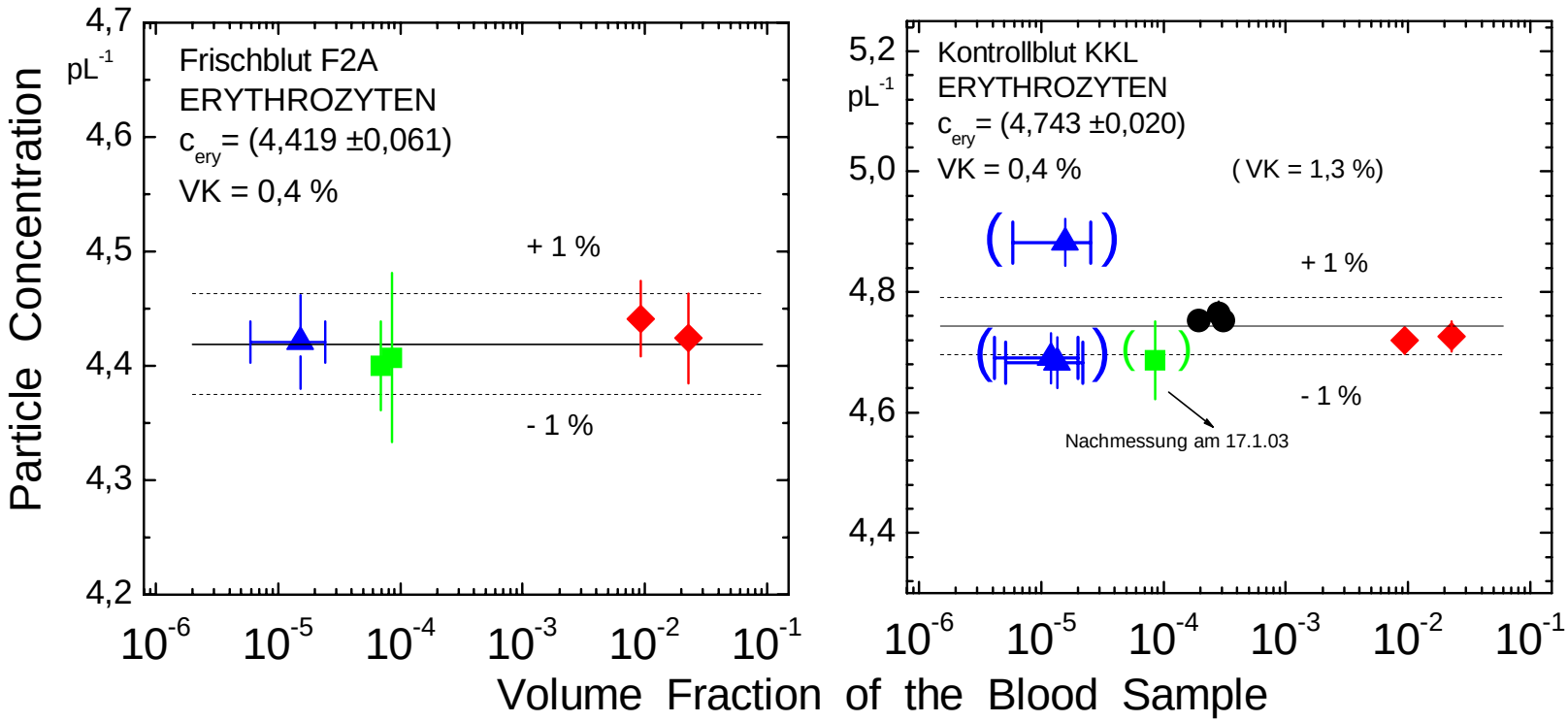


$$\Rightarrow \tau$$

$$N \approx \frac{1}{\phi_i} \cdot \left(\frac{N_{ri}}{1 - \left(\frac{\tau \cdot N_{ri}}{T} \right)} \right)$$

Comparison of Different Reference Instruments Using Integrated Pulse Width Measurement

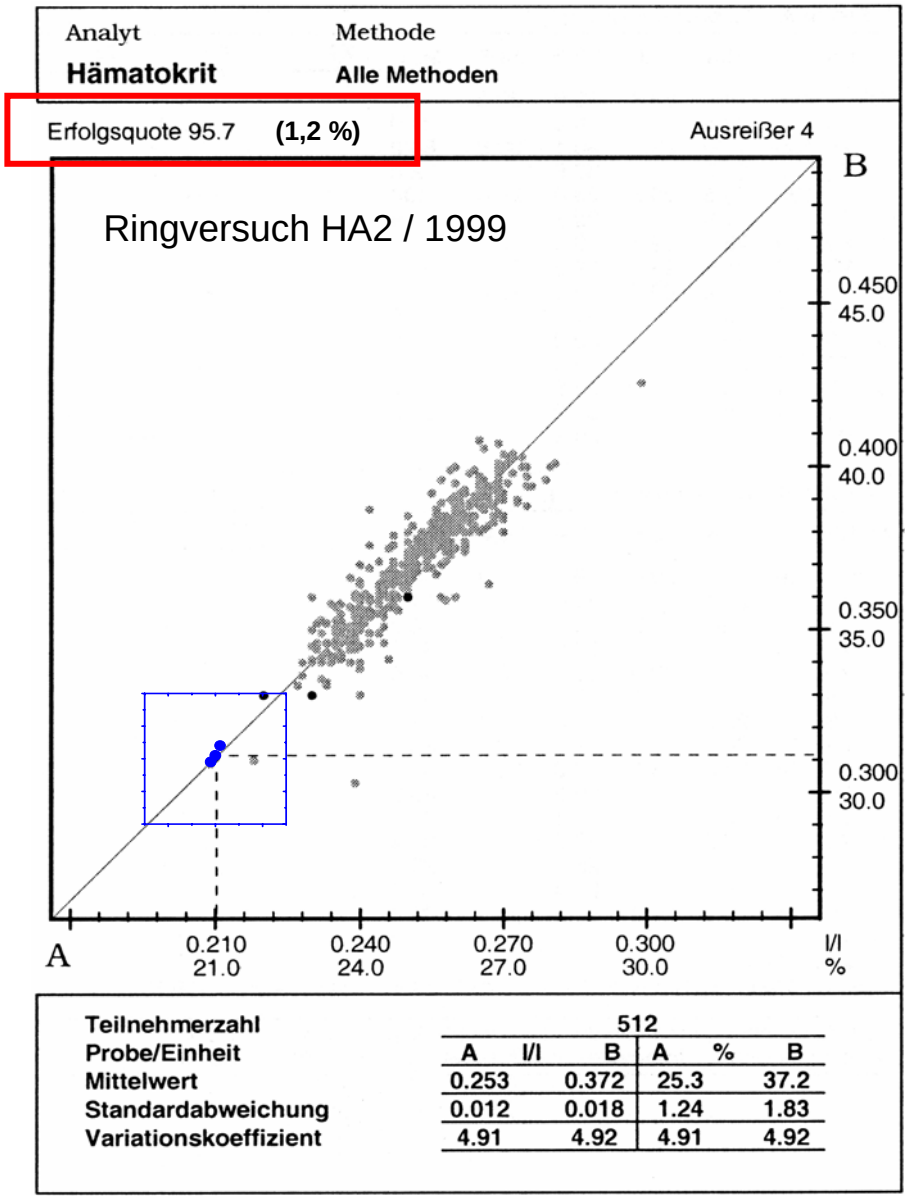
Instruments: ■ EDZ 2 , ● EDZ 1 , ◆ LDZ , ▲ (Coulter ZM, Bonn)



Advantage: Concentration is obtained from a single measurement

Dilution series serves as independent control

PCV / Hkt Determination by Reference and Routine Procedures

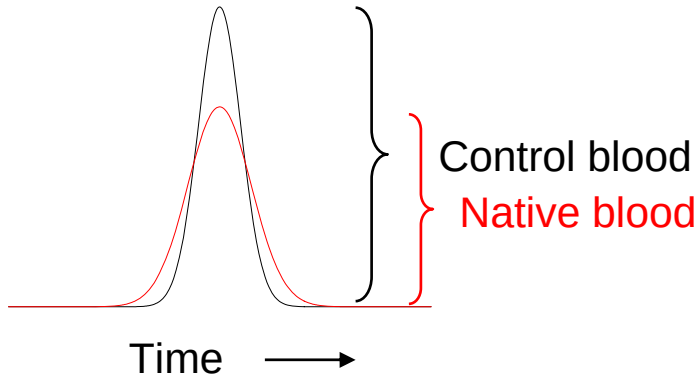


Cetrifugation to determine Hkt – reference values

Routine instruments:

$Hkt = MCV \times C_{ery}$

$MCV \propto$ Amplitude of signal



Analysis of Pathological Blood Samples Requires Dead Time Measurement

Modification of routine instruments is required

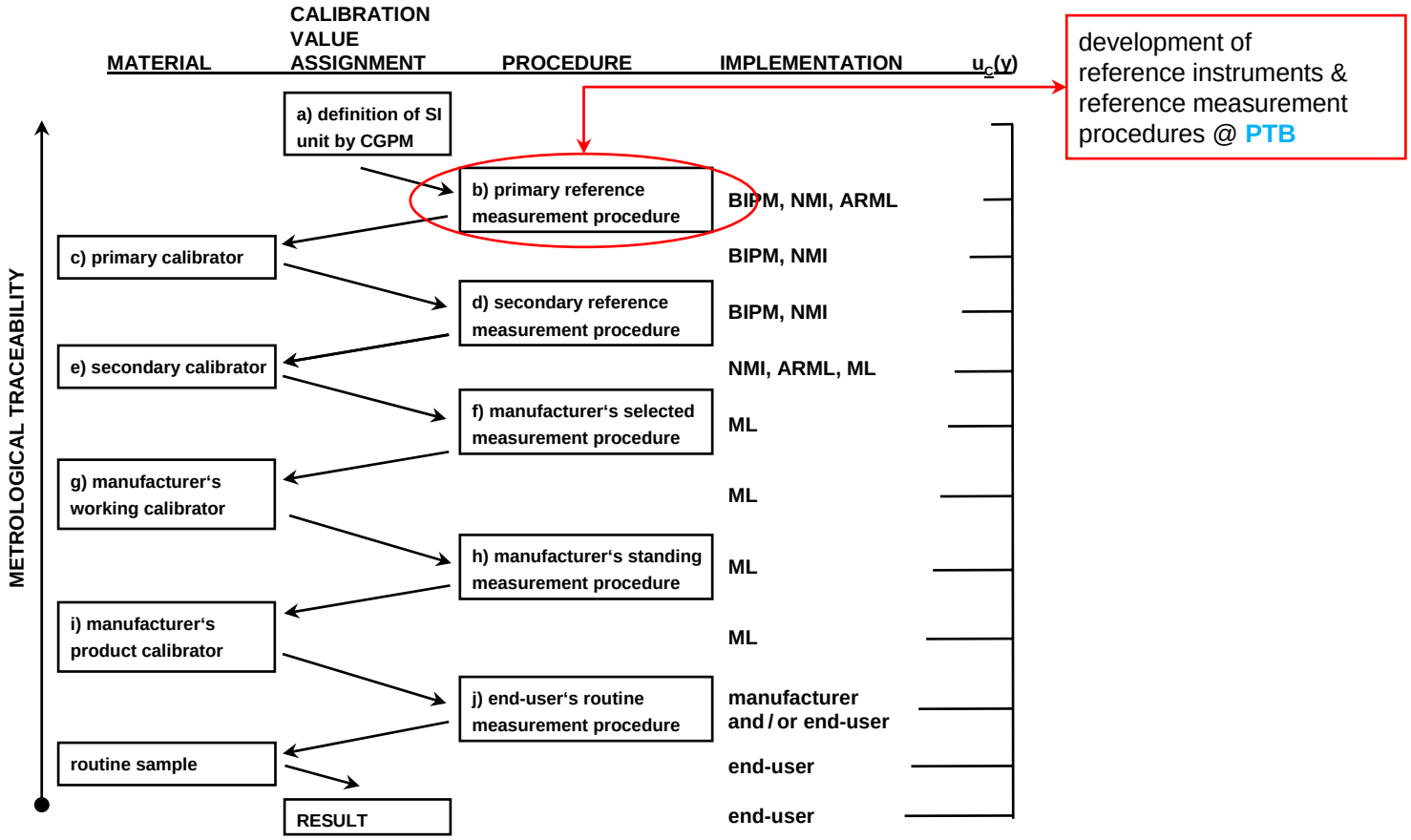
- determination of amplitude & pulse width
- determination of pulse area

$\Rightarrow$ mechanical / rheological properties of cells are determined

- Reference values for PCV / Hkt to evaluate round robin test
- Reliable determination of RBC and PCV for pathological blood samples

Complete Blood Count: Traceability of Results

Comparison of Reference Measurement Procedures with Manufacturer's and End-User's Measurement Procedures (Figure 1, EN ISO 17511, draft 2002: Extensive calibration hierarchy and metrological traceability to SI)



Abbreviations: CGPM: General conference on weights and measures; BIPM: International bureau of weights and measures;
NMI: National metrology institut; ARML: Accredited reference measurement laboratory; ML: Manufacturer's laboratory, uc(y): Combined standard uncertainty of measurement.

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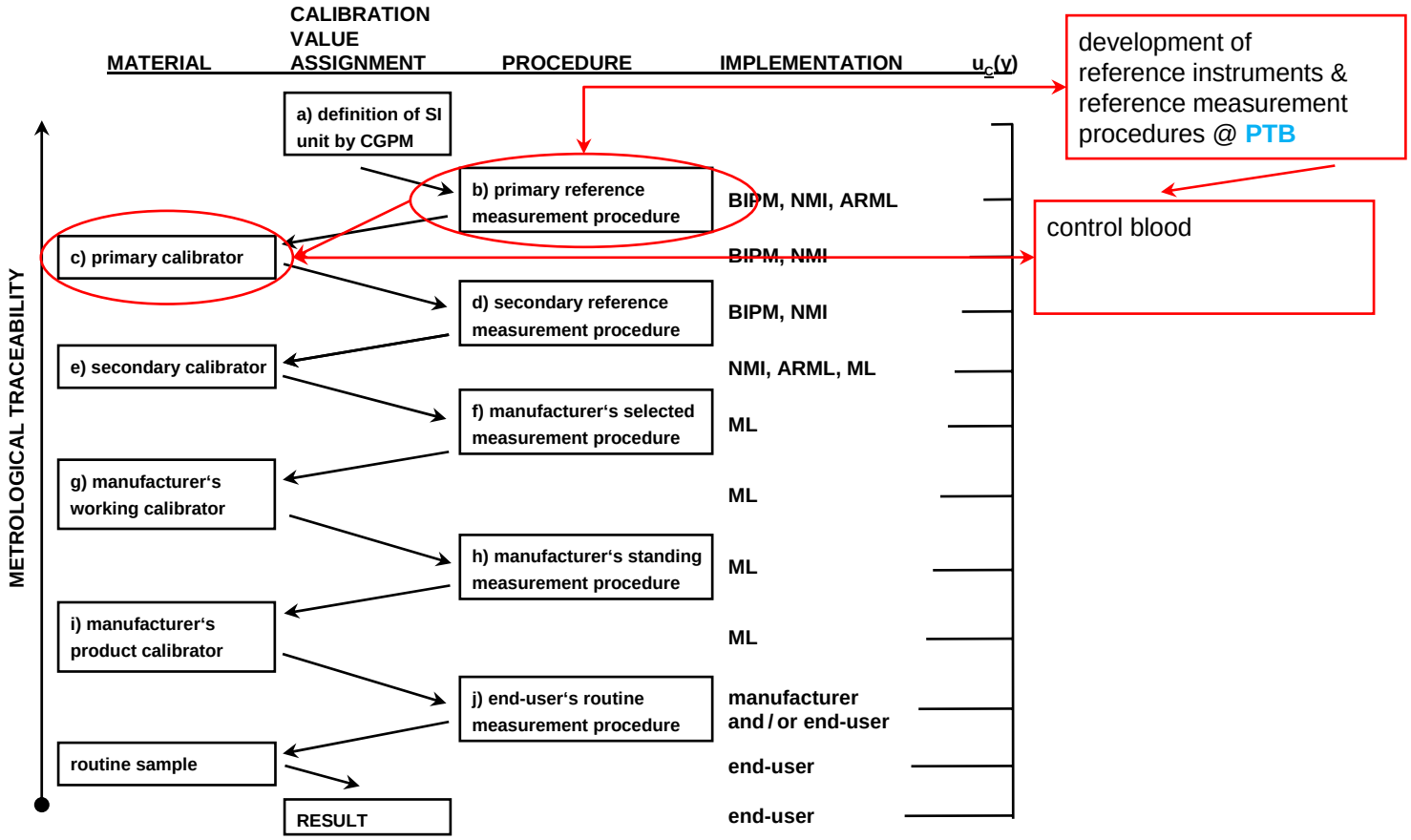

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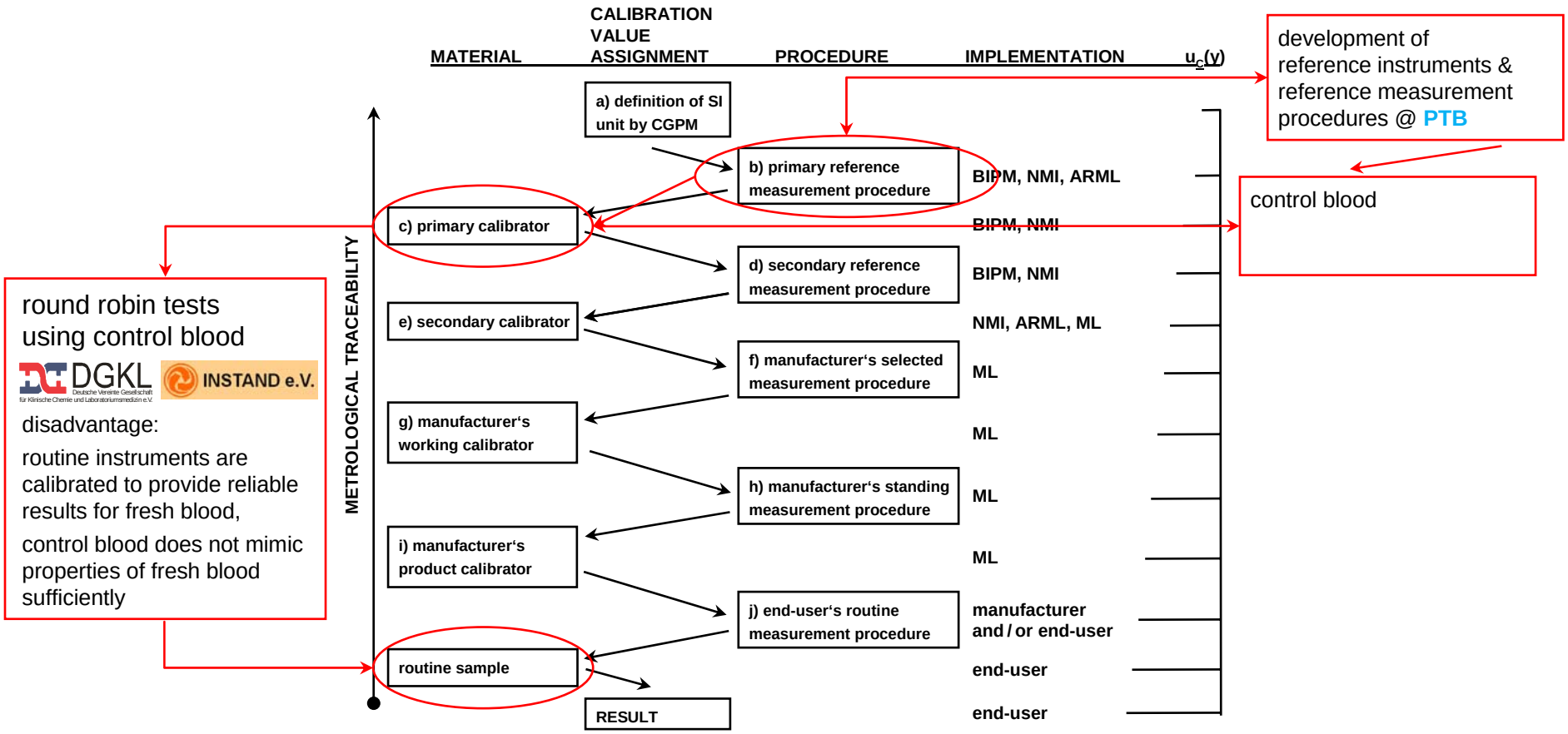

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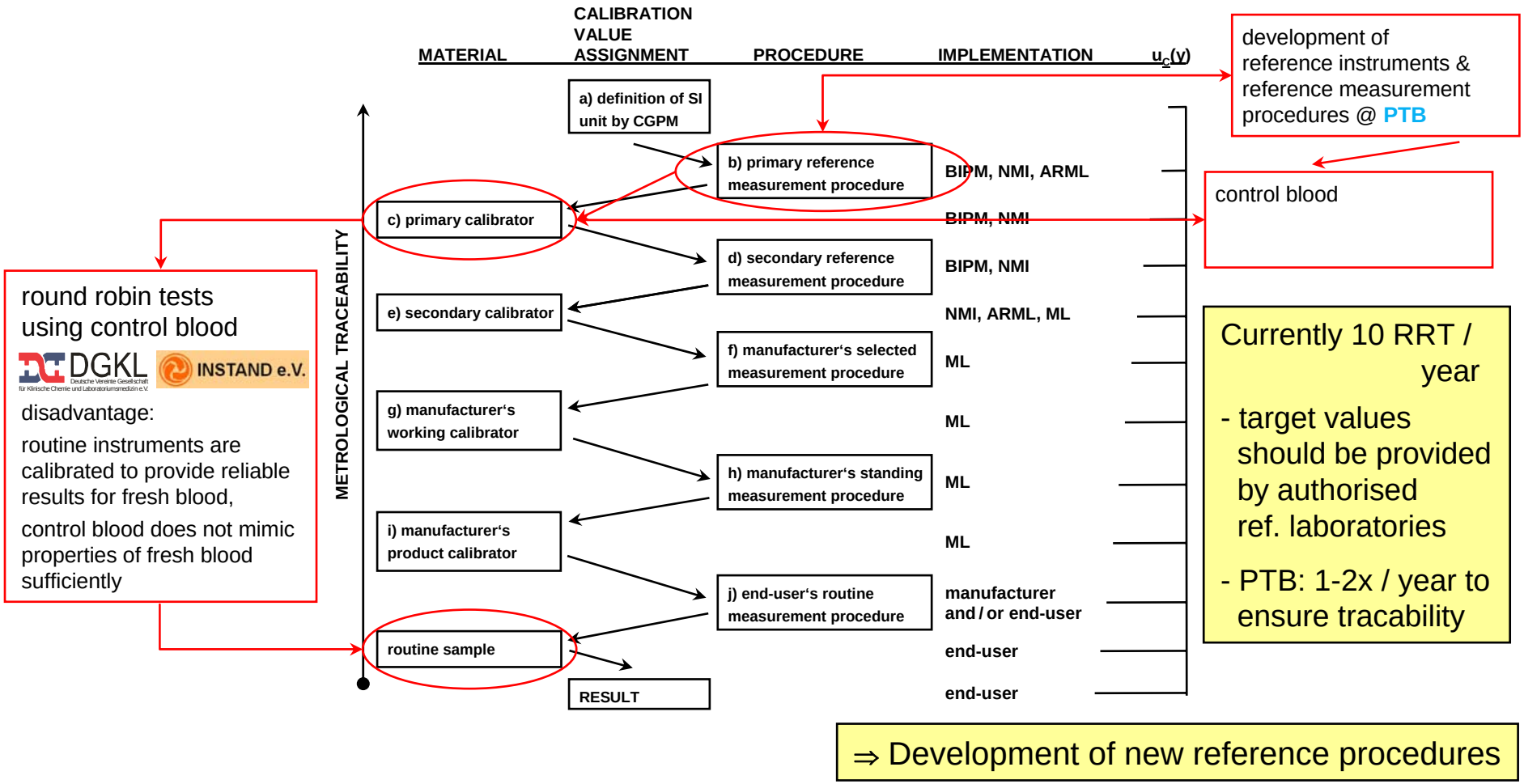

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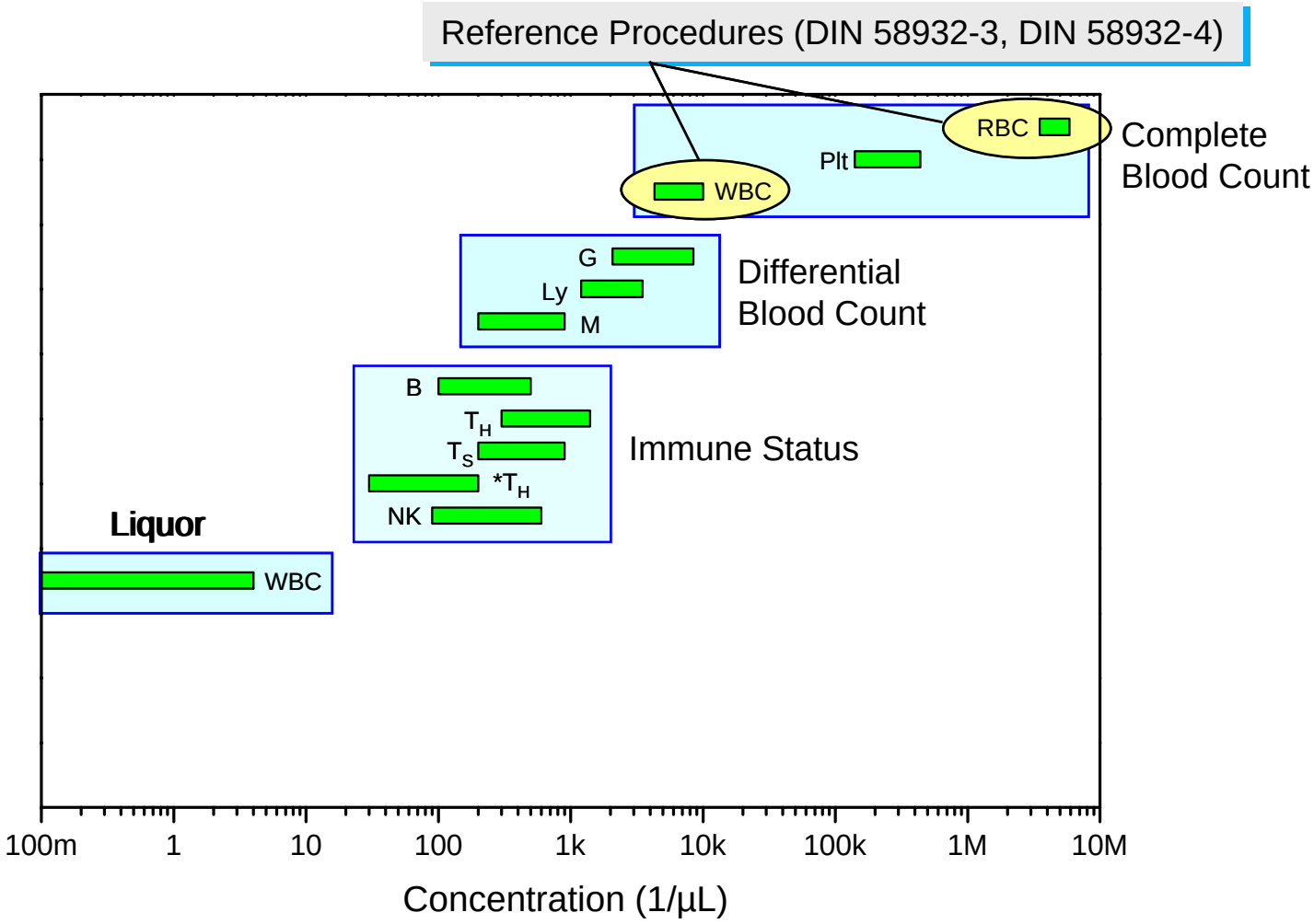
Extension of Measurement Range
Development of Reference Procedures

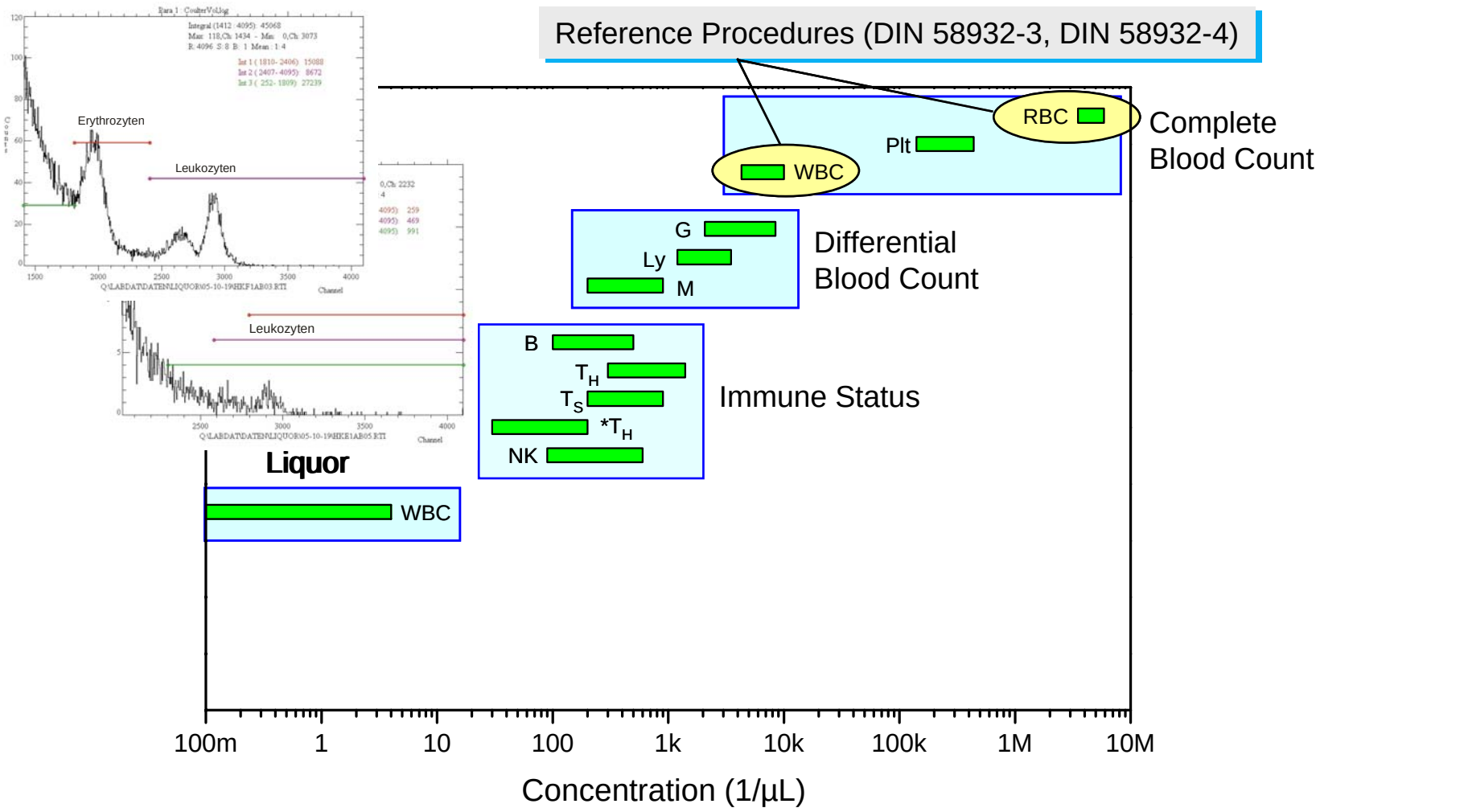


Flow cytometry: Identification - Counting – Sorting

Validation of target cells:

Microscopy (Morphology, Localisation of mAb,) / Polymerase Chain Reaction

Extension of Measurement Range
Development of Reference Procedures



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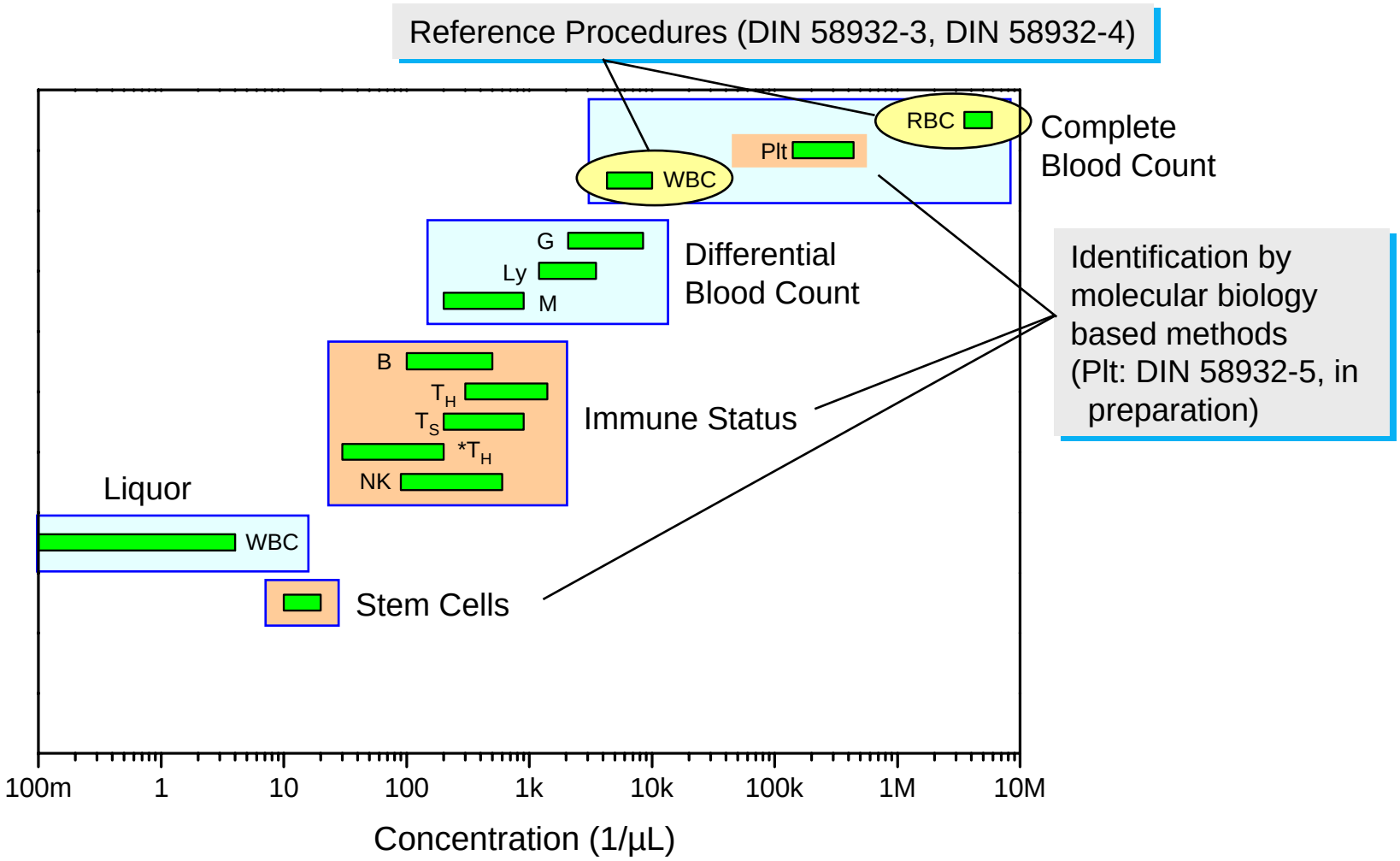

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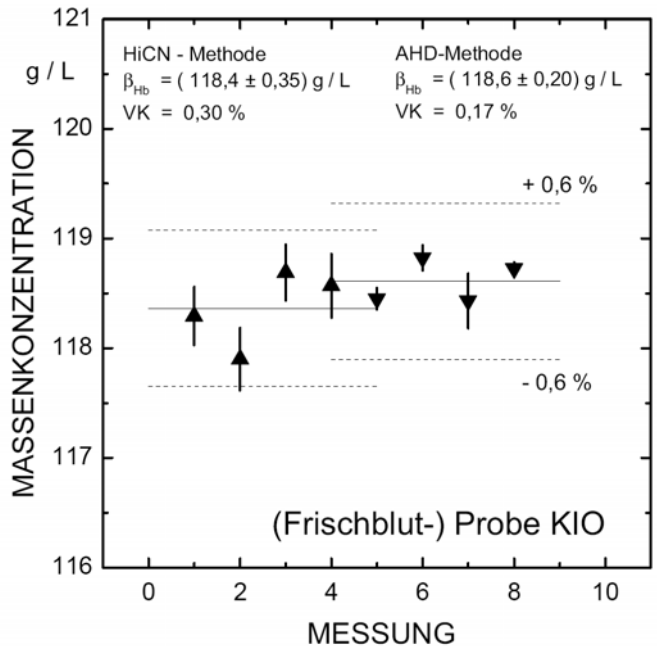
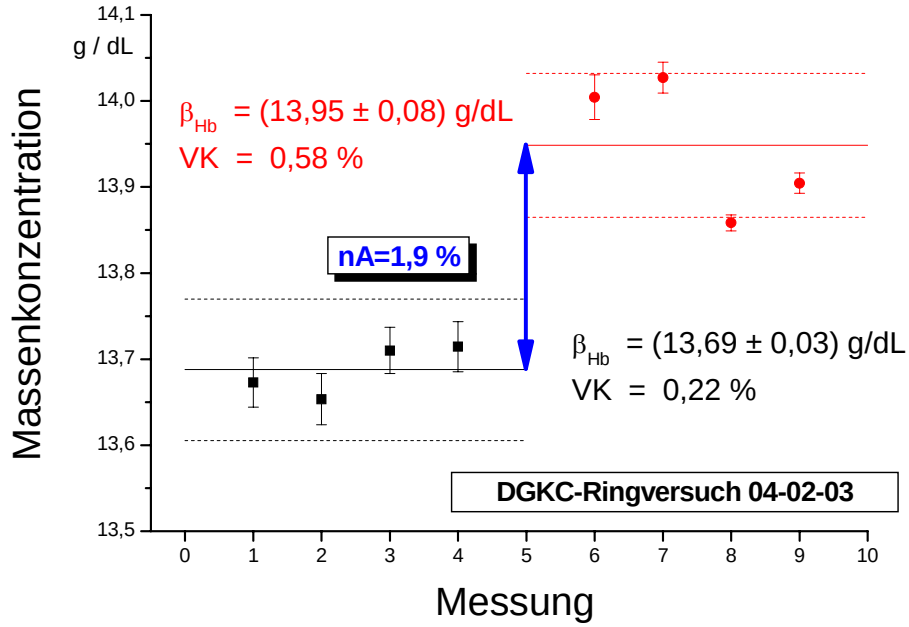
“New” Reference Procedure to Determine Haemoglobin Concentrations ?

Comparison HiCN / AHD* – Methods

Native Blood



Control Blood



Alkaline Haematin D-575 Method; Zander et al., Wolf et al. 1984, *Clin. Chim. Acta*

Comparison Reference / Routine Procedures

Analyte	Control Blood	Native Blood	Influencing Quantities
C_{ery}	✓	✓	Anaemie: shape & rheological properties
C_{leu}	✓	?	Lyse-resistant RBC Lyse-sensitive WBC
Hb (HiCN)	✓	✓	high conc. of WBC
(AHD)	?	✓	–
PCV	Modification of routine instruments	✓	Anaemia

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(AHD)	?	✓	–
PCV	Modification of routine instruments	✓	Anaemia

International Round Robin Tests

The Complete Blood Count (CBC): Reference Procedures and Traceability

Summary:

- RiLiBÄK: Reference values for the leukocyte concentration
- RiLiBÄK: Reference values for the PCV / Hkt ?

  ⇒ Material-independent determination using pulse width measurement !
- Determination of target values by authorised reference laboratories
- Participation of PTB to in selected round robin tests to ensure traceability
- Development & test of new reference procedures
  - WBC / RBC in liquor using flow cytometry & / microscopic validation
  - Hb determination using the AHD method
  - Platelet concentration employing CD41 / CD 61 staining
  - Immune status
  - Stem cell concentration