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VEQ Proteine Specifiche Ciclo 2015

Paolo Chiarugi

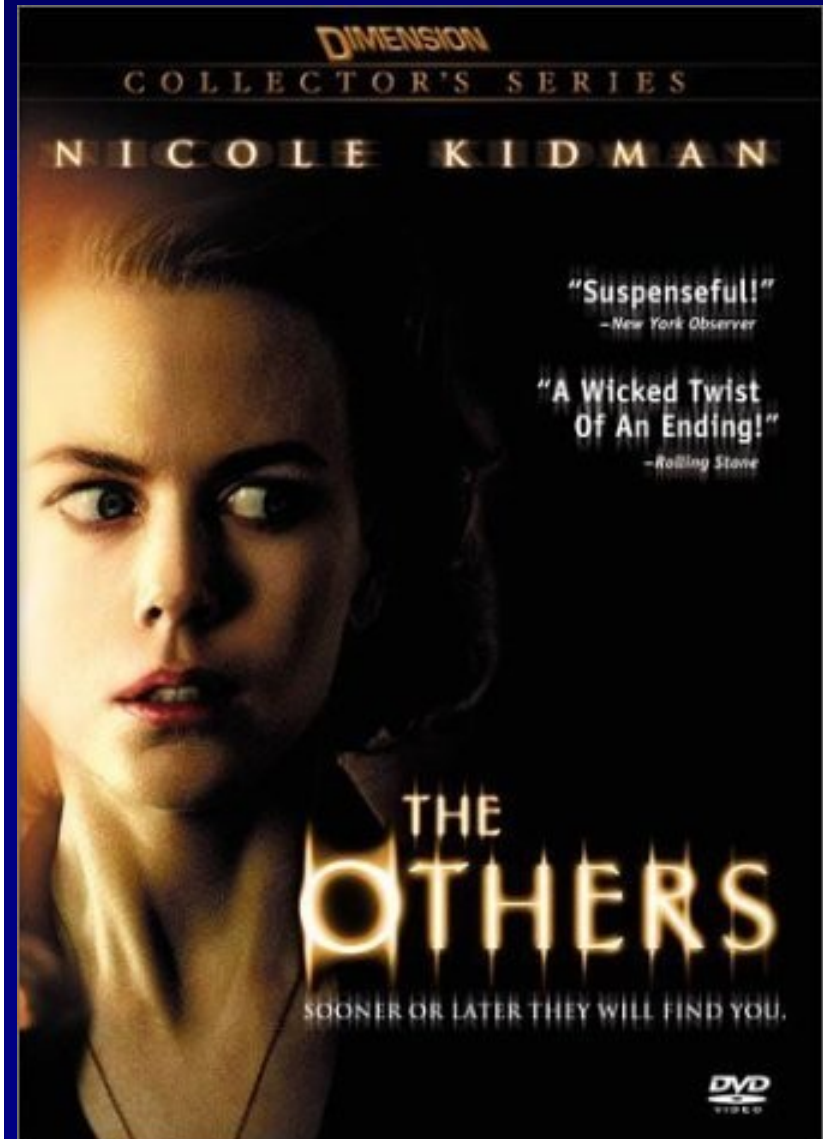
U.O. Analisi Chimico-Cliniche

AOUP
Pisa



Proteine Specifiche o Aspecifiche ?

PROTEINA	Val.Rif. (g/L)	Sintesi
Alfa 1 - Antitripsina	0,9 - 2,0	Fegato
Alfa 1 Glicoproteina Acida	0,5 - 1,2	Fegato
Alfa 2 - Macroglobulina	1,3 - 3,0	Fegato
Aptoglobina	0,3 - 2,0	Fegato
Beta 2 - Microglobulina	0,0008 - 0,0025	Ubiquitaria
C3	0,9 - 1,8	Fegato
C4	0,1 - 0,4	Fegato
Ceruloplasmina	0,2 - 0,6	Fegato
Cistatina C	0,0006 - 0,0014	Ubiquitaria
Fattore Reumatoide	<10 UI/mL	S.I.
IgA	0,7 - 4,0	S.I.
IgE	<0,00043	S.I.
IgG	7 - 16	S.I.
IgM	0,4 - 2,3	S.I.
Kappa-Catena Leggera	2 - 4	S.I.
Lambda-Catena Leggera	1 - 2,4	S.I.
Proteina C Reattiva	< 0,005	Fegato
Prealbumina	0,2 - 0,4	Fegato
Proteina Legante il Retinolo	0,03 - 0,06	Fegato
Recettore Solubile Transferrina	0,0008 - 0,0018	Ubiquitaria
Transferrina	2,0 - 3,6	Fegato



VEQ Proteine Specifiche

UK NEQAS

United Kingdom National External Quality Assessment Service



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Specific Proteins

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Analytes or Clinical Applications Covered

A range of serum proteins assayed by immunochemical means and of clinical relevance, as listed below

IgG

IgA

IgM

Complement component C3

Complement component C4

Alpha1 antitrypsin

Alpha1 acid glycoprotein (Orosomuroid)

Haptoglobin

Transferrin

Alpha2 macroglobulin

Caeruloplasmin

Transthyretin (Prealbumin)

Cystatin C

Reproductive
Science

Chemistry

Genetics

Haematology

Histopathology

Immunology

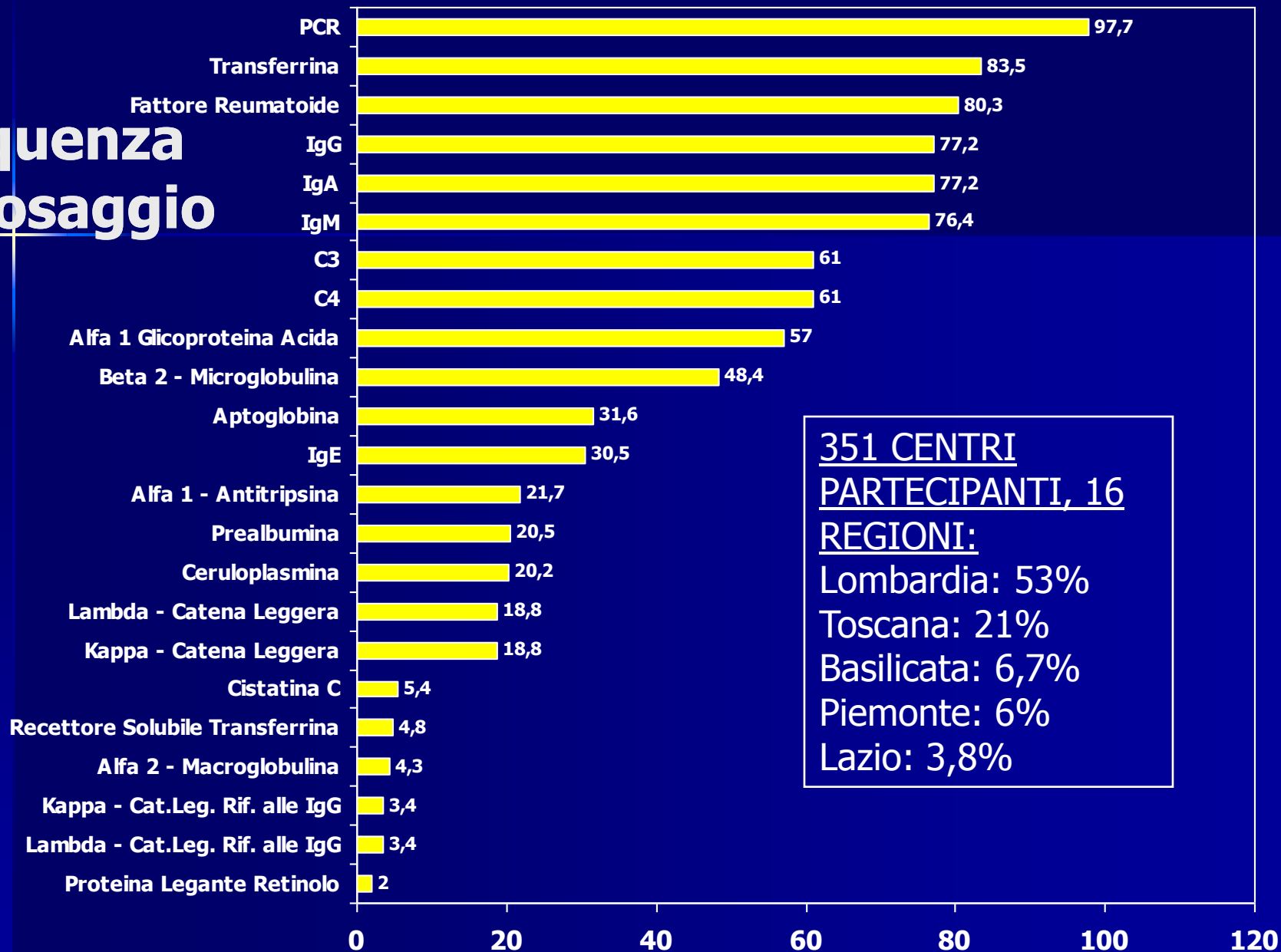
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Frequenza di dosaggio



Metodi usati 20913 test, campioni 7 su 8

METODO	%	VARIANTI	STRUMENTO e/o REAGENTE + USATO	
AGGLUTINAZIONE	0,6	12		SIEMENS
CARD	0,2	4	I-CHROMA	BODITECH
CHEMILUM.	1,7	13	IMMULITE 2000	MEDICAL SYSTEM
COLORIMETRICO	0,03	2	CHEMACOMP 100	G.D.
DRY CHEMISTRY	0,3	2	VITROS	ORTHO
ELFA	1,2	2	VIDAS	BIOMERIEUX
IMMUNODIFF.	2	11		LIOFILCHEM
IMMUNOENZ.	0,2	7	ALISEI	RADIM
IMMUNOFLUOR.	0,6	4	AIA	TOSOH
IMMUNOPREC.	0,1	6	KONELAB	BIODEVICE
IMMUNOTURB.	67,2	110	COBAS	ROCHE
MEIA	0,1	2	AXSYM	ABBOTT
NEFELOMETRICO	25,2	19	B.N.II IMAGE	SIEMENS BECKMAN

% Metodi usati per analisi

	TURBIDIM.	NEFELOM.	RID	ALTRO
Alfa 1 - Antitripsina	40	60	0	0
Alfa 1 Glicoproteina Acida	71	24	5	1
Alfa 2 - Macroglobulina	13	87	0	0
Aptoglobina	58	42	0	0
Beta 2 - Microglobulina	46	20	0	34
C3	72	25	3	0
C4	72	25	3	0
Ceruloplasmina	30	68	2	0
Cistatina C	23	77	0	0
Fattore Reumatoide	77	16	0	7
IgA	77	19	4	0
IgE	11	16	0	73
IgG	76	20	4	0
IgM	76	20	4	0
Kappa - Cat.Leg. Rif. alle IgG	1	99	0	0
Kappa - Catena Leggera	38	62	0	0
Lambda - Cat.Leg. Rif. alle IgG	1	99	0	0
Lambda - Catena Leggera	38	62	0	0
PCR	86	8	0	6
Prealbumina	55	45	0	0
Proteina Legante Retinolo	0	100	0	0
Recettore Solubile Transferrina	2	86	0	12
Transferrina	83	13	3	1

CRM 470: la nuova era delle proteine specifiche

special report

CLIN. CHEM. 40/6, 934-938 (1994)

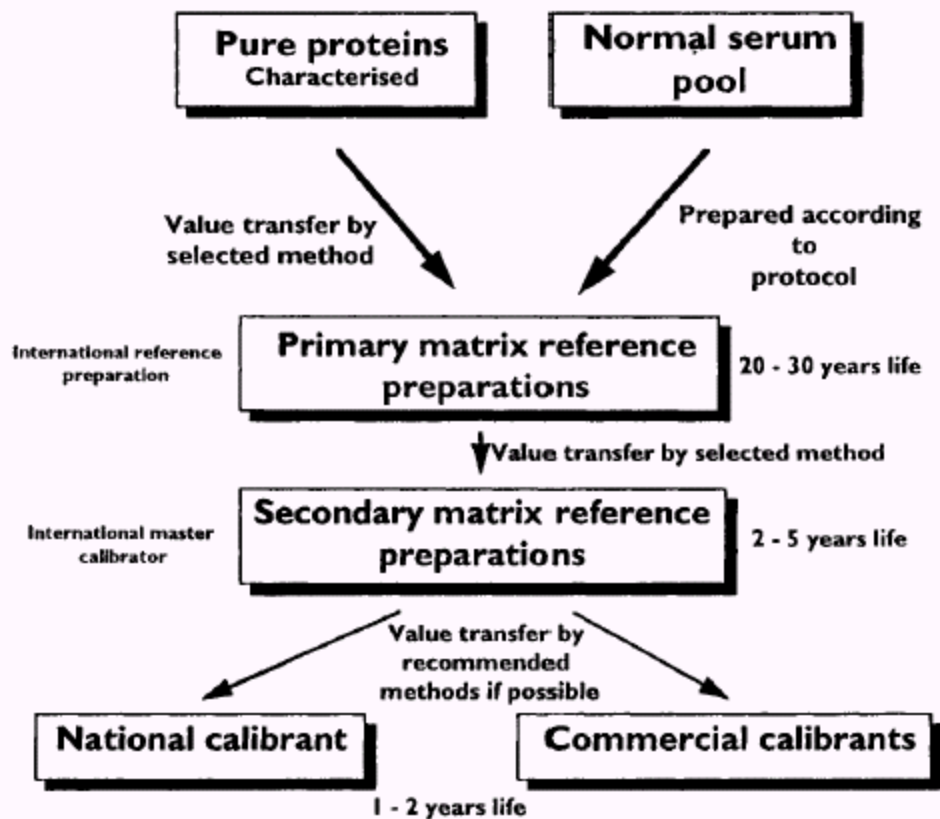
New International Reference Preparation for Proteins in Human Serum (RPPHS)

John T. Whicher,¹ Robert F. Ritchie,² A. Myron Johnson,³ Siegfried Baudner,⁴ Jacques Bienvenu,⁵ Soren Blirup-Jensen,⁶ Anders Carlstrom,⁷ Francesco Dati,⁴ Anthony Milford Ward,⁸ and Per Just Svendsen⁶

Table 1. International reference preparations used in value assignment of the RPPHS.

Protein	USNRP, lot no. 12-0575C	Pure proteins	C-reactive protein, WHO 85/506	Complement, WHO lot no. 5/4	Immunoglobulins WHO 67/86	WHO 6HSP, lot no. 4/2
	mg/L			IU/L		
Transthyretin		●				
Albumin	●					●
α_1 -Acid glycoprotein	●	●				
α_1 -Antitrypsin	●	●				●
Ceruloplasmin	●					●
α_2 -Macroglobulin	●					●
Haptoglobin	●					
Transferrin	●	●				●
C3/C3c	●					●
C4/C4c	●			●		
C-reactive protein			●			
IgG	●				●	
IgA	●				●	
IgM	●				●	

Preparation of CRM 470, the New International Reference Preparation for Proteins in Human Serum.



Cause d'inaccuratezza

- Trasferimento dei valori da CRM a calibratori e controlli
- Eterogeneità delle proteine
- Effetto matrice

Unlike assays for ions and small molecules plasma protein measurements depend entirely on comparison of a test sample with a calibrant, known as a reference material or standard. Owing to the innate molecular heterogeneity of plasma proteins it is often impossible to ensure that the calibrator is identical in its behaviour in measurement systems to the test sample. Despite these fundamental limitations it is clear that internationally agreed calibrants are the only way to improve consistency of reporting of protein values in biological fluids between methods and laboratories. Unfortunately, only international units are not subject to contention and the use of mass values which are not internationally agreed, and are re-ascribed during the life of a standard, has jeopardised the value of international calibrants. If mass values are used it must be accepted that they are, to some extent, arbitrary.

ERM-DA470k/IFCC

Clinical Chemistry 56:12
1880–1888 (2010)

General Clinical Chemistry

Characterization of the New Serum Protein Reference Material ERM-DA470k/IFCC: Value Assignment by Immunoassay

Ingrid Zegers,^{1*} Thomas Keller,² Wiebke Schreiber,³ Joanna Sheldon,⁴ Riccardo Albertini,⁵ Soren Blirup-Jensen,⁶ Myron Johnson,⁷ Stefanie Trapmann,¹ Hendrik Emons,¹ Giampaolo Merlini,⁵ and Heinz Schimmel¹

Table 2. Certified values and uncertainty budget for 12 proteins in ERM-DA470k/IFCC.^a

Protein	Certified value in ERM-DA470, g/L	TF		Certified value and uncertainty in ERM-DA470k/IFCC		Components of the uncertainty budget				
		Mean of means	Relative CV, %	Value, g/L	$U_{CRM} (k = 2)$, g/L	u_{char} , %	u_{cal} , %	u_{bb} , %	u_{lts} , %	u_c , %
A2M	1.64	0.871	2.05	1.43	0.06	0.836	1.53	0.517	0.669	1.93
AAG	0.656	0.941	1.93	0.617	0.013	0.557	0.382	0.484	0.632	1.04
AAT	1.206	0.929	3.03	1.12	0.03	0.874	0.456	0.675	0.410	1.26
ALB	39.7	0.935	2.23	37.2	1.2	0.619	1.01	0.612	0.567	1.45
C3c	1.091	0.920	1.83	1.00	0.04	0.528	1.24	0.557	0.445	1.52
C4	0.151	1.070	3.02	0.162	0.007	0.873	1.66	0.418	0.497	1.98
HPT	0.893	0.995	2.26	0.889	0.021	0.627	0.504	0.637	0.494	1.14
IgA	1.96	0.918	2.36	1.80	0.05	0.632	1.02	0.340	0.357	1.30
IgG	9.68	0.948	1.37	9.17	0.18	0.368	0.516	0.425	0.615	0.98
IgM	0.797	0.908	2.11	0.723	0.027	0.636	1.44	0.745	0.562	1.83
TRF	2.45	0.964	1.60	2.36	0.08	0.483	1.22	0.498	0.646	1.55
TTR	0.243	0.906	1.80	0.220	0.018	0.543	3.71	0.880	0.592	3.89

^a The certified values are valid when the material is reconstituted according to the specified procedure. U_{CRM} , expanded uncertainty of the CRM with a coverage factor k equal to 2; u_{bb} , relative standard uncertainty related to the between-bottle heterogeneity; u_c , combined relative standard uncertainty; u_{cal} , relative standard uncertainty of the calibrant; u_{char} , relative standard uncertainty related to the characterization; u_{lts} , relative standard uncertainty related to the long-term stability of the material; u_{sts} , relative standard uncertainty related to the short-term stability of the material.

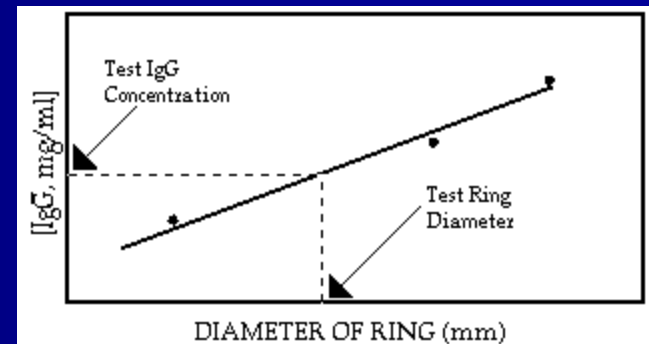
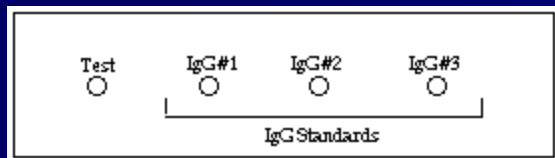
**Principali
metodi
usati**

	%	VARIANTI	STRUMENTO e/o REAGENTI + USATI	
IMMUNODIFFUSIONE RADIALE	2	11		SIEMENS
				LIOFILCHEM
				MEDIC
				PKL POKLER
				BIODEVICE
NEFELOMETRIA	25,2	19	VISTA	SIEMENS
			BN II	SIEMENS
			IMAGE	BECKMAN
			ARRAY 360	BECKMAN
			BNA	SIEMENS
TURBIDIMETRIA	67,2	110	MODULAR	ROCHE
			ARCHITECT	ABBOTT
			COBAS	ROCHE
			DX	BECKMAN
			AU 400	BECKMAN
			AU 600	BECKMAN

Immunodiffusione Radiale



In the 1960's and 1970's, as high quality antisera specific to the individual proteins became available, truly quantitative methods of assay were developed. The prototype of these was radial immunodiffusion (RID), in which a sample of serum or other fluid is placed into a well and allowed to diffuse passively into a gel (usually agar or agarose) containing antibodies to the protein of interest. The diameter of the resulting precipitin ring is proportional to the concentration of the protein of interest. This method revolutionized protein assays. However, it is slow, taking several hours to several days, and demands careful measurement of the relatively small rings.



Nephelometry

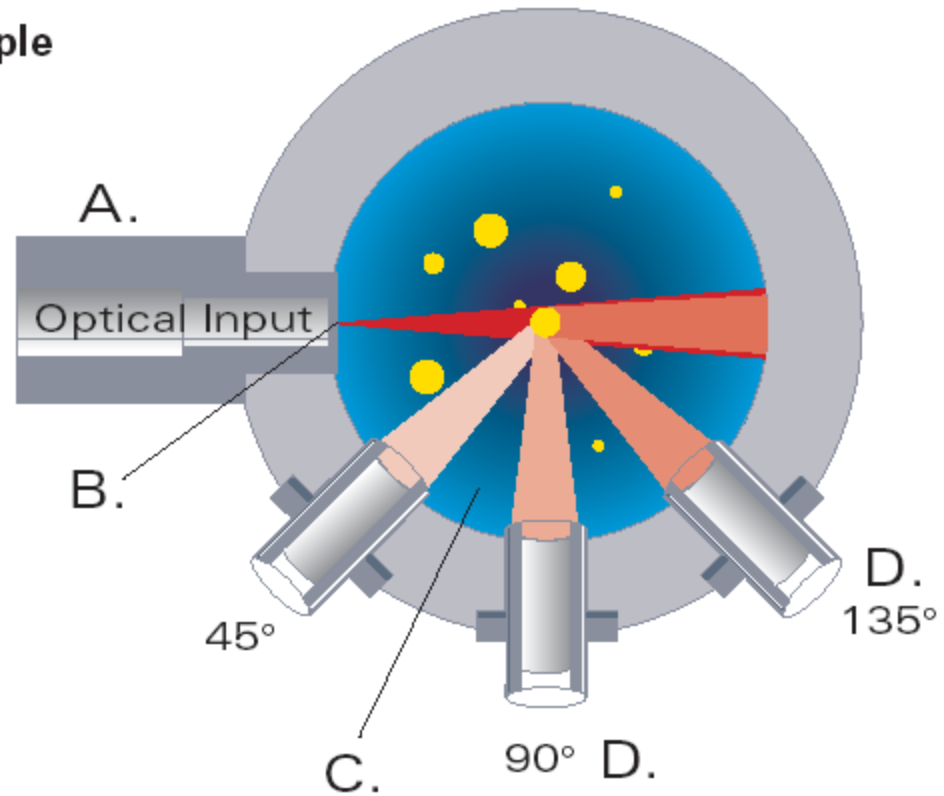
Nephelometry Principle

A. Incoming Light

B. Slit

C. Reaction Cuvette

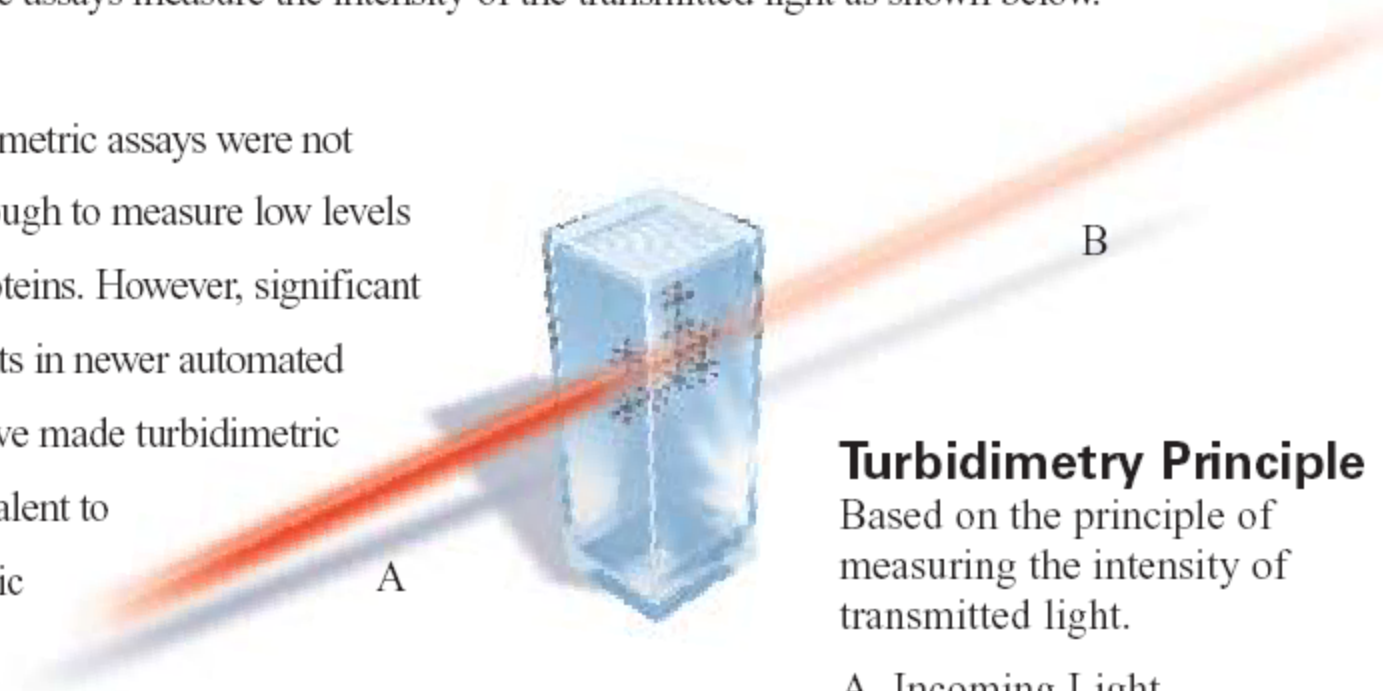
D. Photo Detectors



Turbidimetry

Turbidimetric assays measure the intensity of the transmitted light as shown below.

Early turbidimetric assays were not sensitive enough to measure low levels of serum proteins. However, significant improvements in newer automated analyzers have made turbidimetric assays equivalent to nephelometric analysis.



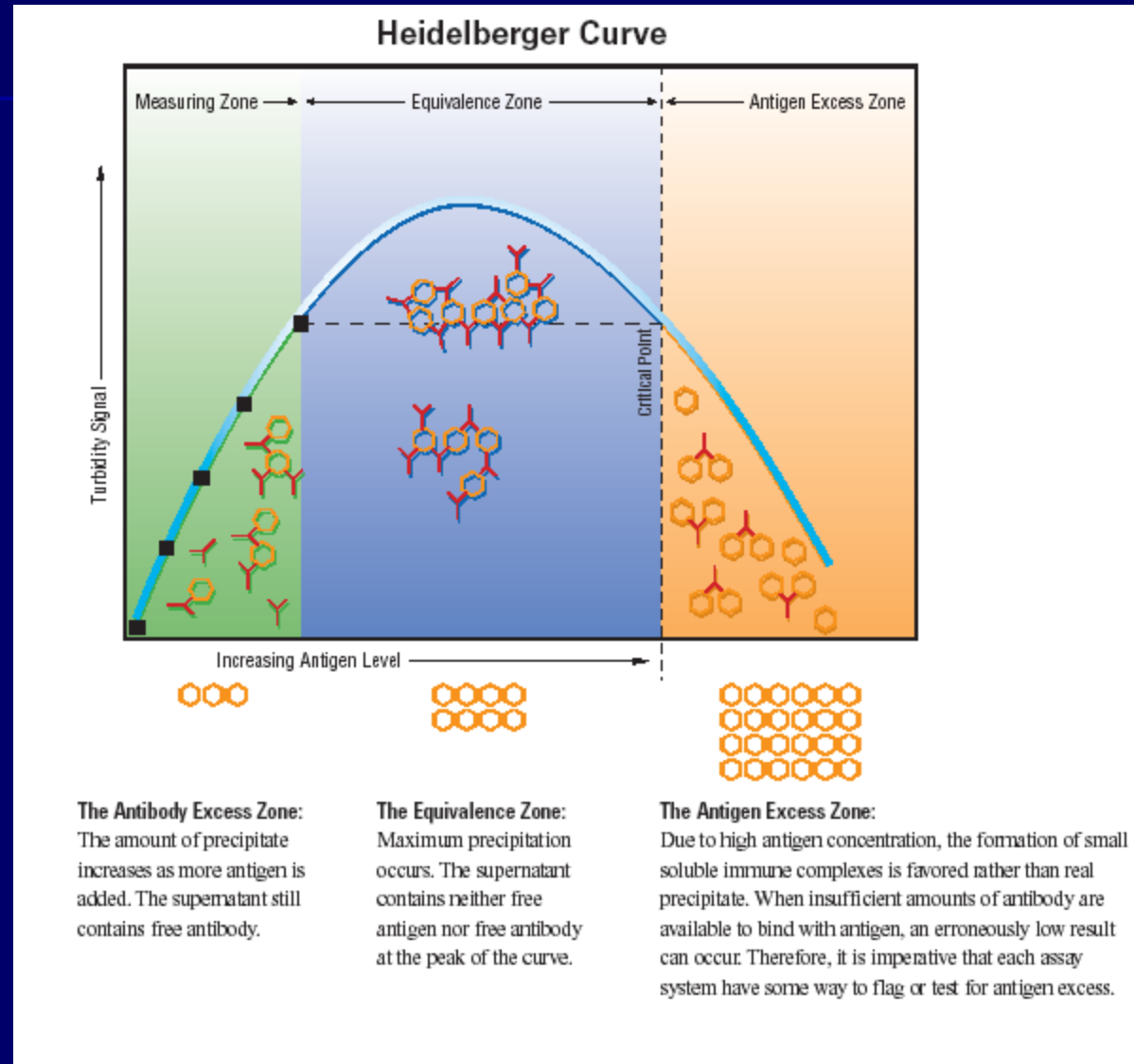
Turbidimetry Principle

Based on the principle of measuring the intensity of transmitted light.

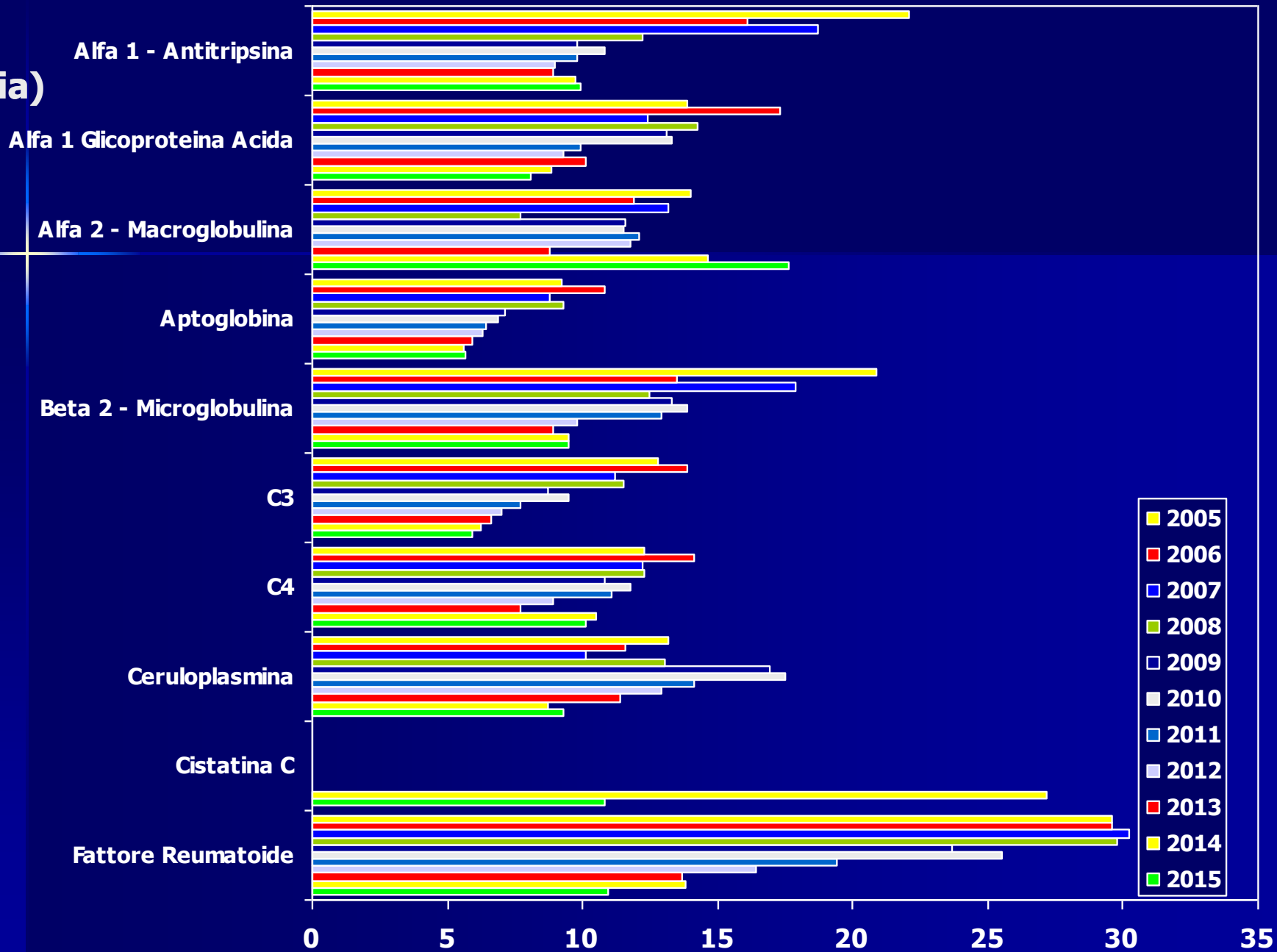
A. Incoming Light

B. Transmitted Light

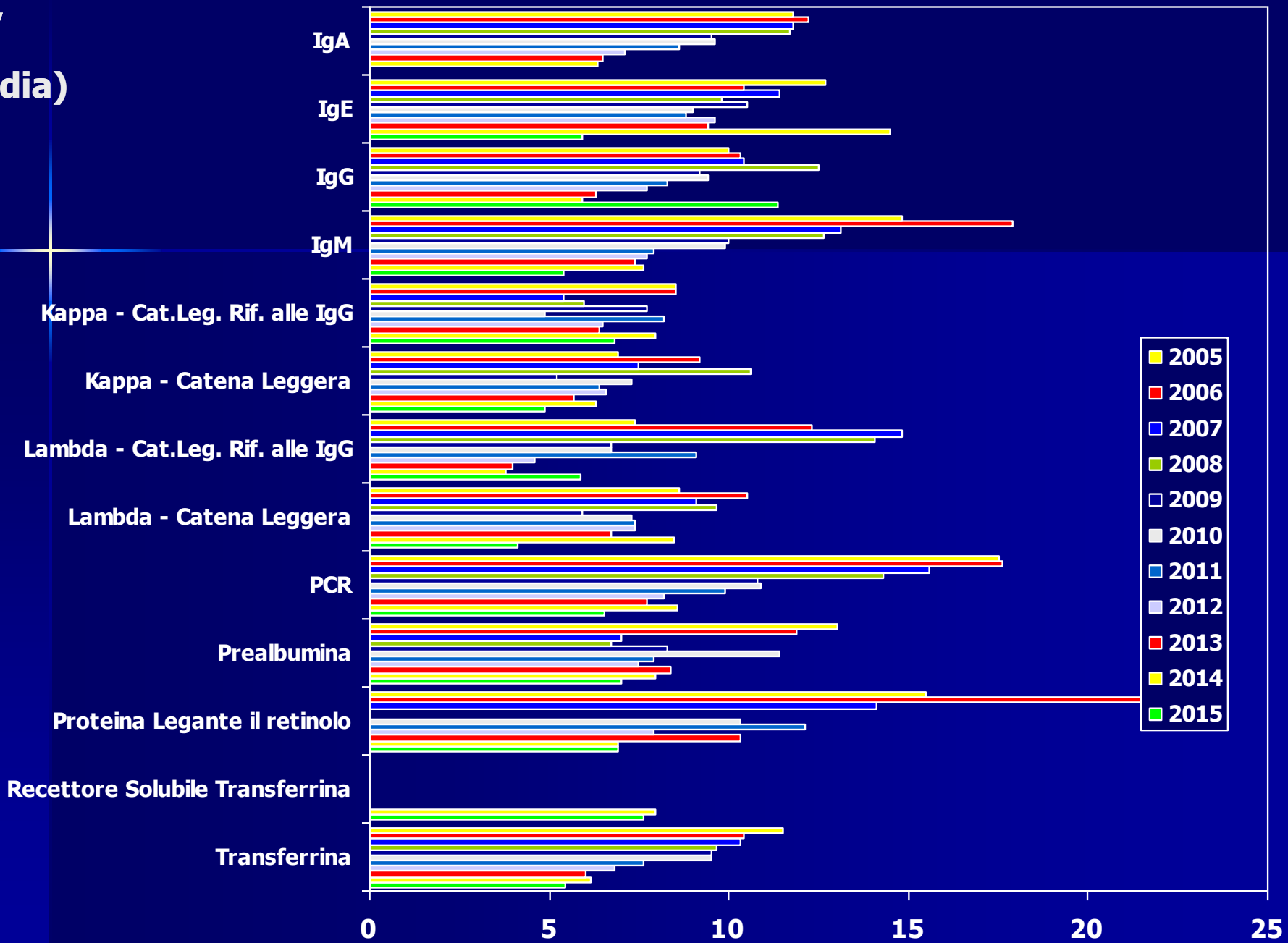
Curva di Heidelberg-Kendall



CV (media)



CV (media)



**CV
2015**

Analita	Media	Min	Max
Alfa 1 - Antitripsina	9,9	2,1	26,9
Alfa 1 Glicoproteina Acida	8,1	2,9	40,4
Alfa 2 - Macroglobulina	17,6	14,7	23,6
Aptoglobina	5,7	1,5	12,2
Beta 2 - Microglobulina	9,5	2,5	20,0
C3	5,9	1,7	9,7
C4	10,1	4,6	18,0
Ceruloplasmina	9,3	2,2	21,1
Cistatina C	10,8	9,1	12,5
Fattore Reumatoide	10,9	3,3	46,0
IgA	5,9	2,2	28,0
IgE	11,4	5,4	20,1
IgG	5,4	1,9	26,7
IgM	6,8	2,1	38,7
Kappa - Cat.Leg. Rif. alle IgG	4,9	3,5	8,2
Kappa - Catena Leggera	5,9	4,4	7,4
Lambda - Cat.Leg. Rif. alle IgG	4,1	3,0	6,1
Lambda - Catena Leggera	6,5	5,0	9,4
PCR	7,0	2,4	19,4
Prealbumina	6,9	3,3	10,5
Recettore Solubile Transferrina	7,6	4,2	11,1
Transferrina	5,5	2,2	27,9

**CV
2015**

Metodo	MediaDiCV	MinDiCV	MaxDiCV
AGGLUTINAZIONE	37,4	26,8	46,0
CHEMILUM.	13,5	9,4	18,2
DRY CHEMISTRY	12,9	6,7	19,4
ELETTROCHEMIL ROCHE	7,4	5,8	8,7
ELFA	12,7	9,1	20,1
IMMUNODIFF.	24,2	11,1	40,4
IMMUNOTURB.	9,4	3,6	18,7
IMMUNOFLUOR.	8,1	7,3	8,7
IMMUNOT.ROCHE COBAS 6-8000/M	4,6	1,5	10,0
IMMUNOTURB SIEMENS	5,1	3,3	6,9
IMMUNOTURB BECKMAN RF LATEX	5,1	3,5	6,5
IMMUNOTURB. ABBOTT ARCHITEC	3,7	2,4	5,6
IMMUNOTURB. ADVIA SIEMENS	4,4	3,0	6,8
IMMUNOTURB. BECKMAN AU	5,5	3,0	9,2
IMMUNOTURB. BECKMAN LX/CX/D	5,2	1,9	18,0
IMMUNOTURB. I.L./BIOKIT	7,6	3,0	17,6
IMMUNOTURB. INTEGRA	12,9	8,8	17,3
IMMUNOTURB. SCLAVO	11,5	5,1	15,6
IMMUNOTURB.BECKMAN AU	2,7	2,2	3,2
NEFELOMETRICO	6,1	2,2	18,8
NEFELOM. BECKMAN	6,9	1,7	15,0
NEFELOM. BECKMAN IMAGE	4,6	3,5	6,7
NEFELOM. SIEMENS	7,4	4,2	17,0
NEFELOM. SIEMENS BN	8,1	3,4	23,6
NEFELOMETRICO BECKMAN	8,3	3,9	21,1

CV 2015

Analita	Metodo	MaxDiCV
Fattore Reumatoide	AGGLUTINAZIONE	46,0
Alfa 1 Glicoproteina Acida	IMMUNODIFF.	40,4
IgM	IMMUNODIFF.	38,7
IgA	IMMUNODIFF.	28,0
Transferrina	IMMUNODIFF.	27,9
Fattore Reumatoide	IMMUNOTURB.ABBOTT ARCHITECT	27,8
Alfa 1 - Antitripsina	NEFELOMET.- BECKMAN	26,9
IgG	IMMUNODIFF.	26,7
Alfa 2 - Macroglobulina	NEFELOM. SIEMENS BN	23,6
Fattore Reumatoide	IMMUNOTURB SIEMENS	22,6
Ceruloplasmina	NEFELOMETRICO BECKMAN	21,1
IgE	ELFA	20,1
Beta 2 - Microglobulina	ELFA	20,0
PCR	DRY CHEMISTRY	19,4
Fattore Reumatoide	NEFELOMETRICO	18,8
IgM	IMMUNOTURB.	18,7
Beta 2 - Microglobulina	CHEMILUM.	18,2
IgA	NEFEL. BECKMAN IMAGE	18,1
C4	IMMUNOTURB. BECKMAN LX/CX/DX	18,0
IgG	IMMUNOTURB. I.L./BIOKIT	17,6

VEQ Immunoglobuline

Evaluation of Proficiency Survey Results for Serum Immunoglobulins Following the Introduction of a New International Reference Material for Human Serum Proteins, *Thomas B. Ledue,^{1*} A. Myron Johnson,² Linda A. Cohen,¹ and Robert F. Ritchie¹* (¹ Foundation for Blood Research, Scarborough, Maine 04070 and ² University of North Carolina School of Medicine, Chapel Hill, North Carolina; * address for correspondence: 69 U.S. Route 1, P. O. Box 190, Scarborough, ME 04070-0190; fax 207 883-1527; e-mail tledue@fbr.org)

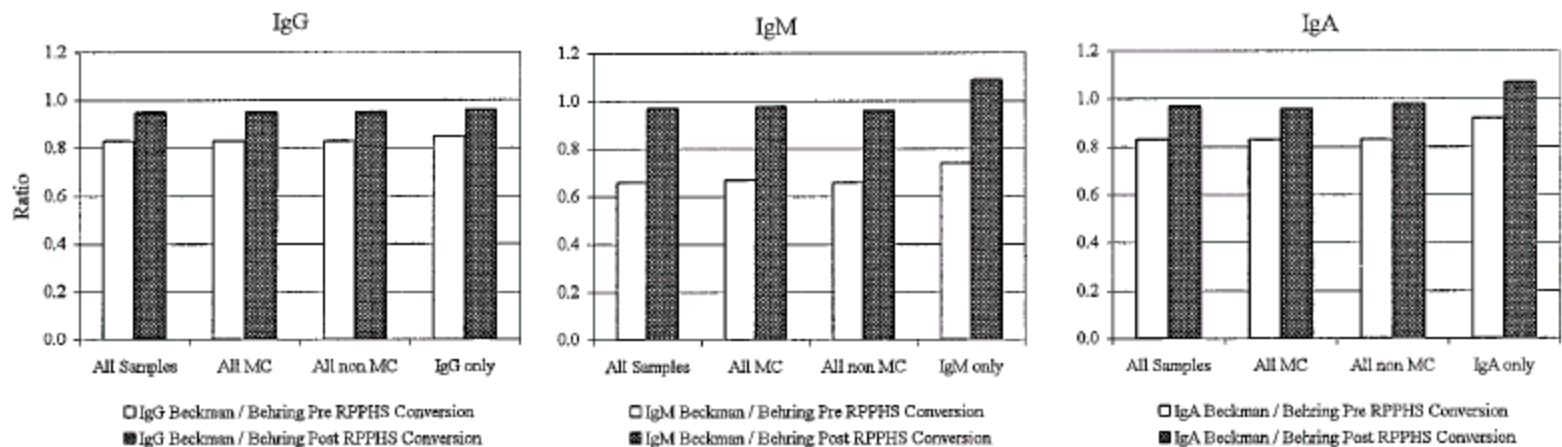
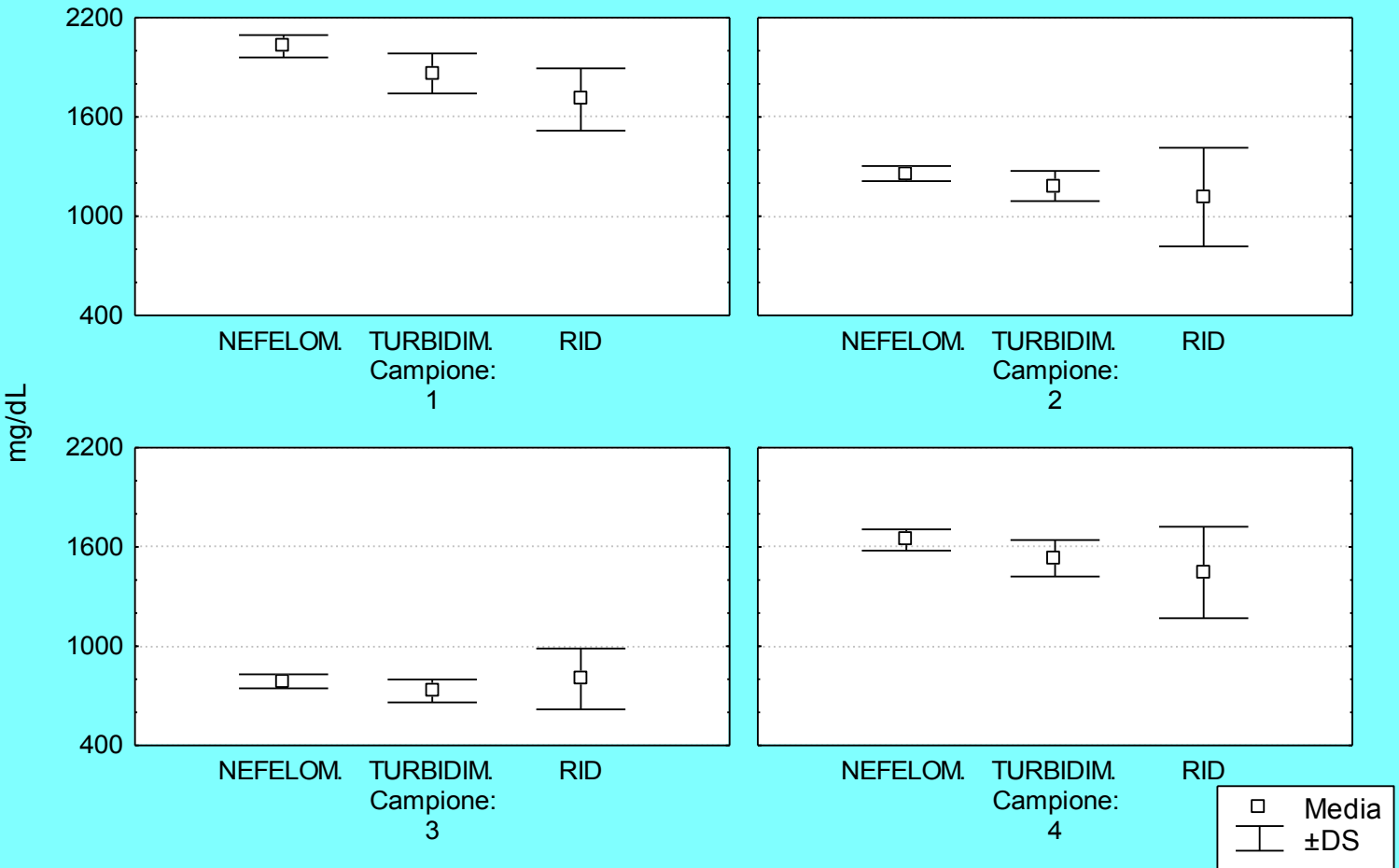
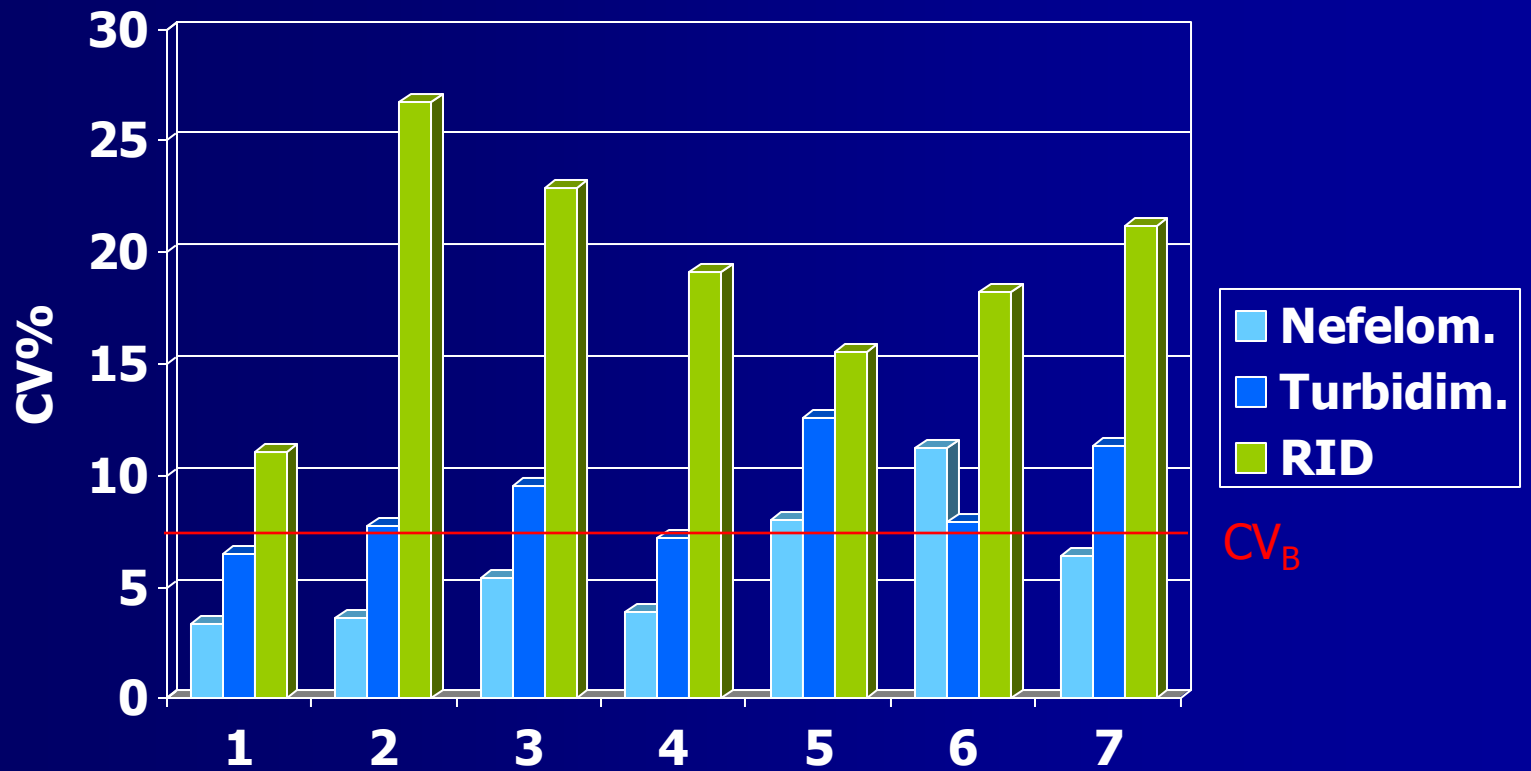


Fig. 1. Average ratios (Beckman to Behring systems) for immunoglobulin results before and after introduction of the new RPPHS. Results were extracted from the CAP Diagnostic Immunology Survey Reports for the period 1992-96. (MC, M-component).

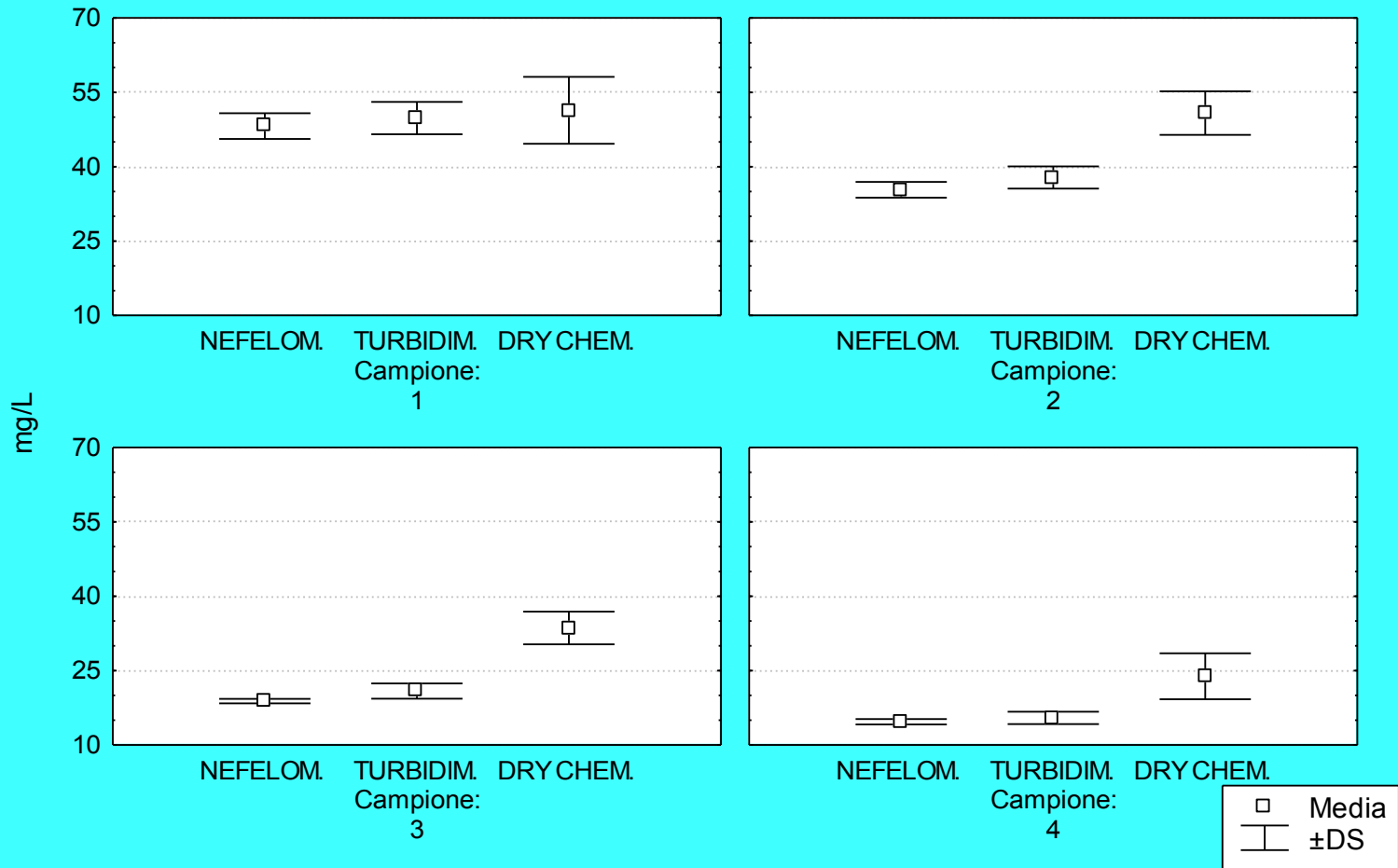
IgG



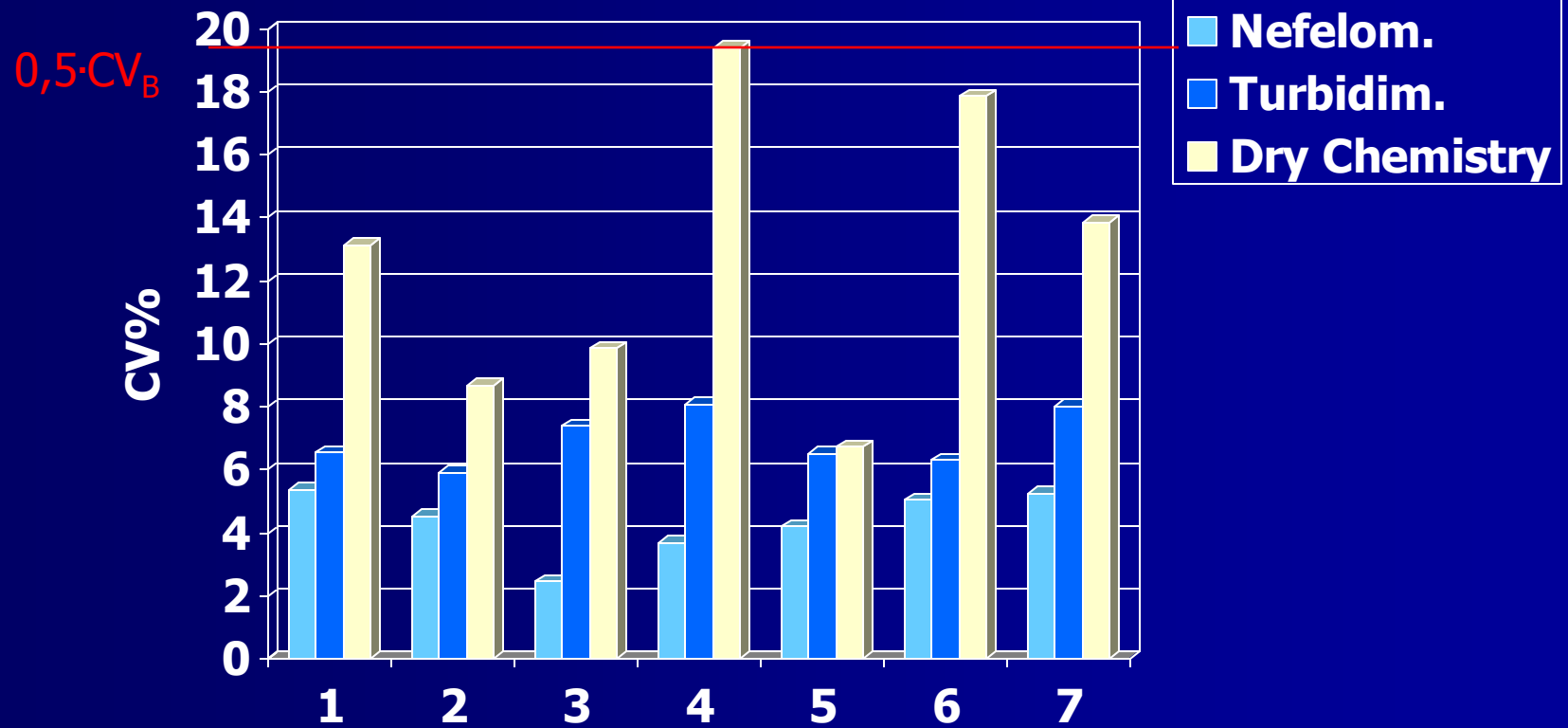
IgG



PCR



PCR



PCR ultrasensibile

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Reproductive Science

Chemistry

Genetics

Haematology

Histopathology

Ultrasensitive C-Reactive Protein (CRP)

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Analytes or Clinical Applications Covered

Prognostic indicator of cardiovascular disease and risk assessment for coronary artery disease. Monitoring of the acute phase response in neonates



Clinical Chemistry 47:3
403–411 (2001)

Review

High-Sensitivity C-Reactive Protein: A Novel and Promising Marker of Coronary Heart Disease

NADER RIFAI^{1,2,4*} and PAUL M. RIDKER^{2,3,5}

Table 1. RR estimates for future coronary events in men and women associated with quintiles of hs-CRP and TC:HDL-C ratio.^a

Quintile of TC:HDL-C ratio	Men	Women	Quintile of hs-CRP, mg/L				
			1 (<0.7)	2 (0.7–1.1)	3 (1.2–1.9)	4 (2.0–3.8)	5 (3.9–15.0)
1	<3.4	<3.4	1	1.2	1.4	1.7	2.2
2	3.4–4.0	3.4–4.1	1.4	1.7	2.1	2.5	3
3	4.1–4.7	4.2–4.7	2	2.5	2.9	3.5	4.2
4	4.8–5.5	4.8–5.8	2.9	3.5	4.2	5.1	6
5	>5.5	>5.8	4.2	5	6	7.2	8.7

^a RR estimates and TC:HDL-C ratio were derived from the PHS (23) and the WHS (24) databases. hs-CRP concentrations were derived from ongoing population-based surveys.

Fattore Reumatoide

Analita: *Fattore Reumatoide* UI/mL

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	254	3	44.53	24.3	10.8	46.50
Tuo Metodo	10	0	18.65	8.0	1.5	18.40

Campione	4 (Scad. 22/05/2015)
Tuo risultato	19.3

	Diff. S	Diff. %
Tutti	-2.33	-56.66
Tuo Metodo	0.43	3.49

Campioni							
1	2	3	4	5	6	7	8
		○	○				

○ = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati numerici	254
N. risultati semiquantitativi/qualitativi	7

Riepilogo x Metodo risultati numerici (> 7 Centri)							
Metodo	N.	Out	M.	C.V.	Pos	Ne	Du
IMMUNOTURB.	73	3	48.40	13.2	1		
IMMUNOT.ROCHE COBAS 6-6000/MODULAR	45	2	46.57	3.3			
IMMUNOTURB.ABBOTT ARCHITECT	29	1	43.43	8.5	1		
IMMUNOTURB BECKMAN RF LATEX	22	1	47.35	5.4			
IMMUNOTURB. I.L./BIOKIT	14	0	48.11	10.9	1		
NEFELOMETRICO	14	0	18.64	11.0			
IMMUNOTURB SIEMENS	13	0	54.90	9.6			
AGGLUTINAZIONE	12	0	37.63	41.8	1		
NEFELOM. SIEMENS BN	10	0	18.65	8.0			
NEFELOMETRICO/BECKMAN	10	0	48.80	9.9			

Tuo Metodo
NEFELOM. SIEMENS BN

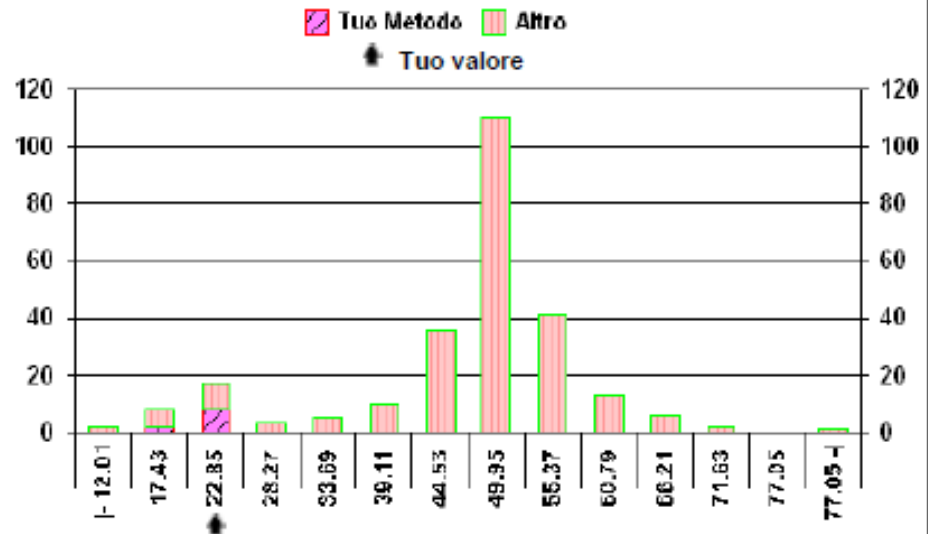
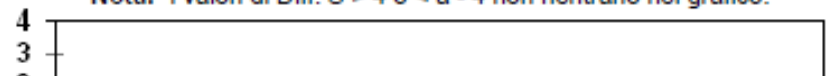


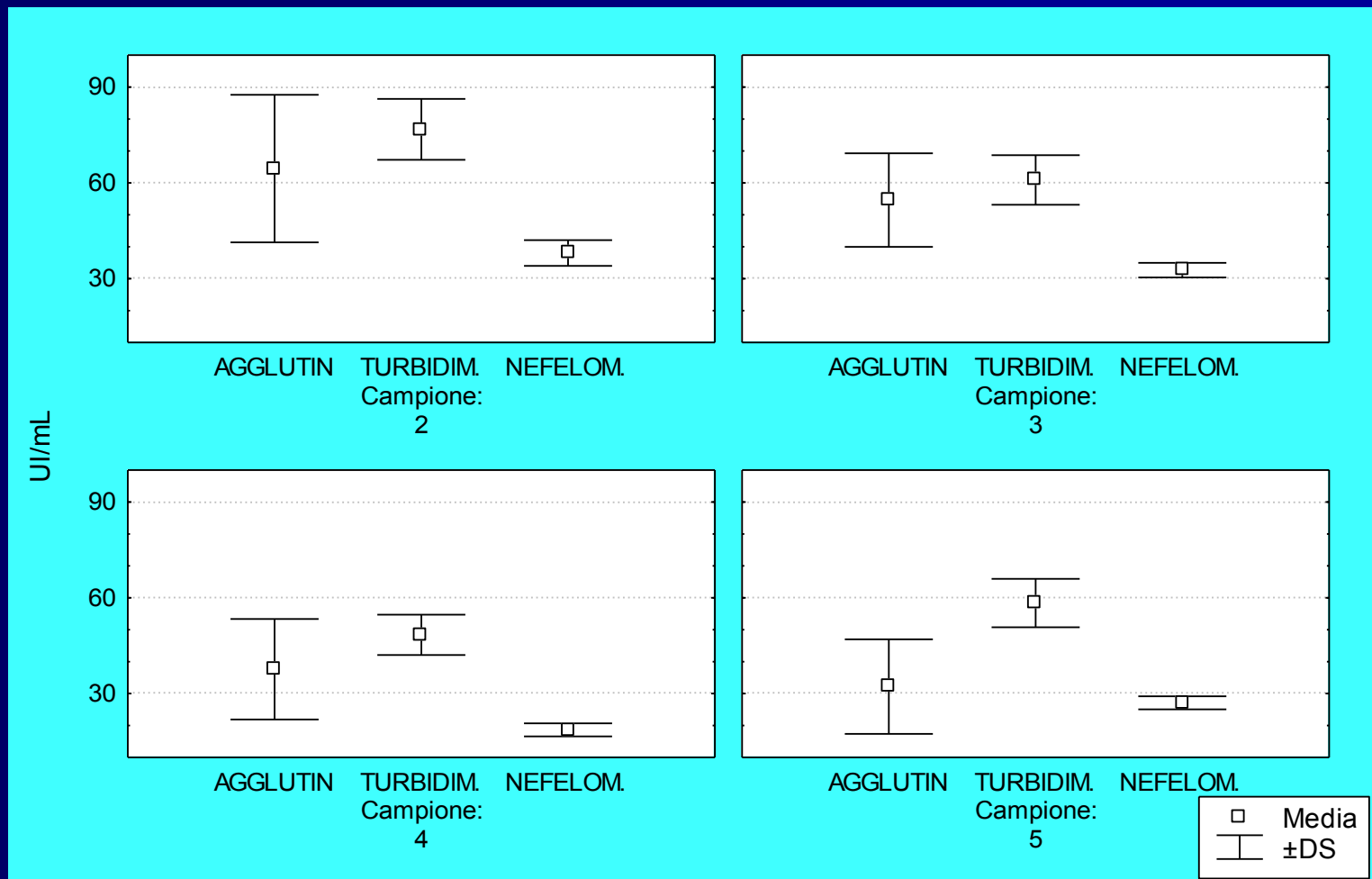
Grafico dati semiquantitativi/qualitativi	Tuo Metodo
+++	0/1
+-	0/1
DUBBIO	0/1
NEGATIVO	0/1
POSITIVO	0/2
< 64.0	0/1

◆ Tuo Metodo ○ Tutti

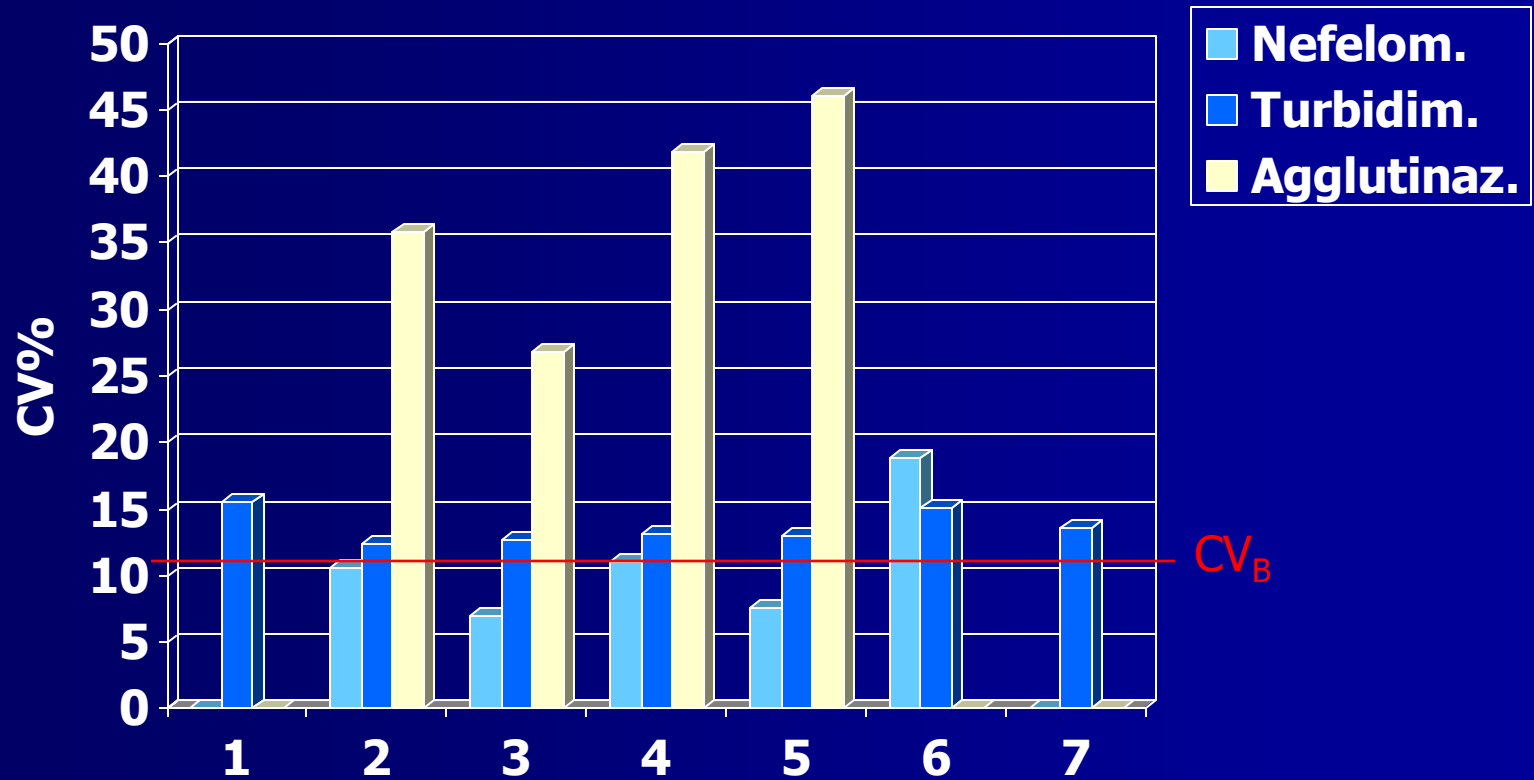
Nota: I valori di Diff. S > 4 o < -4 non rientrano nel grafico.



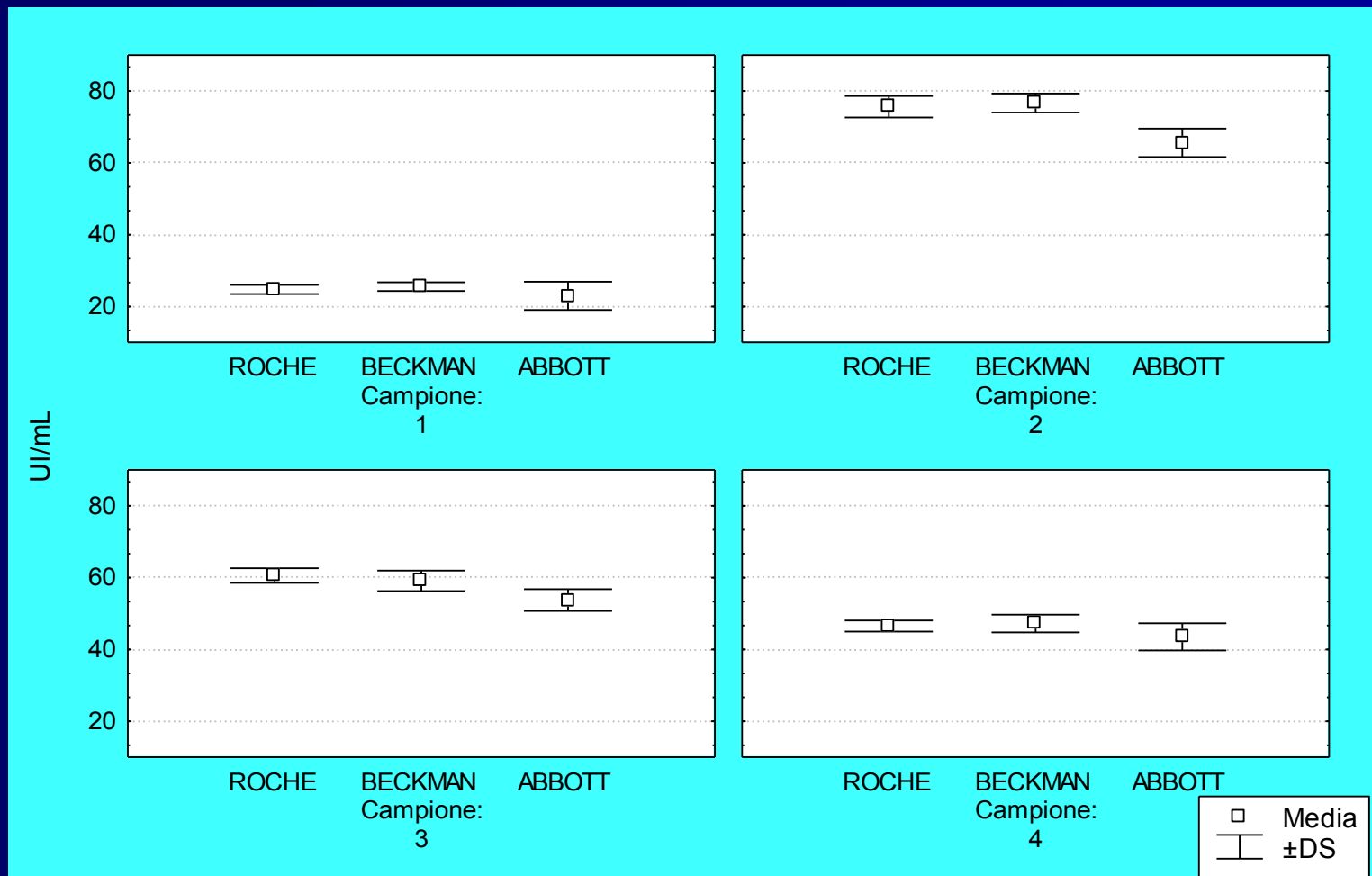
Fattore Reumatoide



Fattore Reumatoide



Fattore Reumatoide (Turbidim.)



Fattore Reumatoide

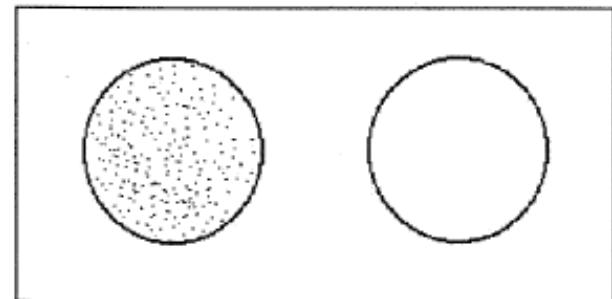
- Standardizzazione non ERM-DA470
- Non dosabile come IgM in unità di massa
- Persistenza di metodi qualitativi - semiquantitativi

Semi-quantitative Test:

The titer of the serum is the reciprocal of the highest dilution, which exhibits a positive reaction.

An estimate of the RF concentration in the specimen can be expressed in IU/ml by using the following equation:

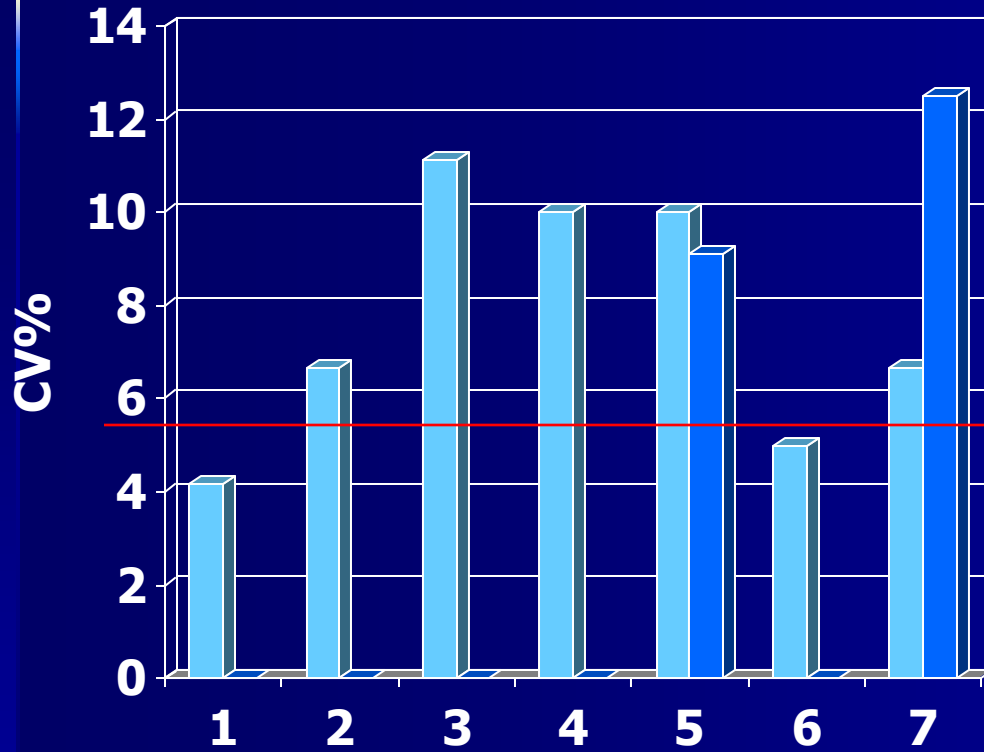
$$\text{IU/ml of specimen} = \text{IU/ml control} \times \text{specimen titer}$$



Positive

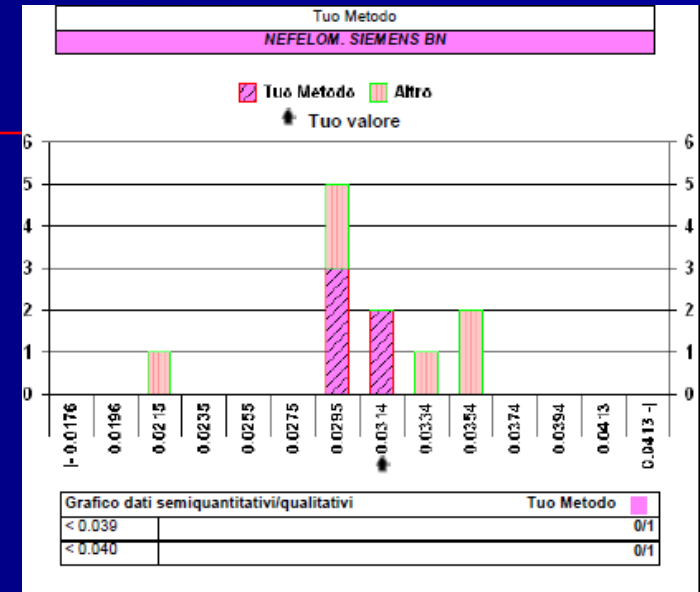
Negative

Esordienti

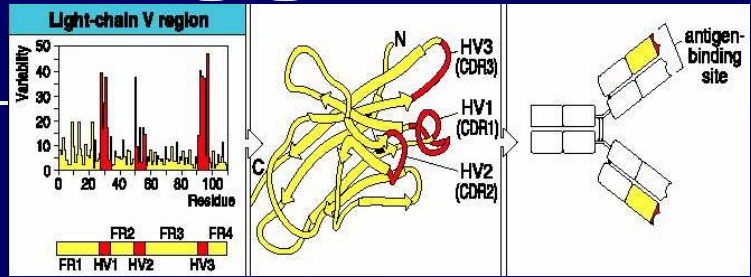


NEFELOMETRICO SIEMENS

■ Recettore Solubile
 Transferrina
■ Cistatina C



Leggere le catene leggere



CATENE LEGGERE

LEGATE
K(2-4)g/L
 λ (1-2,4)g/L

LIBERE
K(3-19)mg/L
 λ (6-26)mg/L

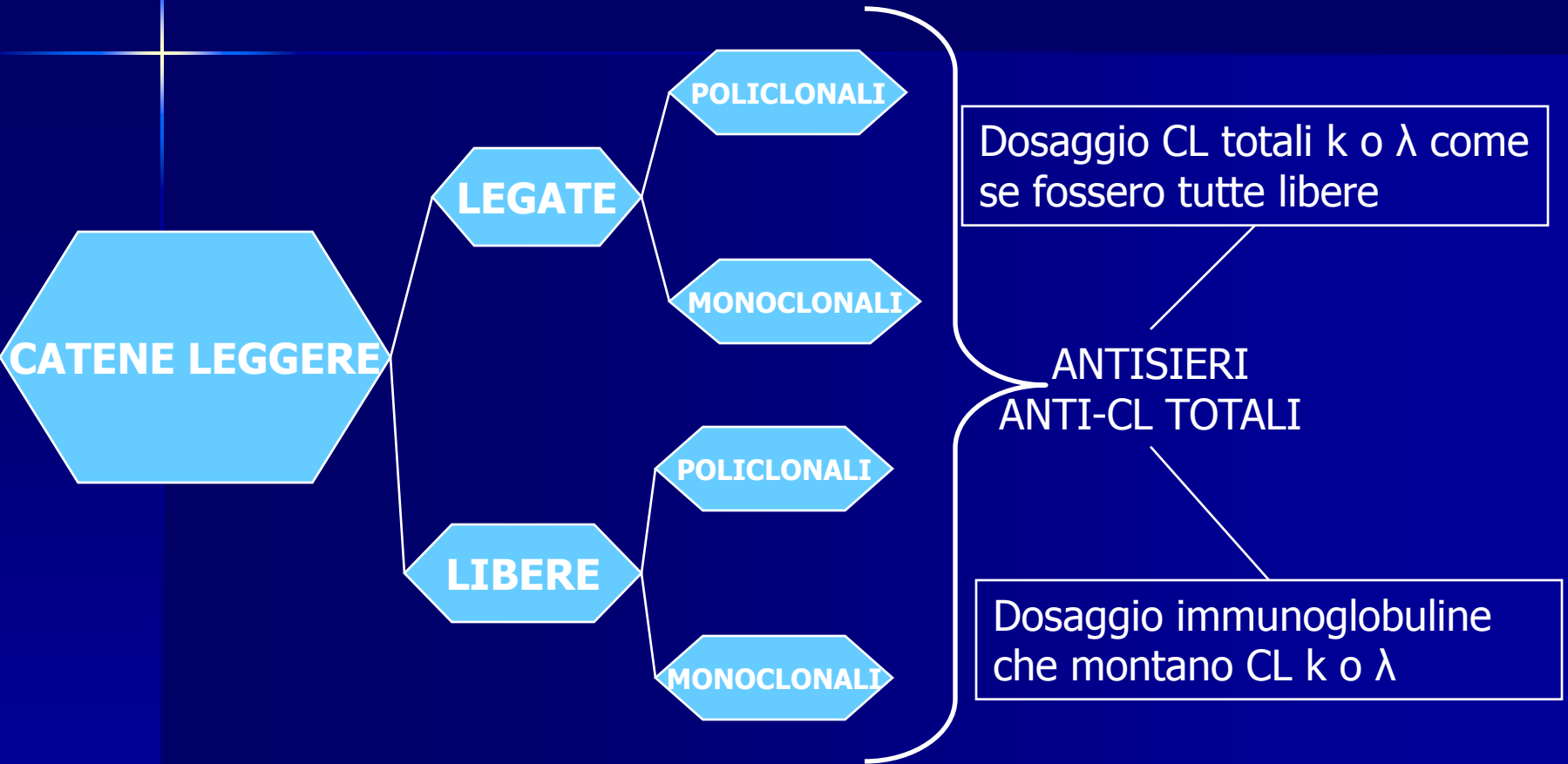
POLICLONALI
MONOCLONALI

POLICLONALI
MONOCLONALI

ANTISIERI
ANTI-CL TOTALI

ANTISIERI
ANTI-CL LIBERE

Leggere le catene leggere



$$\text{Fattore di conversione} = 150 (\text{PM}_{\text{IgG}}) / 46 (2 \times \text{PM}_{\text{CL}}) \approx 3$$

Leggere le catene leggere

Analita: **Kappa - Catena Leggera** mg/dL

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	56	1	336.7	6.8	22.9	333.0
Tuo Metodo	15	1	328.1	3.6	11.7	327.0

Campione	2 (Scad. 25/02/2005)					
Tuo risultato	324					

	Diff. S	Diff. %
Tutti	-0.55	-3.76
Tuo Metodo	-0.35	-1.25

Campioni			
1	2	3	4
0	0		

0 = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati quantitativi	56
N. risultati semiquantitativi/qualitativi	1

Riepilogo x Metodo risultati quantitativi (> 7 Centri)				
Metodo	N.	Out	M.	C.V.
NEFELOMETRICO	37	0	339.5	7.8
IMMUNOTURB.	15	1	328.1	3.6

Analita: **Kappa - Cat.Leg. Rif. alle IgG** mg/dL

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	22	0	1128.00	9.7	109.5	1140.00
Tuo Metodo	20	0	1137.60	9.6	108.8	1145.00

Campione	2 (Scad. 25/02/2005)					
Tuo risultato	1140					

	Diff. S	Diff. %
Tutti	0.11	1.06
Tuo Metodo	0.02	0.21

Campioni			
1	2	3	4
0	0		

0 = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati quantitativi	22
N. risultati semiquantitativi/qualitativi	

Riepilogo x Metodo risultati quantitativi (> 7 Centri)				
Metodo	N.	Out	M.	C.V.
NEFELOMETRICO	20	0	1137.60	9.6

Tuo Metodo
IMMUNOTURB.

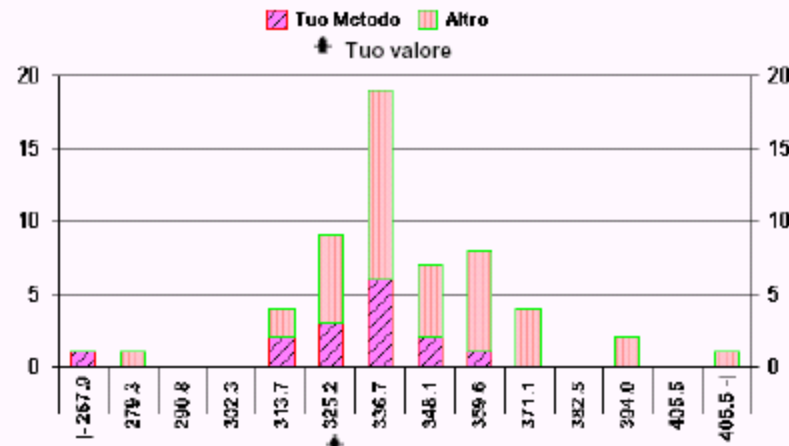
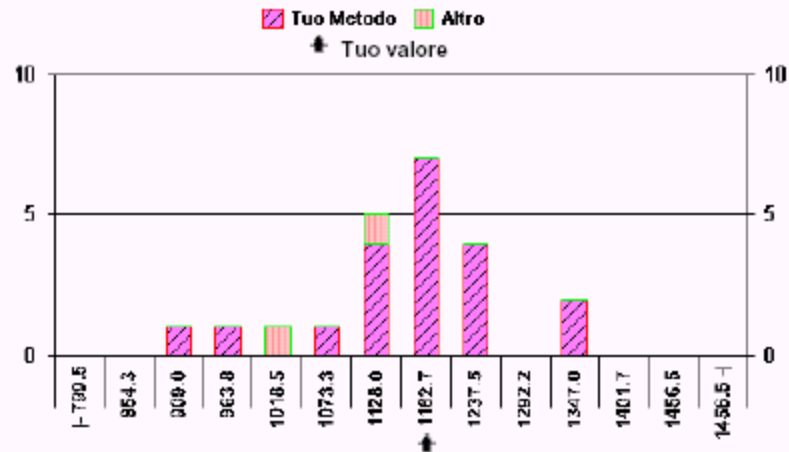
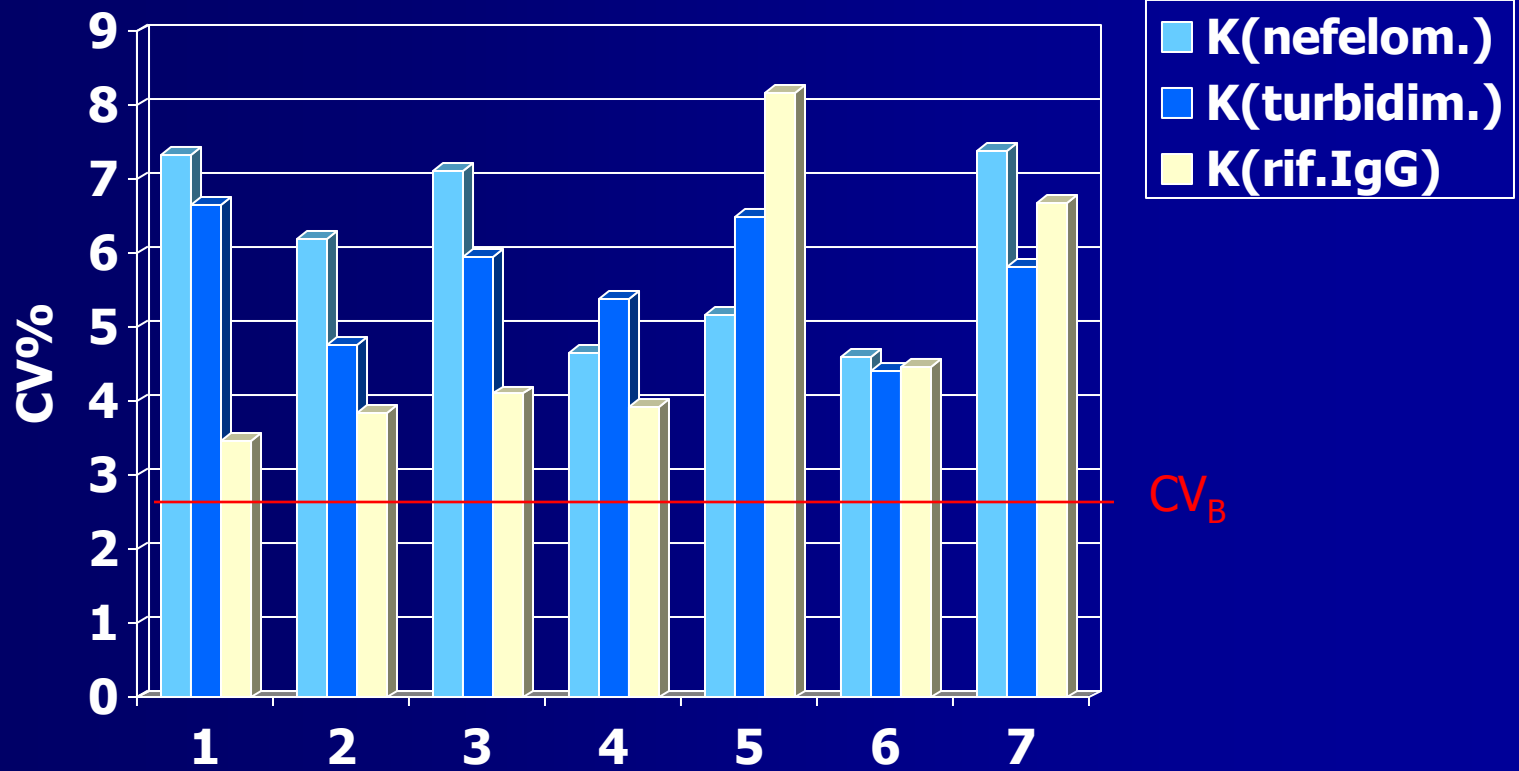


Grafico dati semiquantitativi/qualitativi
< 10 1

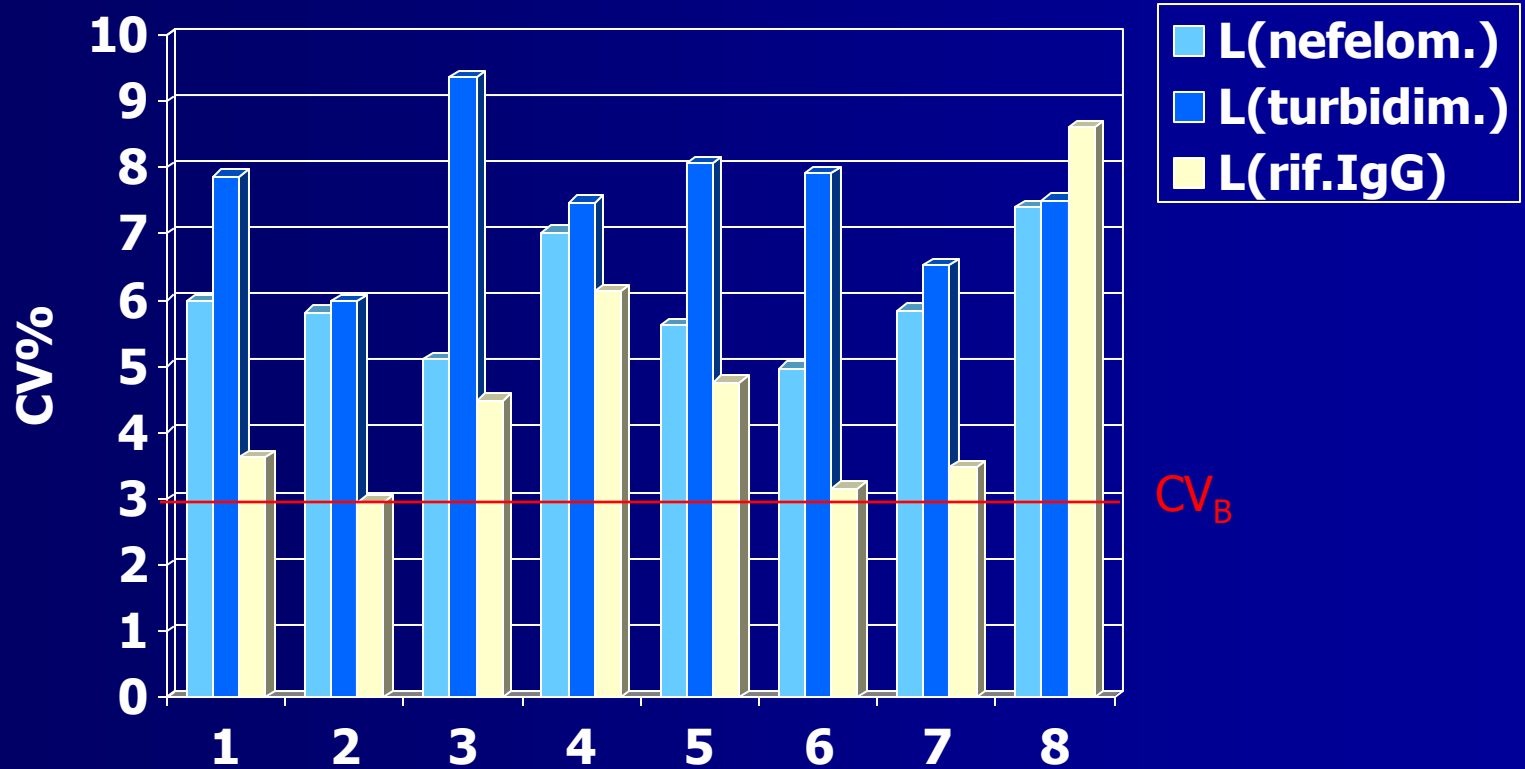
Tuo Metodo
NEFELOMETRICO



Kappa catena leggera



Lambda catena leggera



CL totali

As might be expected the values obtained from the different approaches for the certification of light chains differ markedly. For both K and L pooled monoclonal light chains provided a higher value than light chains derived from polyclonal immunoglobulins. Values in both cases differed significantly from those obtained by calculation (14) and values obtained by radial immunodiffusion differed from those of nephelometry and turbidimetry. All three sets of light chain values are incompatible with the total amount of light chains theoretically present on the basis of the ascribed values for IgG, IgA and IgM. We conclude that purified light chains, whether monoclonal or polyclonal, cannot be used to assign values for light chains bound to immunoglobulins accurately.

Dosaggio CL totali. Utilità clinica:

- Rapporto k/λ come indizio di monoclonalità
- Parametro di follow-up per CM già tipizzate

Pure & Appl. Chem., Vol. 68, No. 10, pp. 1851–1856, 1996.
Printed in Great Britain.
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CLIN. CHEM. 37/11, 1917–1921 (1991)

Use of Immunoglobulin Heavy-Chain and Light-Chain Measurements in a Multicenter Trial to Investigate Monoclonal Components: I. Detection

R. G. Jones, F. Aguzzi,¹ J. Bienvenu,² P. Bianchi,¹ C. Gasparro,¹ M. R. Bergami,¹ A. Perinet,² H. Bernon,² G. M. Penn,³ I. Keller,⁴ and J. T. Whicher

We assessed the combined use of serum protein electrophoresis (SPE) and nephelometric measurement of immunoglobulin heavy- and light-chain components for detecting serum monoclonal immunoglobulins (monoclonal components, MC) in 4788 unselected samples from 4173 patients. MC were detected in 514 samples from 390 patients. In 356 these were detected by SPE; the other 34 had a normal SPE pattern but an abnormal kappa:lambda light-chain ratio (KLR). Only 208 of the 356 (58%) samples with bands by SPE had abnormal KLRs. Samples with MC concentrations >5 g/L had a higher proportion of abnormal KLRs (75%) than those with concentrations <5 g/L (42%). The KLR was abnormal in 13% of samples in which no MC were visible by SPE or immunofixation electrophoresis (IFE). Compared with quantitative measurements of immunoglobulin heavy and light chains, high-quality SPE remains the method of choice for the detection of MC. Quantitative methods, however, are able to detect additional MC, especially those containing free light chains, and in the absence of SPE and IFE will detect about 75% of MC present at >5 g/L.

Highly Sensitive, Automated Immunoassay for Immunoglobulin Free Light Chains in Serum and Urine

ARTHUR R. BRADWELL,^{1*} HUGH D. CARR-SMITH,² GRAHAM P. MEAD,² LIAN X. TANG,²
PAUL J. SHOWELL,² MARK T. DRAYSON,¹ and ROGER DREW²

Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance

S. Vincent Rajkumar, Robert A. Kyle, Terry M. Therneau, L. Joseph Melton III, Arthur R. Bradwell, Raynell J. Clark, Dirk R. Larson, Matthew F. Plevak, Angela Dispenzieri, and Jerry A. Katzmann

We hypothesized that the presence of monoclonal free kappa or lambda immunoglobulin light chains in monoclonal gammopathy of undetermined significance (MGUS), as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy. Of 1384 patients with MGUS from Southeastern Minnesota seen at the Mayo Clinic from 1960 to 1994, baseline serum samples obtained within 30 days of diagnosis were available in 1148. At a median

follow-up of 15 years, malignant progression had occurred in 87 (7.6%) patients. An abnormal FLC ratio (kappa-lambda ratio < 0.26 or > 1.65) was detected in 379 (33%) patients. The risk of progression in patients with an abnormal FLC ratio was significantly higher compared with patients with a normal ratio (hazard ratio, 3.5; 95% confidence interval [CI], 2.3-5.5; $P < .001$) and was independent of the size and type of the serum monoclonal (M) protein. Patients with an abnormal

serum FLC ratio, non-immunoglobulin G (non-IgG) MGUS, and a high serum M protein level (≥ 15 g/L) had a risk of progression at 20 years of 58% (high-risk MGUS) versus 37% with any 2 of these risk factors (high-intermediate risk), 21% with one risk factor (low-intermediate risk), and 5% when none of the risk factors were present (low risk). (*Blood*. 2005; 106:812-817)

CL libere

Freelite™ & Hevylite™



Freelite™ Serum Free Light Chain Assays

Freelite is a major breakthrough for the detection and monitoring of Multiple Myeloma (MM) and other B-cell dyscrasia. There is now significant clinical evidence indicating the benefit of serum free light chain assays in initial screening for monoclonal gammopathies, identifying AL amyloidosis and Nonsecretory MM patients missed by conventional electrophoretic methods, as a prognostic indicator for progression in myeloma*, for risk stratification of MGUS patients* and rapid evaluation of treatment efficacy.

Freelite assays were developed by [Binding Site](#) to measure free lambda and free kappa immunoglobulin light chains. Our expertise in the manufacture of antibodies has enabled us to provide a quantifiable, highly specific, automatable free light chain assay for serum.

Review the [clinical evidence](#) for the utility of **Freelite** in detecting and monitoring plasma cell disorders and compare the results from [laboratory protocols](#) with and without **Freelite**.

* In the USA diagnostic use of this product is restricted to those stated in the product insert

Hevylite™ - See what the **Hevylite** ratio offers you.

Latest News 09 April 2009

Binding Site sponsors Free Light Chain Disease workshop at Euromedlab 2009

A panel of eminent speakers from Austria, USA and the UK will discuss recent advances in the management of free light chain disease at an Industry Sponsored Workshop as part of the scientific program at the Euromedlab conference in Innsbruck this year. The workshop includes a review of clinical applications of serum free light



This site is for Healthcare Professionals only

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Still requesting urine?

Latest News Headlines

- Binding Site sponsors Free Light Chain Disease workshop at Euromedlab 2009
09 April 2009
- Further study indicates only serum measurements needed for MM diagnosis or exclusion
10 February 2009
- Publication of largest Light Chain Escape – Multiple Myeloma Study
24 January 2009

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Keyword

4.3. Comparison of free light chain immunoassays on different instruments

sFLC kits are made for many laboratory instruments. A complete list of those currently available is given in Chapter 27. Comparison of FLC results using a Hitachi 911 and a Siemens Dade Behring BNII are shown in Figure 4.15 and the characteristics of four different instruments are shown in Table 4.4. Comparisons of normal ranges are discussed in Chapter 5. There are no significant differences between the results on different instruments [38].

	Siemens BN™II	Beckman Coulter IMMAGE®	Roche Cobas Integra®	Binding Site SPAPLUS
Sensitivity (mg/L)	κ 0.30 (1/5): λ 0.25 (1/5)	κ 3.0 (1/5): λ 2.4 (1/5)	κ 0.6 (neat): λ 1.3 (neat)	κ 0.4 (neat): λ 0.5 (neat)
Precision (Intra-)	3.1-8.4%	2.0-8.1%	0.7-5.8%	1.6-3.4%
(CV) (Inter-)	4.7-8.4%	5.8-11.7%	0.7-2.7%	0.0-4.2%
Antigen excess	Good	Good	Good	Good
Analytical time	18 minutes	10 minutes	10 minutes	15 minutes
Time to run 20 normal/20 MM samples	52/127 minutes	40/84 minutes	33/75 minutes	33/68 minutes
Higher dilutions	Automatic	Off-line if high	Automatic & off-line if very high	Automatic & off-line if very high
Utility	Closed systems	Open system	Closed system, FLC channels on standard menu	Closed system

(C.V. = coefficient of variation in precision studies. Numbers in brackets refer to sample dilutions). See Chapter 27 for more details.

CL libere: discrepanza tra letteratura e pratica

*Conoscere e capire
il dosaggio delle
Catene Leggere
Libere nel Siero*



International Myeloma Foundation
12650 Riverside Drive, Suite 206
North Hollywood, CA 91607 USA

Telephone:

800-452-CURE (2873)

(USA & Canada)

818-487-7455

Fax: 818-487-7454

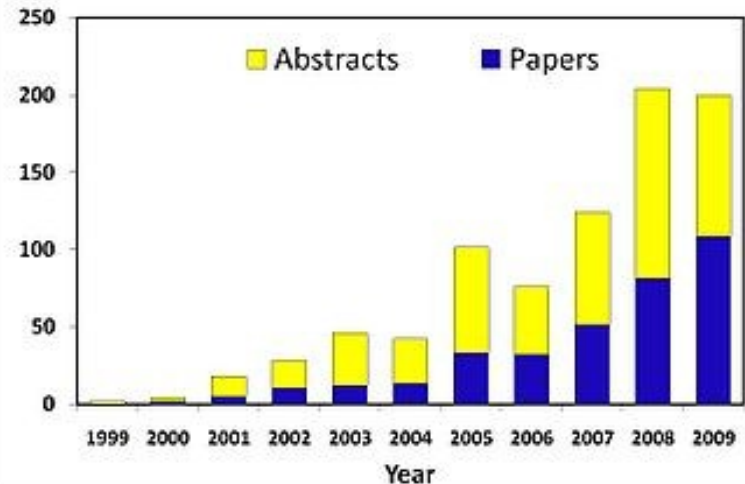
TheIMF@myeloma.org

www.myeloma.org



10/07

Serum free light chains publications by year



Nonostante il crescente numero di pubblicazioni in materia e l'adattabilità del metodo sui principali strumenti, la maggior parte dei laboratori ignora il dosaggio delle CLL nel siero continuando ad utilizzare il dosaggio di CL totali, equivoco e destinato ad obsolescenza.

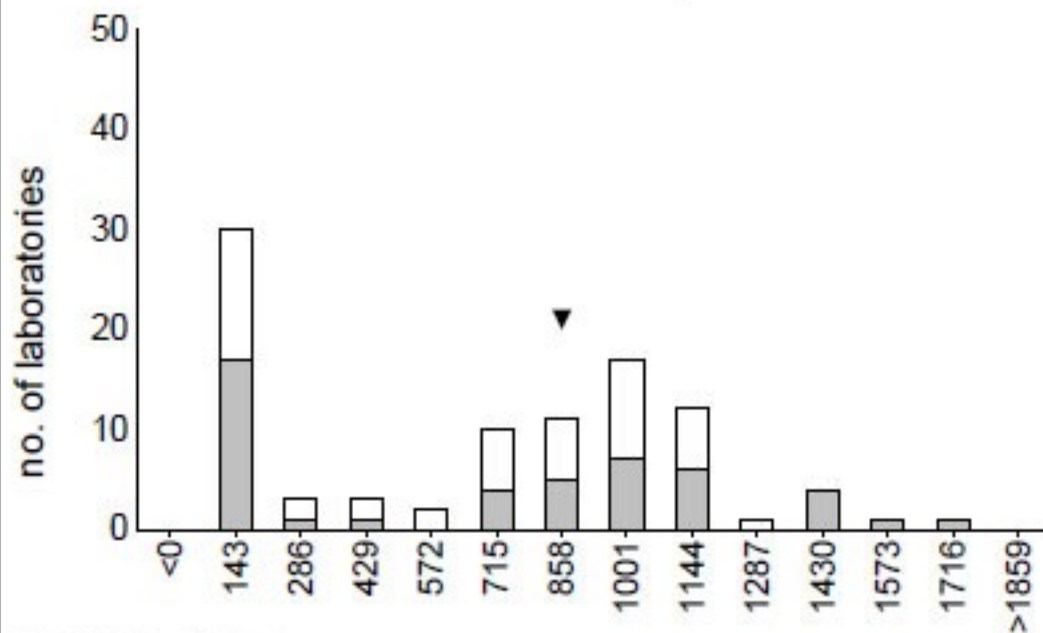


Monoclonal Protein Identification

Reproductive

- All Analysers
- Your result
- Your Analyser (Dade Behring) %CV= 81.05

sFLC data determined using Freelite™

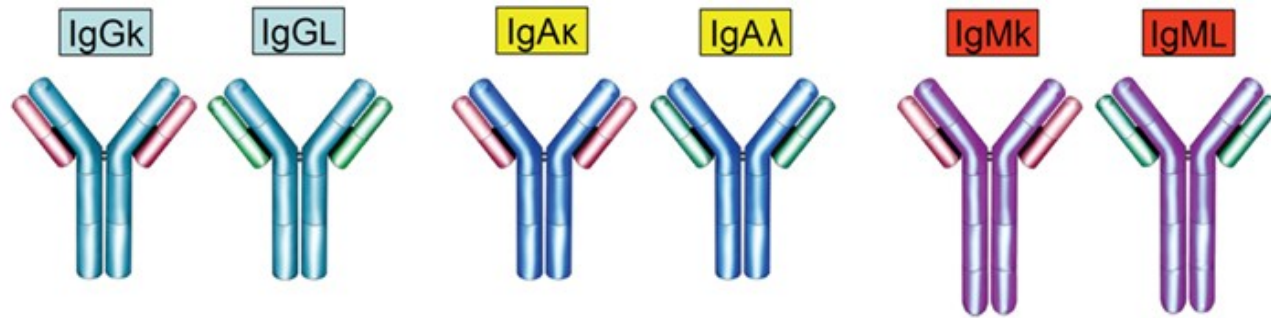
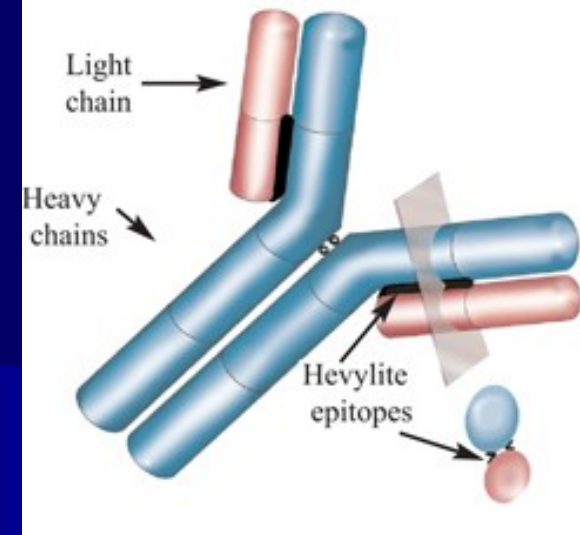


CL libere:
VEQ

Variabilità fra
strumenti

Problema dell'eccesso
di antigeni

Coming soon: Hevylite



Analysis of immunoglobulin heavy chain/light chain (HLC) pairs - Hevylite™

Hevylite (HLC), a new assay to be launched by Binding Site in 2009, promises to transform the way patients with B cell dyscrasia are managed, just as the **Freelite** assay has done.

In summary **Hevylite** assays offer:

- ⇒ higher sensitivity than serum protein electrophoresis for quantifying monoclonal immunoglobulins
- ⇒ a numerical result for patients at a sensitivity as high as or better than immunofixation electrophoresis
- ⇒ clinical value when monitoring patients with monoclonal gammopathies
- ⇒ HLC ratios which have a greater range of changes than monoclonal immunoglobulin measurements because the non-tumour immunoglobulin allows assessment of immunosuppression
- ⇒ HLC ratios which are not affected by changes in blood volume, haematocrit and variable metabolism (via FcRn receptors for IgG) that affect current assays for serum immunoglobulins
- ⇒ HLC ratios that provide information about the tumour selective killing rate versus non-tumour plasma cell kill rates. The assessment of selective tumour killing rates may help with decision making regarding effective chemotherapies



Proteine specifiche: conclusioni

Variabilità inter- e intra-metodo:

- Limiti di standardizzazione
- Metodi manuali

Vecchie e nuove proteine:

- ✓ Nuove esigenze cliniche
- ✓ Svecchiamento del repertorio

