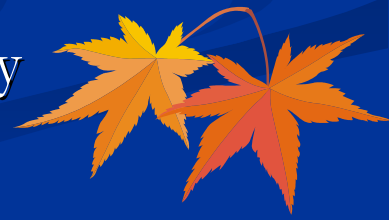
Joint Committee for Traceability in Laboratory Medicine (JCTLM) Meeting

Blood Cell Counting Standardization

November 15, 2005

Keiji FUJIMOTO
Vice President, Dept. of Scientific Affairs
Sysmex Corporation
Member of ICSH Expert Panel on Cytometry



Contents

- Traceability of Blood Cell Counting
  - ICSH Reference Methods
- Reference Method for Red Blood Cell Counting
  - Measurement Principle
  - Traceability Chain
  - Estimation of Uncertainty in Measurement
- Present Situation of Standardization
  - 6 manufacturers' comparison study in Japan
- Summary

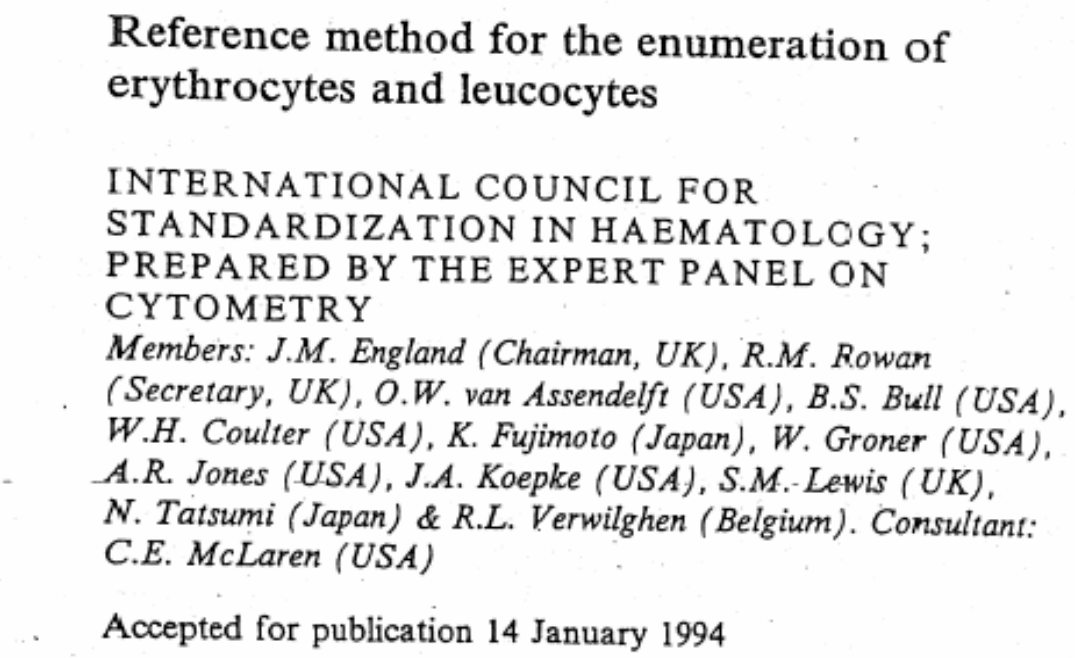
List of Reference Methods(1 / 4)

- RBC & WBC

  Reference method for the enumeration of erythrocytes and leucocytes

  International Council for Standardization in Haematology; Prepared by the Expert Panel on Cytometry

  - Clinical Laboratory Haematology, 16, 131-138 (1994)



Reference method for the enumeration of erythrocytes and leucocytes

INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY; PREPARED BY THE EXPERT PANEL ON CYTOMETRY

Members: J.M. England (Chairman, UK), R.M. Rowan (Secretary, UK), O.W. van Assendelft (USA), B.S. Bull (USA), W.H. Coulter (USA), K. Fujimoto (Japan), W. Groner (USA), A.R. Jones (USA), J.A. Koepke (USA), S.M. Lewis (UK), N. Tatsumi (Japan) & R.L. Verwilghen (Belgium). Consultant: C.E. McLaren (USA)

Accepted for publication 14 January 1994

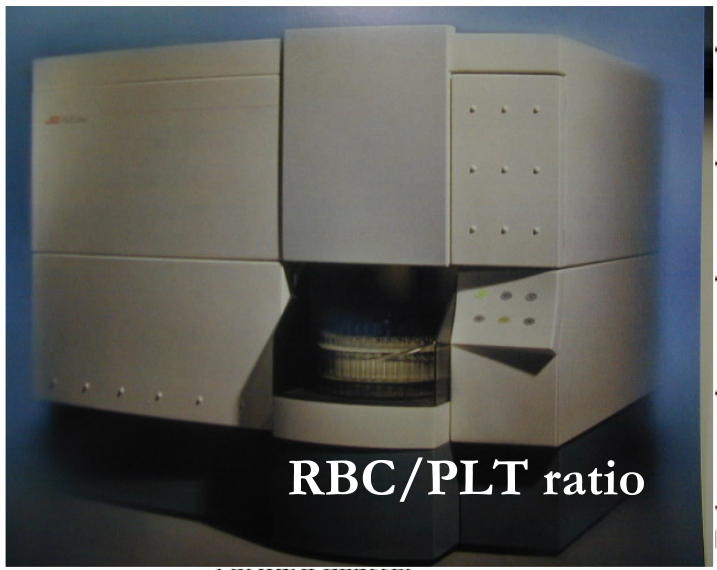
List of Reference Methods(2 / 4)

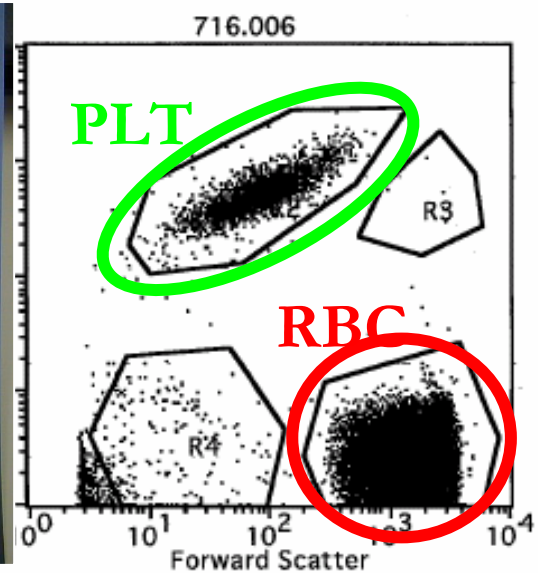
- Platelet

Platelet Counting by the RBC/Platelet Ratio Method
A Reference Method (CD41/61)

International Council for Standardization in Haematology Expert Panel on Cytometry and International Society of Laboratory Hematology Task Force on Platelet Counting

- American Journal of Clinical Pathology 115:460-464 (2001)





List of Reference Methods(3 / 4)

Hemoglobin

Recommendations for reference method for haemoglobinmetry in human blood (ICSH standard 1995) and specifications for international haemiglobincyanide standard (4th edition)

International Council for Standardization in Haematology: Expert Panel on Haemoglobinometry
- Clinical Laboratory Haematology, 16, 131-138 (1994)

Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood ; Approved Standard – Third Edition
NCCLS H15-A3 (HiCN method)

>> registered in "List-I, Reference Procedures of JCTLM"

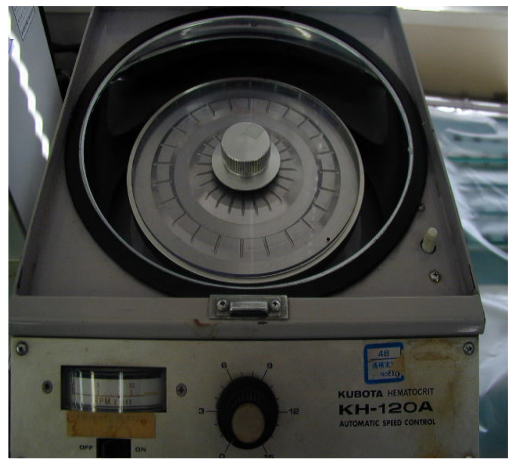
List of Reference Methods(4/4)

- PCV (Hematcrit)

  Recommendations for Reference Method for the Packed Cell Volume (ICSH Standard 2001)

  International Council for Standardization in Haematology: Expert Panel on Cytometry
  - Laboratory Haematology 7, 148 -170 (2001)

  Procedure for Determining Packed Cell Volume by the Microhematocrit Method ;
  Approved Standard – Third Edition
  NCCLS H7-A3



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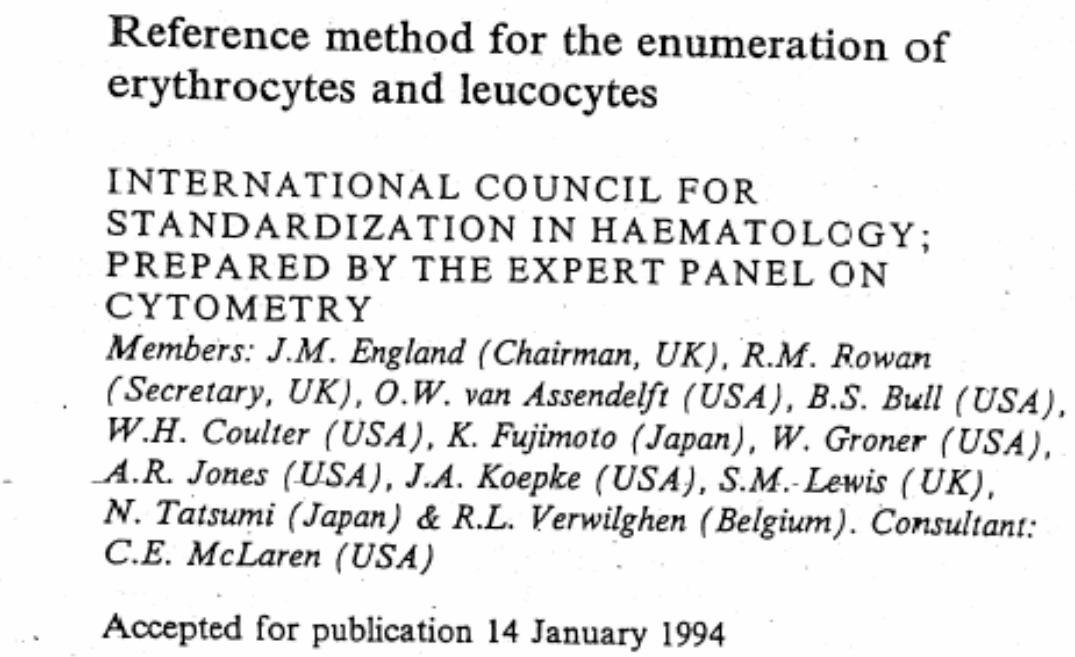

Reference method for the enumeration of erythrocytes and leucocytes

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Accepted for publication 14 January 1994

Instrument Specifications by ICSH

- **Measurement System**

  The probability of "re-circulation" of particles through the sensing zone must be low.

  ⇒semi-automated single channel aperture-impedance particle counter

- **Volume displaced during the counting period**

  "within an accuracy of 1% traceable" to a national or international metrological standard

  ⇒ only achieved by "Mercury column"

  thermal effects should not be greater than 0.1% per ℃

- **Diameter of Orifice**

  80～100μm

- **Threshold setting**

  signal to noise ratio is greater than 100:1

Specifications recommended by ICSH

- **Glassware**

  Positive displacement pipetts

  calibrated to an accuracy of ±0.5%, validated by a process traceable to a primary metrological standard

  Volumetric Flasks : Grade A

  borosilicate glass, each with a stated volume which has been tested by an appropriate national standard body

- **Counting Vials**

  a minimum volume of 10ml, sufficient height that the entry orifice of the electronic cell counter is at approx. half the depth of the fluid before counting commences. no adherence of cells

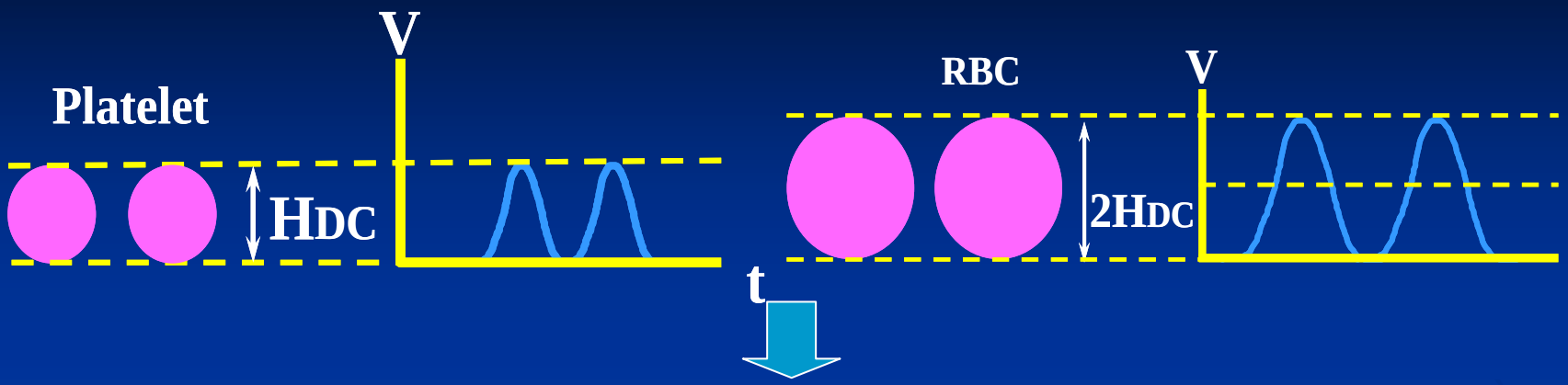
- **Diluent**

  sterile, non-toxic, buffered salt solution, tonicity:280 ±15mOsms, must not cause cell lysis nor alter cell volume by > 2fl over a 20 min period

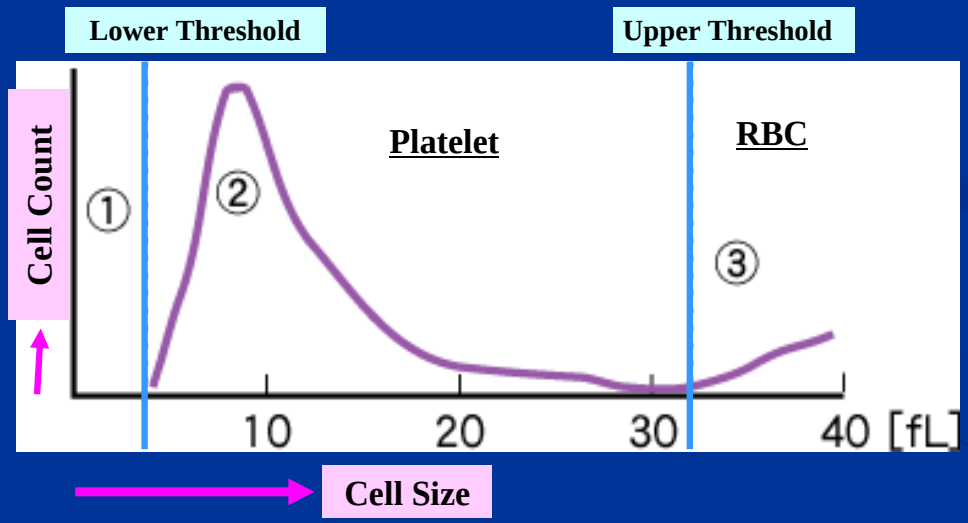
Analysis of Error (by ICSH protocol)

- **Maximum Permissible Bias**
  - —RBC counting ; 2.0%
  - —WBC counting ; 4.0%
- **Sampling Error**
  - —Poor Mixing, hemorheological effects
  - —Inaccuracy of Dilution
- **Transport Error**
  - —Sedimentation, cell loss and/or Recirculation
  - —Inaccuracy of the displaced volume
- **Counting Error**
  - —Improper discrimination
  - —Inaccurate correction for coincidence loss
  - —Imprecision of reference cell count <=1%

Electronic Cell Counting Method



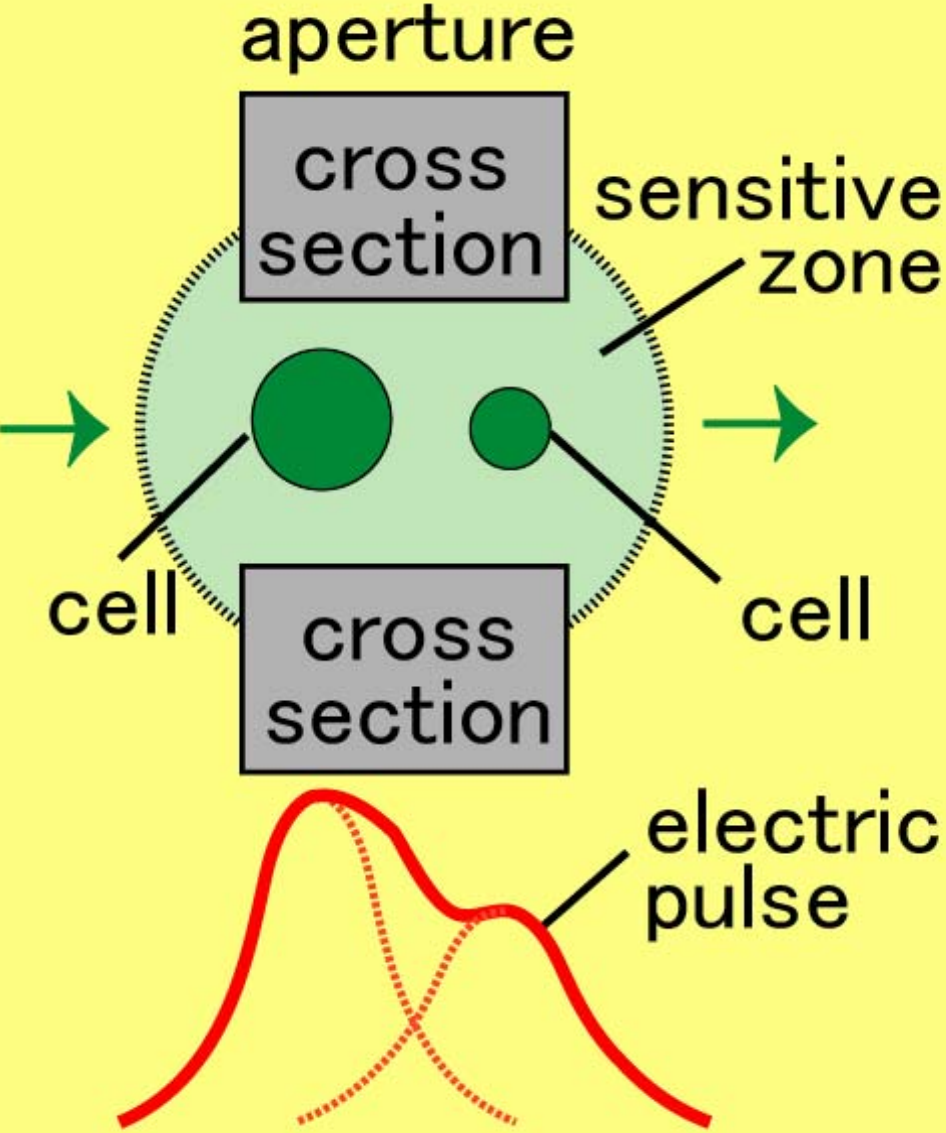
Histogram



★Pulse size is proportional to Cell size.

★Pulse count corresponds to Cell count.

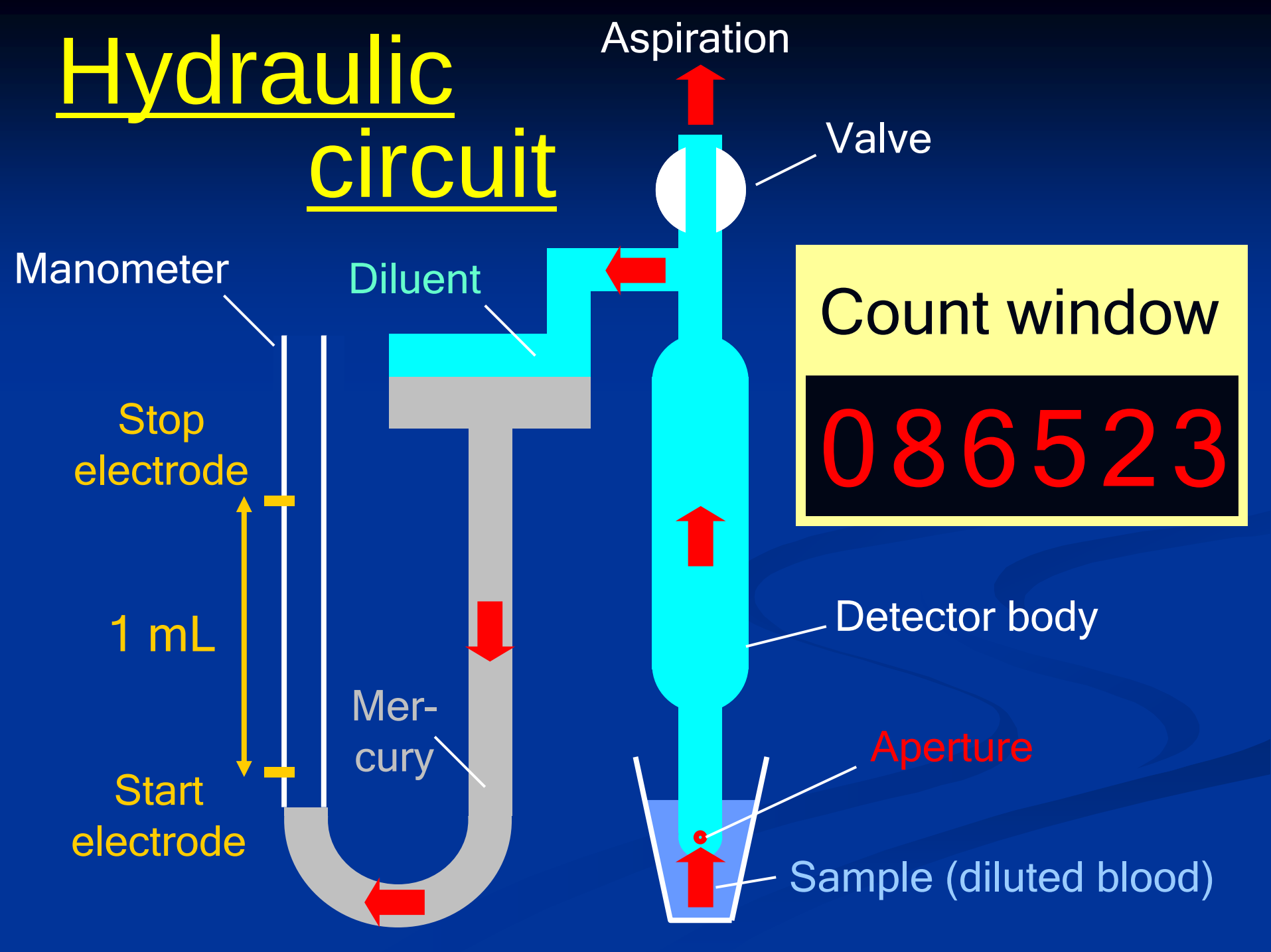
What is "coincidence"?



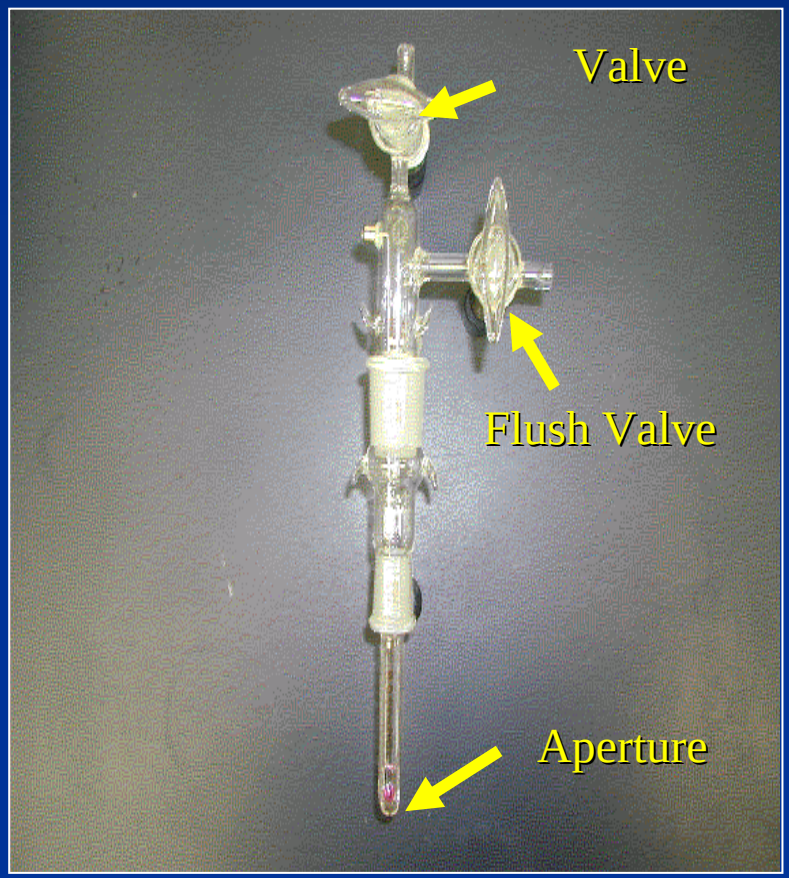
It's the coincidence that a couple of cells run through the aperture sensitive zone at a time.

A couple of cells are observed as a single electric pulse and then counted as a single cell, so one of these cells is miscounted.

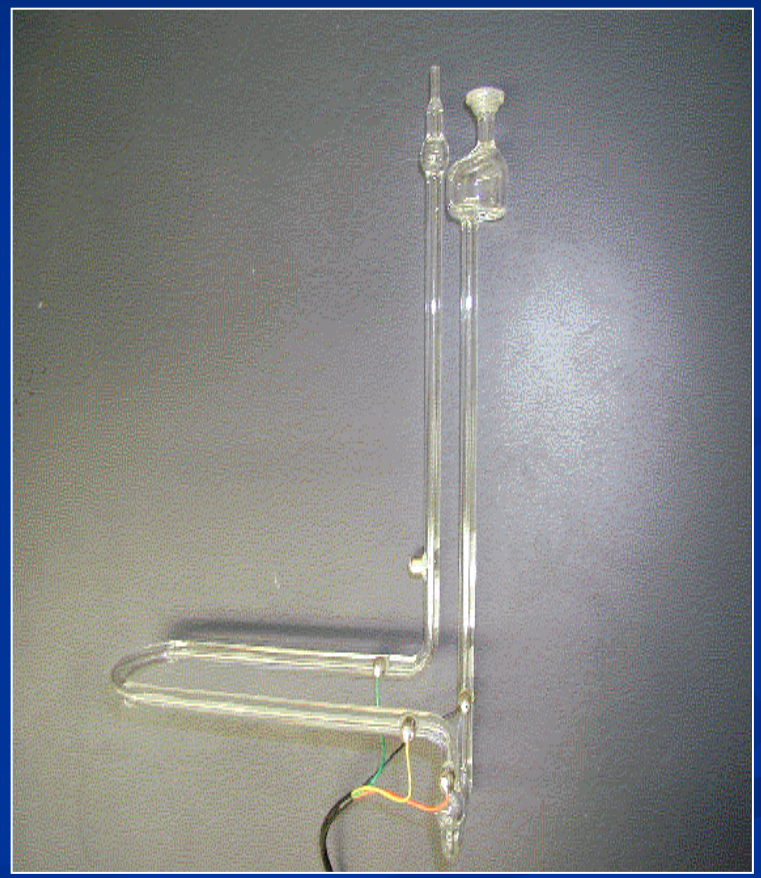
Hydraulic circuit



Quartz Glass Parts



Detector Unit



Mercury Manometer

Manometer volume was certified by a neutral body

DER MINISTER
FÜR WIRTSCHAFT UND VERKEHR
DES LANDES SCHLESWIG-HOLSTEIN
– Amt für das Eichwesen –

P r ü f b e s c h e i n i g u n g

für eine Einrichtung zur Volumenbestimmung von vorverdünnten Blutproben als Bestandteil von Blutkörperchenzählgeräten

Hersteller : TOA Medical Electronics Co. Ltd.
Kitano 314-2, Noguchi-Cho
Khogawa-City
Hyoogo Prefecture
Japan

Fabr.-Nr. : 87

Antragsteller: "Sysmex" Toa Medical Electronics (Europe) GmbH,
Tarpen 15 a, 2000 Hamburg 62

Aufbau und Wirkungsweise der Einrichtung:
Nach dem Einschalten des Geräts wird Quecksilber im U-Rohr-Manometer durch eine Vakuumpumpe unter die Startelektrode gesaugt. Nach Betätigung des Startschalters läuft das Quecksilber über je eine im Glaskörper eingeschlossene Start- und Stop-Elektrode und saugt dabei Flüssigkeit über einen Rüssel an. Als Volumen gilt das vom Quecksilber geschaltete Teilstück zwischen den beiden Elektroden.

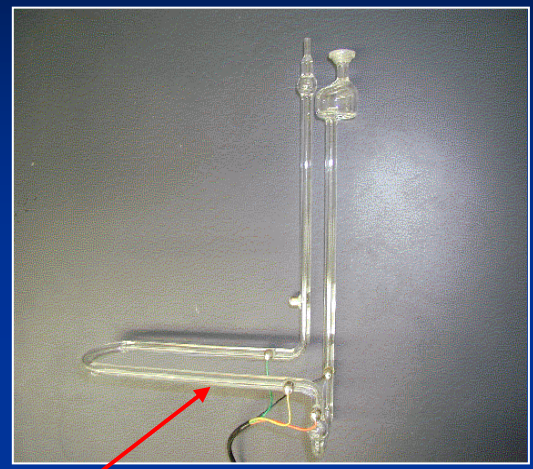
Prüfeinrichtung

a) Manometer-Prüfstand und Manometer-Steuer- u. Anzeigeelektronik SCC-1, TOA Medical Electronics, Japan
b) Analysenwaage Typ AND ER-182 A, Japan mit integriertem Datenausgang
c) Computer NEC PC 9801 F2
d) Interface MI-1 zum Datenabruf von der Waage

Meßergebnis
Das Volumen im Glasrohr zwischen der Start- und Stop-Elektrode beträgt bezogen auf die Bezugstemperatur von 20 °C,

V = 1,010 ml ± 0,004 ml

Das Volumen ist der Mittelwert aus 30 Messungen, die angegebene Meßunsicherheit gilt für ein Vertrauensniveau von 95 %.
Prüfgut: Destilliertes Wasser



Volume = 1.010 ml +/- 0.004 mL

Bezeichnungen und Stempelstellen:
Auf dem Glaskörper ist die Fabr.-nr. eingeätzt. Weitere Kennzeichen wurden nicht aufgebracht.

Hinweis:
Das Gerät ist eichamtlich sondergeprüft.

Es darf nicht für Messungen nach § 3 des Eichgesetzes verwendet oder bereit gehalten werden (Gesetz über das Meß- und Eichwesen -Eichgesetz- in der Fassung der Bekanntmachung vom 22. Februar 1985 -Bundesgesetzblatt I Seite 410-).

Die Einrichtung muß an das Hauptgerät so eingebaut, angebaut oder angeschlossen werden, daß Rückwirkungen auf die Meßsicherheit vernachlässigbar klein sind und die ordnungsgemäße Verwendung und Wirksamkeit des Gesamtgeräts nicht beeinträchtigt wird.

Prüfbescheinigungen ohne Unterschrift und ohne Dienststempel haben keine Gültigkeit. Prüfbescheinigungen dürfen nur unverändert weiterverbreitet werden. Auszüge oder Änderungen bedürfen der Genehmigung des Amtes für das Eichwesen in Kiel.

I. A. des
Langniß

DER MINISTER FÜR WIRTSCHAFT UND VERKEHR DES LANDES SCHLESWIG-HOLSTEIN – AMT FÜR DAS EICHWESEN –

Kiel, den 21. März 1988

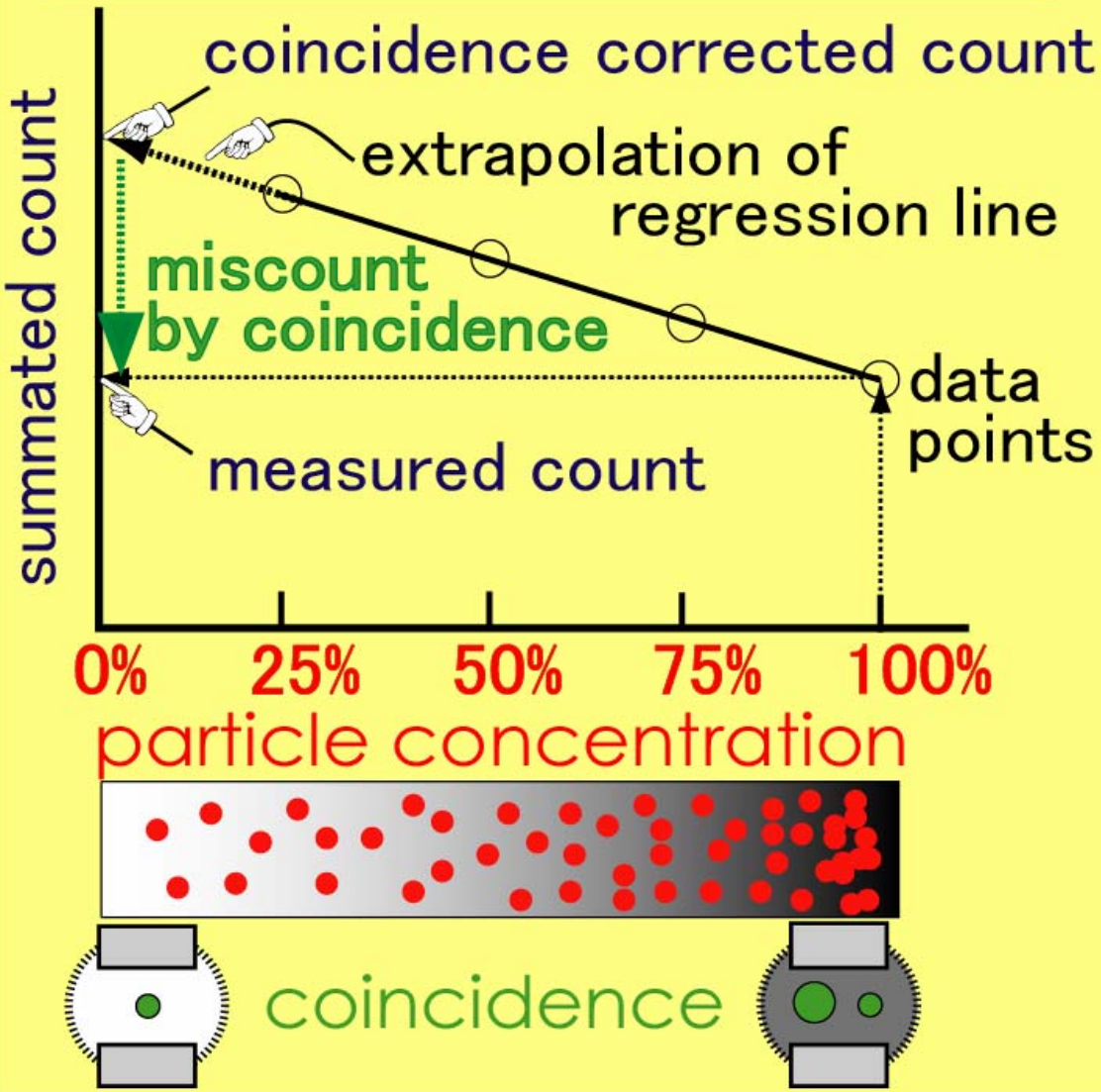
Prüfer:
Florschütz

Reference Counter & Macro-dilution

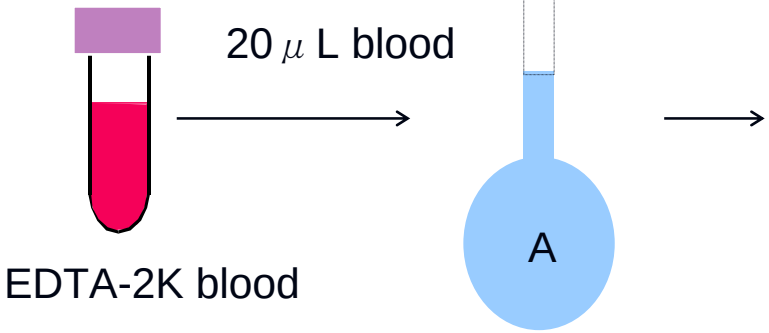
Reference Counter

Preparing 50,000-fold dilution

Extrapolation of the regression line for the coincidence correction



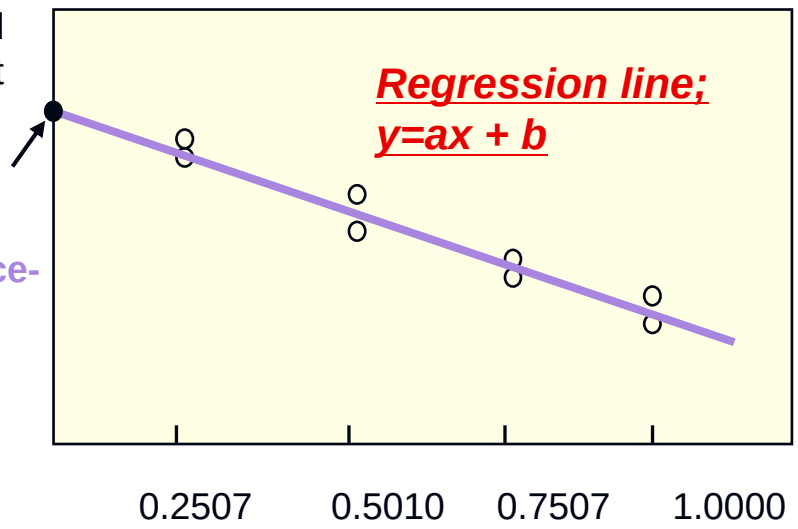
RBC Reference Method - Macro-dilution-



	Ratio	(A)	Diluent	DilRatio	# of counts
(A)	1.0000	100ml	0ml	50,001	3
(B)	0.7507	75ml	25ml	66,606	4
(C)	0.5010	50ml	50ml	99,802	6
(D)	0.2507	25ml	75ml	199,446	12

1000ml Reagent
(50,001-fold
dilution)

Summated
Count

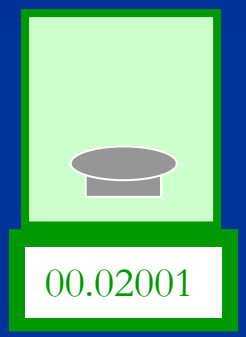
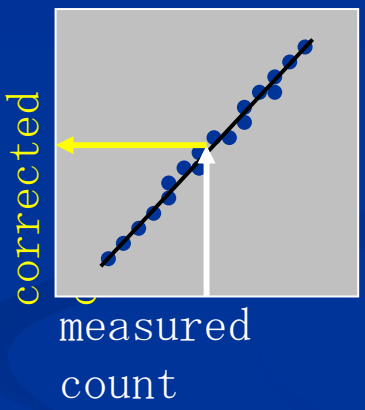
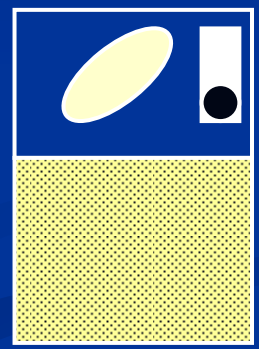


RBC True Value ← Y-axis(b) ;

Coincidence-corrected count

$$\frac{(\text{Yaxis} / 3) \times 50001(\text{fold})}{\text{Certified Manometer volume } (\mu \text{ l})}$$

Uncertainty of RBC count

Precision Balance	Micro-Dispenser	Flask	Mano-meter	Coincidence Correction	Reference Counter
					
u_B	u_D	u_F	u_M	u_{CC}	u_R

$$u_c^2 = u_B^2 + u_D^2 + u_F^2 + u_M^2 + u_{CC}^2 + u_R^2$$

RBC count = 4.728 +/- 0.057 (x 10^{12}/L) , k = 2

code	factor of uncertainty	value of the factor	value of the unc.	type	probability distribution	divisor	standard unc.	conversion coefficient	std. unc. in required unit
u_B	calibration of balance	20 mg	0.130 mg	B	normal	2	0.065 mg	0.239 x 10^{15}/g	0.0155 x 10^{12}/L
u_D	repeatability of dispensing volume of flask	20.12 uL	0.042 uL	A	—	1	0.042 uL	0.238 x 10^{18}/L^2	0.0100 x 10^{12}/L
u_F		1000 mL	0.3 mL	B	rectangular	1.73	0.173 mL	0.005 x 10^{15}/L^2	0.0008 x 10^{12}/L
u_M	volume of manometer	1.010 mL	0.004 mL	B	normal	2	0.002 mL	4.735 x 10^{15}/L^2	0.0095 x 10^{12}/L
u_{CC}	coincidence correction	110.2 x 10^3count	0.856 x 10^3count	B	normal	2	0.428 x 10^3count	0.043 x 10^9/L	0.0186 x 10^{12}/L
u_R	repeatability of measuring	4.782 x 10^{12}/L	0.005 x 10^{12}/L	A	-	1	0.005 x 10^{12}/L	1 -	0.0050 x 10^{12}/L
u_c	combined std. uncertainty				normal				0.0283 x 10^{12}/L
U	expanded uncertainty				normal (k = 2)				0.057 x 10^{12}/L

RBC count = 4.728 +/- 0.057 (x 10^{12}/L) , k = 2

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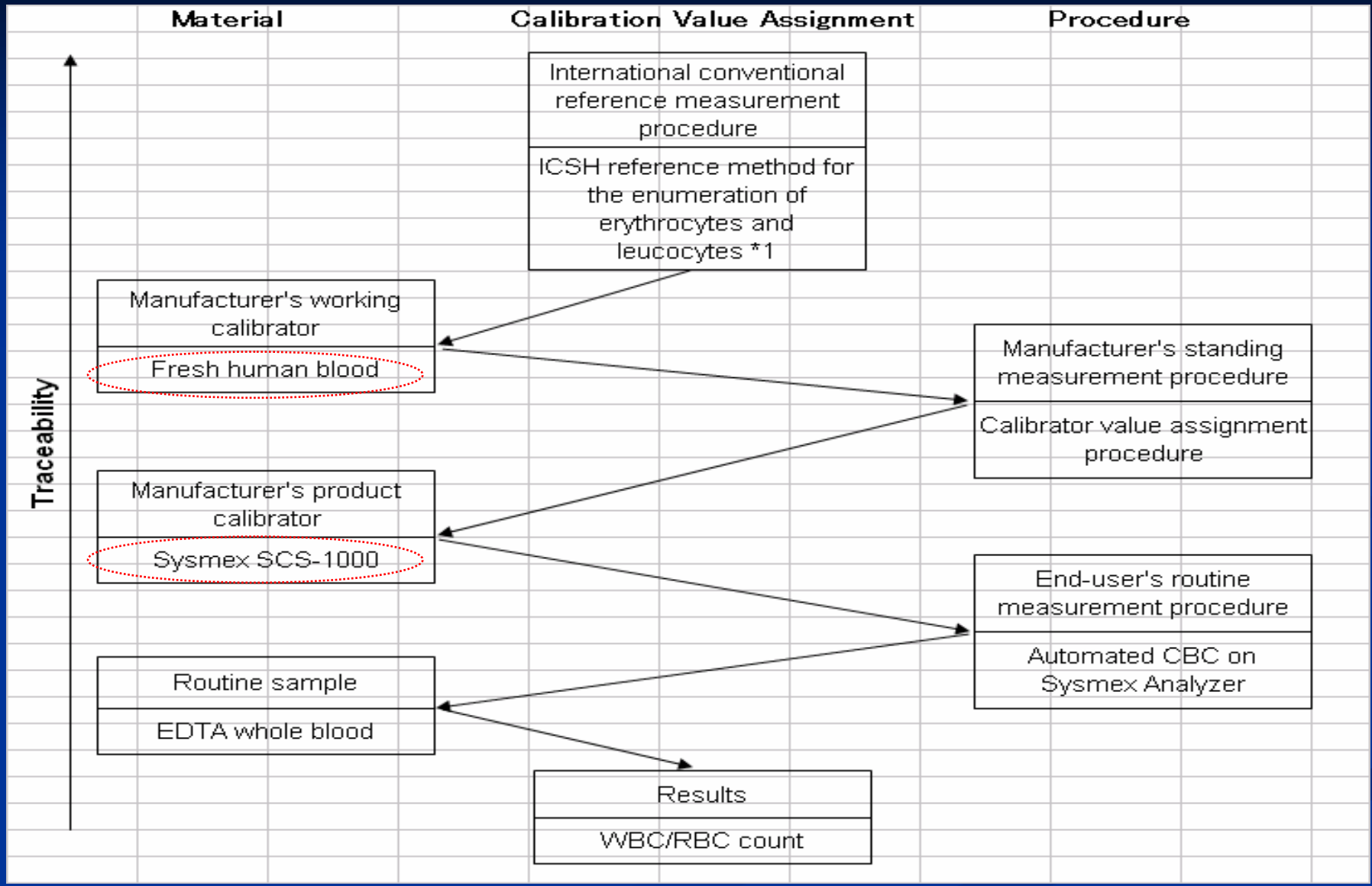
u_M							0.002	4.735	0.0095 x 10^{12}/L
u_{CC}	correction	10^3 co	10^3 count	B					0186 x 10^{12}/L
u_R	repeatability of measuring	4.782 x 10^{12}/L	0.005 x 10^{12}/L	A					0050 x 10^{12}/L
u_c	combined std. uncertainty				normal				0.0283 x 10^{12}/L
U	expanded uncertainty				normal (k = 2)				0.057 x 10^{12}/L

Measurand :Mean of 10 times measurements by the reference counter

Expanded uncertainty : U_c is doubled to express 95 % confidence interval of measurand

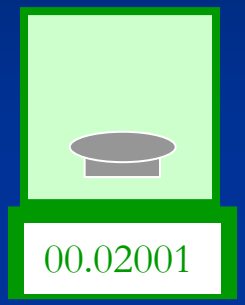
Traceability Chain for RBC count calibration



* ***ISO 17511:2003*** (Cases with international conventional reference measurement procedure (which is not primary) but no international conventional calibrator and without metrological traceability to SI)

Uncertainty in RBC count on SCS-1000

Precision balance

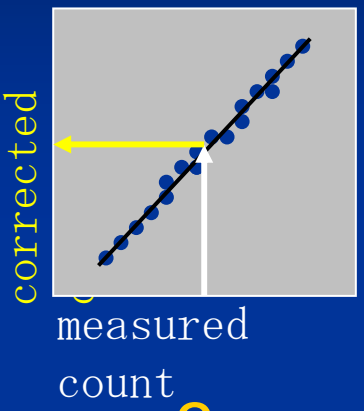


Micro-dispenser

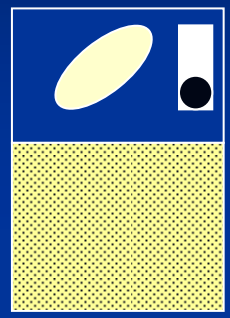
Flask

Mano-meter

Coincidence correction



Reference counter



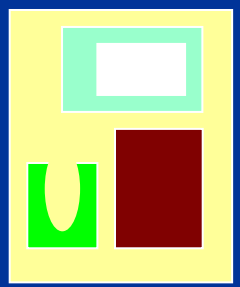
Flesh blood

$$u_c^2 = u_1^2 + u_2^2 + u_3^2 + \ldots\ldots\ldots + u_n^2$$

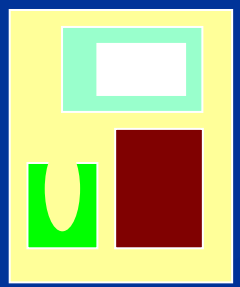
Flesh blood

Standard analyzers

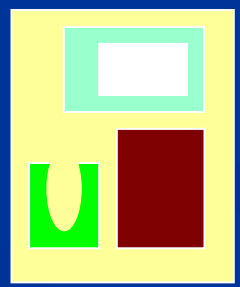
Kobe



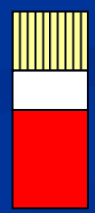
Ono



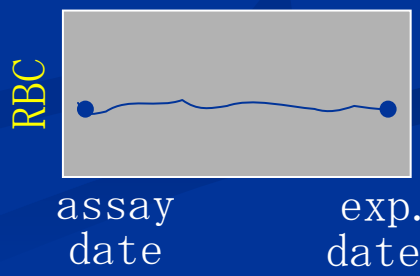
kakogawa



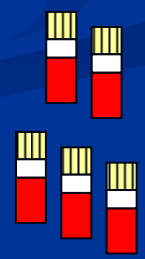
SCS-1000



Instability



Vial to Vial



Traceability & Uncertainty for Calibrator

Traceability and Uncertainty

SCS-1000 Sysmex Calibrator System

XE-2100, Automated Hematology Analyzer

LOT NO: **51580525**

EXP. DATE: **11-Jul-05**

Traceability

Uncertainty

June 07, 2005

Paramter	Reference Method	Reference Method	Assigned Value	Uncertainty*	Unit
WBC	*1	-	6.721	0.089	10^9/L
RBC	*1	-	4.785	0.062	10^{12}/L
PLT	*2	-	209.9	14.4	10^9/L
HGB	*3, *4	-	14.76	0.11	g / d L
HCT	*5, *6	-	42.17	0.85	%

* : This uncertainty (expanded undertainty: k=2 was calculated in accordance with the "Guide to the expression of Uncertainty in Measurement" (GUM: 1993).

*1: ICSH Expert Panel on Cytometry, Clinical Laboratory Haematology, 16, 131-138, 1994
"Reference method for the enumeration of erythrocytes and leucocytes"

*2: ICSH Expert Panel on Cytometry and International Society of Laboratory Hematology Task Force on Platelet Counting,
American Journal of Clinical Pathology, 115, 460-464, 2001
"Platelet Counting by the RBC/Platelet Ratio method – A reference method"

*3: NCCLS, H15-A3
"Reference and selected procedures for the quantitative determination of hemoglobin in blood – 3rd edition; Approved standard"

*4: Journal of Clinical Pathology, 49, 271-274, 1996
"Recommendation for reference method for haemoglobinometry in human blood (ICSH standard 1995) and

*5: NCCLS H7-A3
"Procedure for Determining Packed Cell Volume by the Microhematcrit Method – 3rd edition; Approved Standard"

*6: Laboratory Hematology, 7, 148-170, 2001
"Recommendations for reference method for the packed cell volume (ICSH Standard 2001)"

Contents

- Traceability for Blood Cell Counting
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- Summary

6 Manufacturers' Comparison Study in Japan

- Purpose:

  To evaluate hematological values obtained with Standard Automated Hematology Analyzers of 6 manufactures.

- Method:

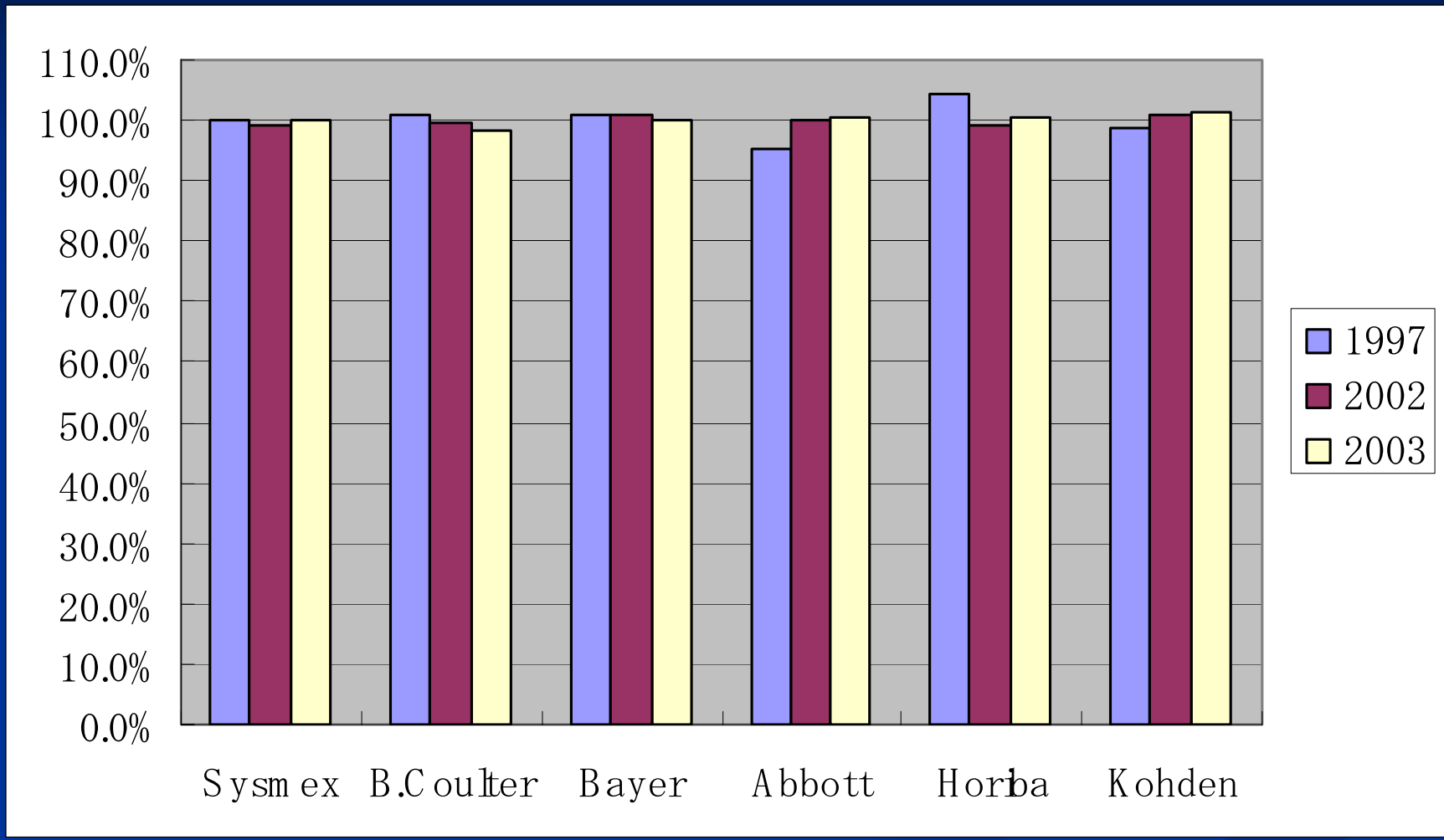
  Prepare fresh whole blood samples from 3 healthy doners.

  Measure 3 samples on standard automated analyzers of each manufacture at the same time within 6 hours after drawing.

  Compare each data obtained by each manufacture.

  This study was done in 1991, 1997, 2002 ,2003 and 2005 (still under investigation).

Difference among 6 manufactures for RBC

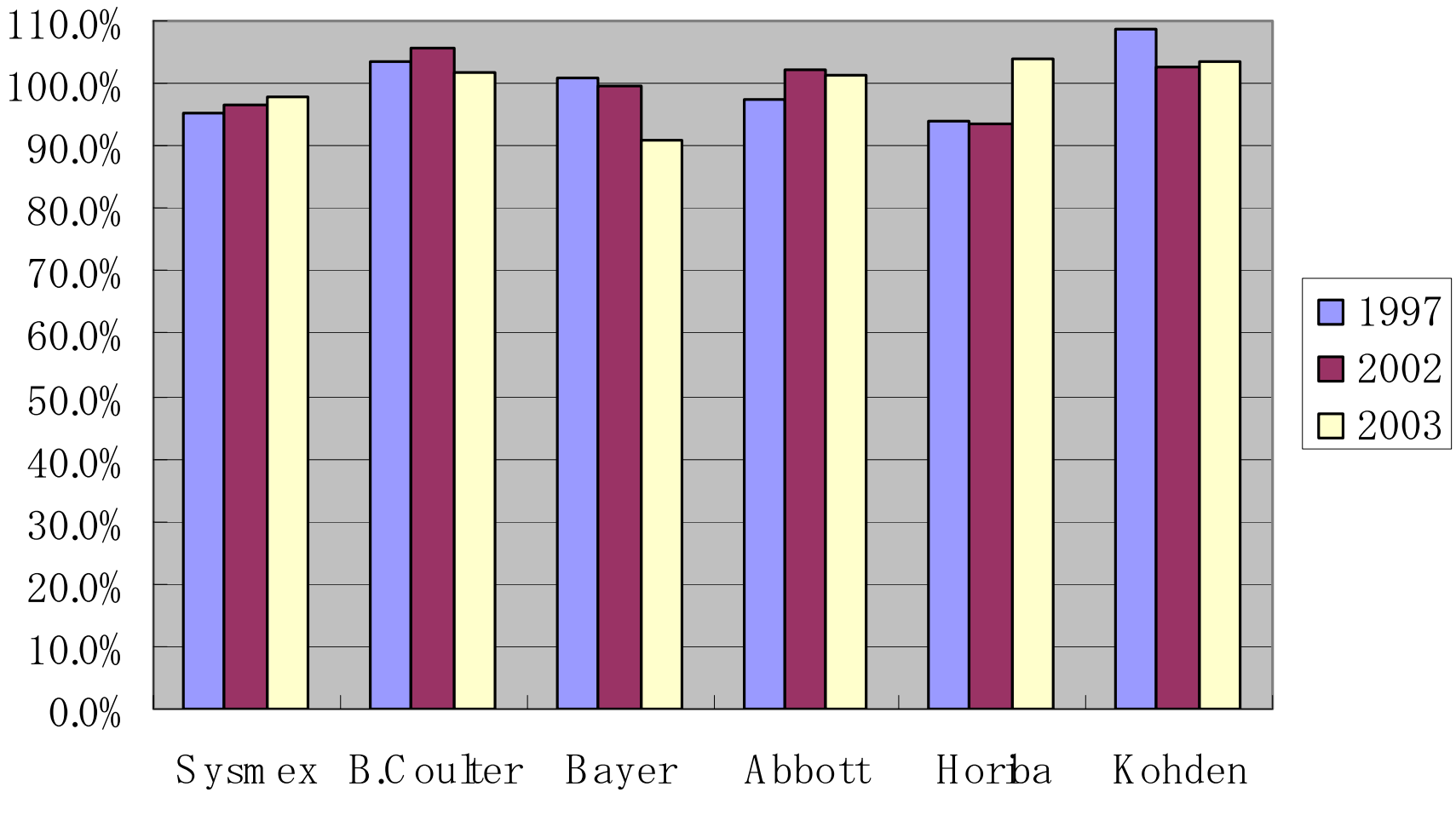


* 100% represents mean of all 6 manufacturers' data.

<RBC >

6 manufactures data showed well-harmonized (within +/- 1.5%) in 2003 study

Difference among 6 manufactures for WBC

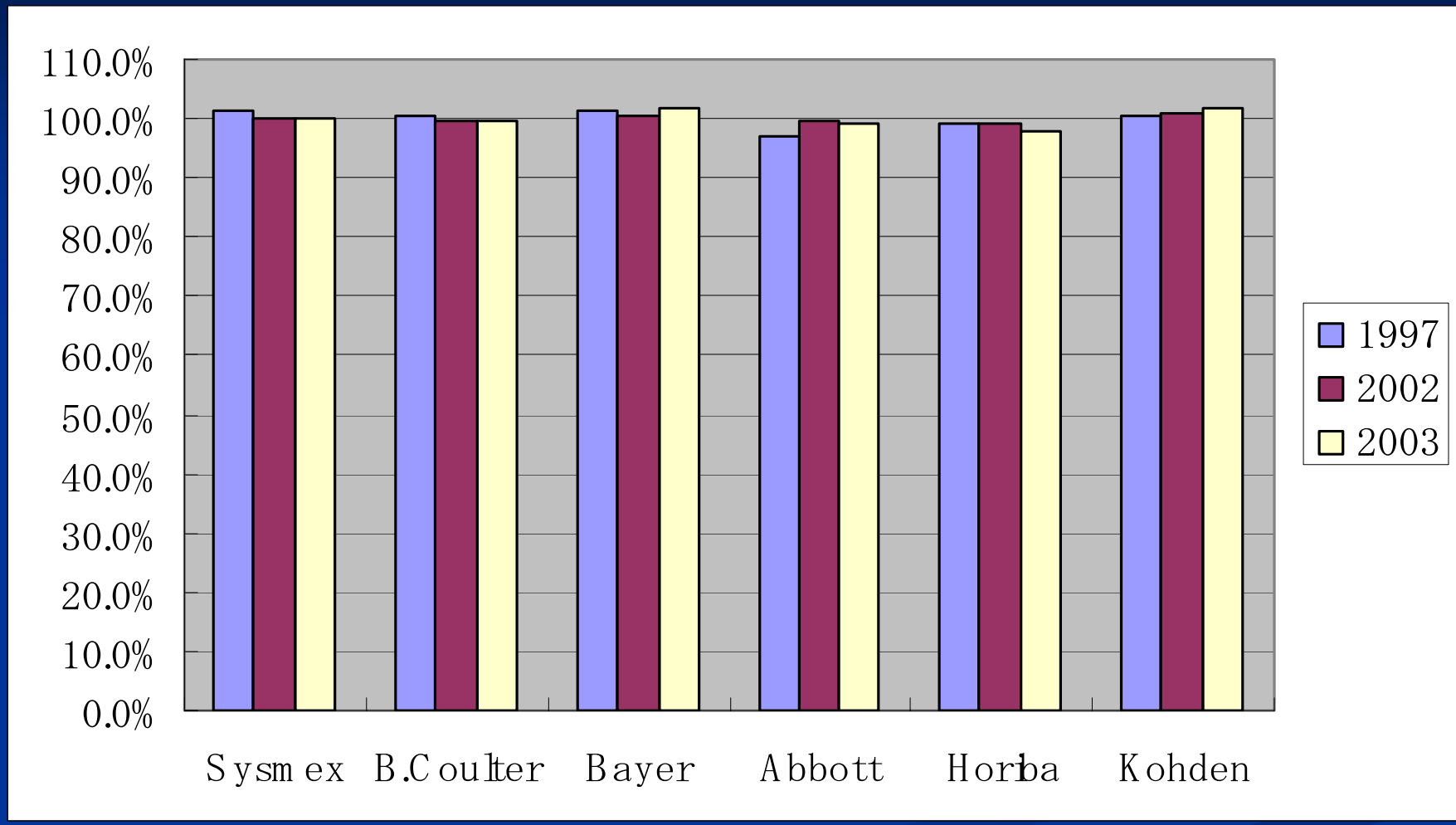


* 100% represents mean of all 6 manufacturers' data.

<WBC >

6 manufactures data showed wide variation (> +/- 5%) in 2003 study.

Difference among 6 manufactures for HGB

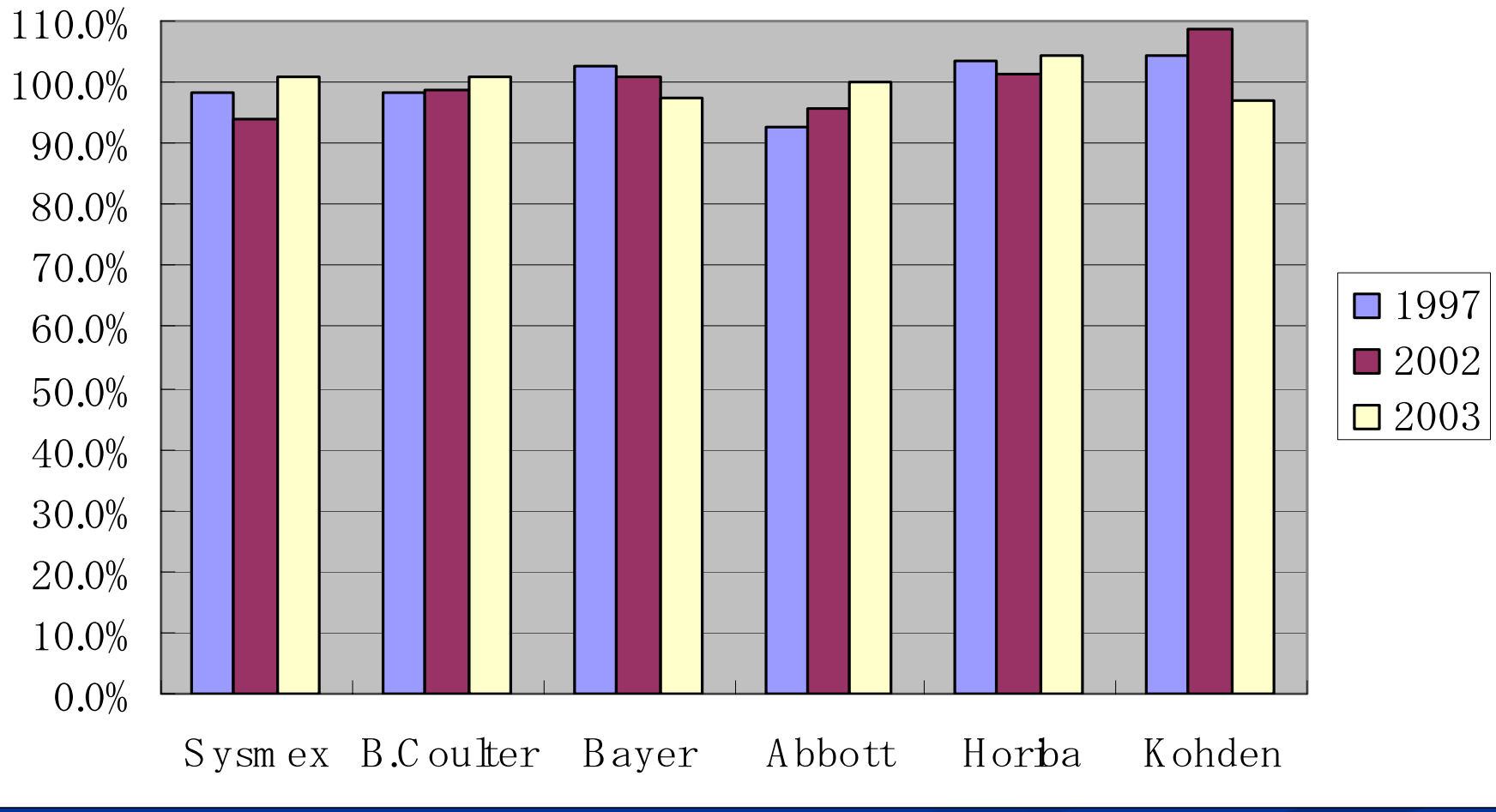


* 100% represents mean of all 6 manufacturers' data.

<HGB >

6 manufactures data showed well-harmonized (within +/- 1.5%) in 2003 study.

Difference among 6 manufactures for PLT

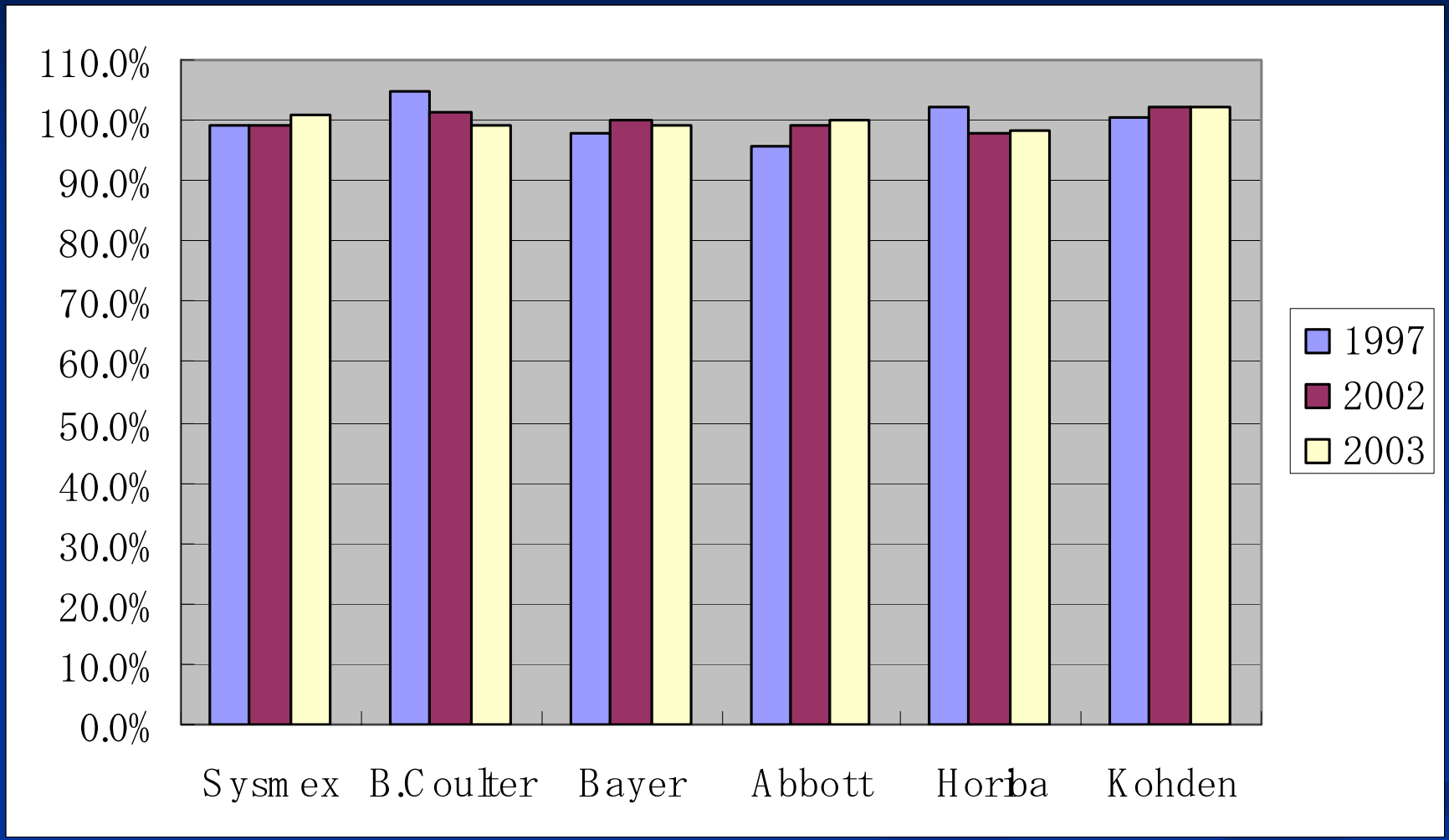


* 100% represents mean of all 6 manufacturers' data.

<PLT >

6 manufactures data showed wide variation (> +/- 5%) in 2003 study.

Difference among 6 manufactures for HCT



* 100% represents mean of all 6 manufacturers' data.

<HCT>

6 manufactures data showed well-harmonized (within +/- 2%) in 2003 study.

Summary

- Reference Method and Traceability has been established by using ICSH recommended protocol, which each manufacturers agreed.
- Discrepancy of fresh whole blood data among major manufacturers has been less than +/- 1% for RBC and Hgb.
- Announcement of Uncertainty for Calibrator is ready for end-users.

Thank you for your attention !