Challenges in methods of creatinine measurement

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This Talk

- Creatinine methods
- Development History

  Jaffe : end point Jaffe, Jaffe-kinatic, compensated &

  Enzymatic

  Dry chemistry
- Problems

  Specificity

  Analytical performance

Jaffe Reaction

$$\text{Creatinine + picric acid} \xrightarrow{\textit{alkaline pH}} \text{Orange-red (Janovski) complex}$$

Read @ 510 nm

- **Jaffe end point**
- **Jaffe Kinetic**

  - Prone to "protein error"
  - Negative interference from Bilirubin
  - Keto Acids, Glucose and other metabolites
  - Drugs

R Vaishya, Indian Journal of Clinical Biochemistry, 2010 / 25 (1) 64-66

Table 1. Substances added to serum (70 µmol/L creatinine) to test for interference with Roche Jaffe and enzymatic creatinine assays on an Hitachi 917 analyser (data from authors' laboratory).

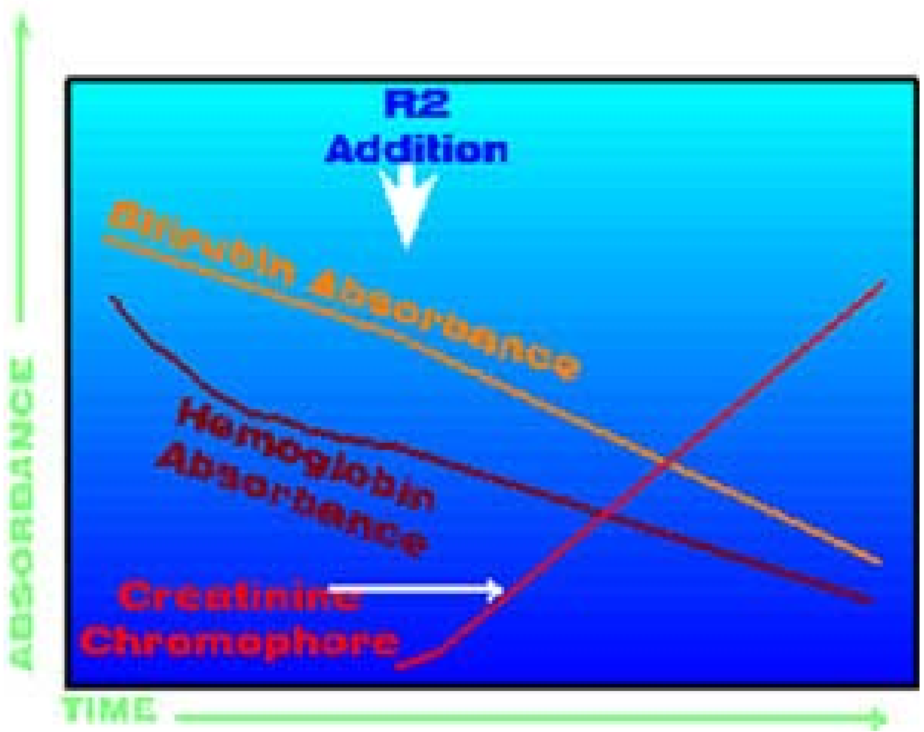
Interferent	Concentration	Measured Bias (µmol/L)	
		Jaffe Rate Blank Method	Enzymatic Method
Ascorbate	10 mmol/L	+ 37	- 13
Pyruvate	2 mmol/L	+ 31	n.s.
Albumin	40 g/L	+ 21	n.s.
Glucose	50 mmol/L	+ 19	n.s.
Creatine	2 mmol/L	+ 16	n.s.
Bilirubin ditaurate	500 µmol/L	- 18	-11
Haemoglobin F	10 g/L	- 66	n.s.
Haemoglobin adult	10 g/L	n.s.	n.s.
Acetoacetate	2 mmol/L	n.s.	n.s.
Intralipid	12 g/L triglyceride	n.s.	n.s.
Dopamine	1 mmol/L	- 15	- 60
Cephalosporins		Variable (see below)	
Cefpirome	1 mmol/L	+ 213	n.s.
Cefoxitin	1 mmol/L	+ 47	n.s.
Cephalothin	1 mmol/L	+ 24	n.s.
Cephazolin	1 mmol/L	+ 20	n.s.
Ceftriaxone	1 mmol/L	n.s.	n.s.
Ceftazidime	1 mmol/L	n.s.	n.s.
Cefotaxime	1 mmol/L	n.s.	n.s.

n.s.= not significant

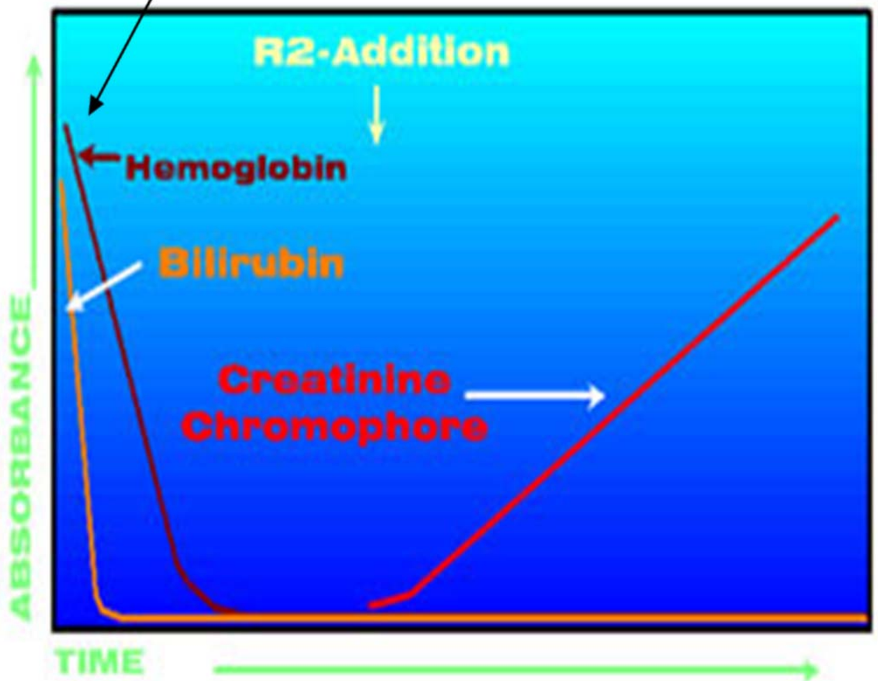
Jaffé Reaction – Modifications
O'Leary + Kinetic

❑ Kinetic........Interference

- Rapid – e.g. acetoacetate – within 20 secs
- Slow – e.g. protein – 80 – 100 secs
- Measure absorbance change between 20 and 80 secs



Potassium ferricyanide oxidises bilirubin to biliverdin and Hb to metHb



Jaffé Reaction - Modifications

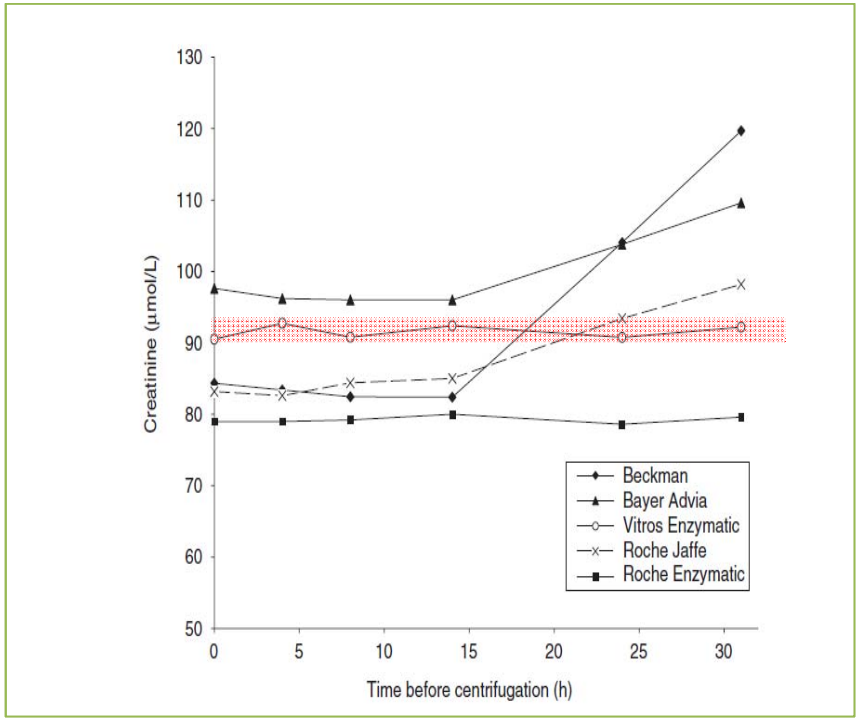
- **Compensated**
  - Interference
    - Protein – Positive difference of about 27 µmol/L
    - adjust calibrator set point to take account of pseudo-creatinine contribution of protein

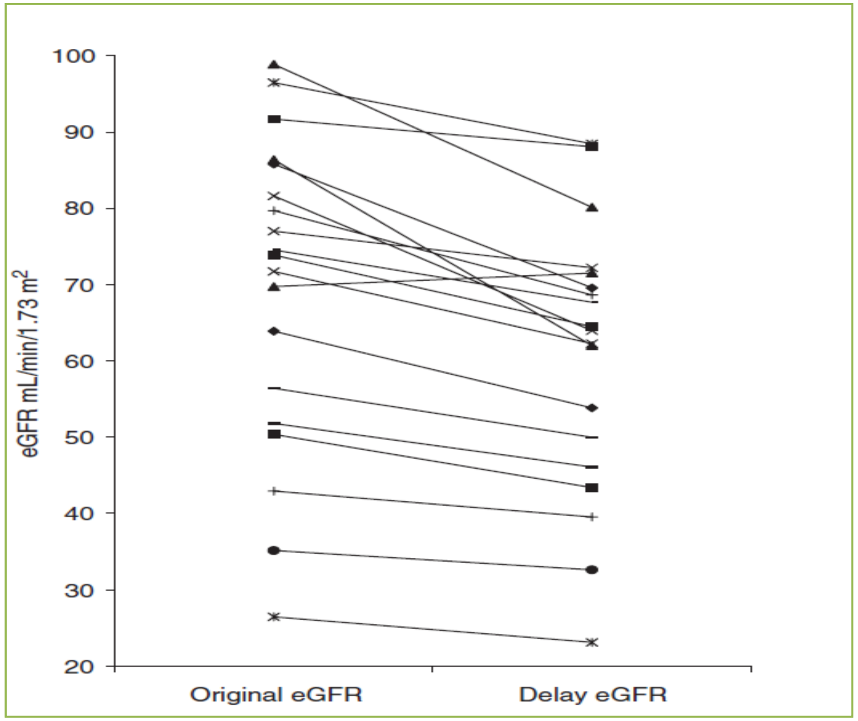
- **Enzymatic methods**
    - 2008: EU-wide study …17% users
    - 2017: EU –wide study …..23% users

Stability of creatinine with delayed separation of whole blood and implications for eGFR

Impact of separation delay on creatinine

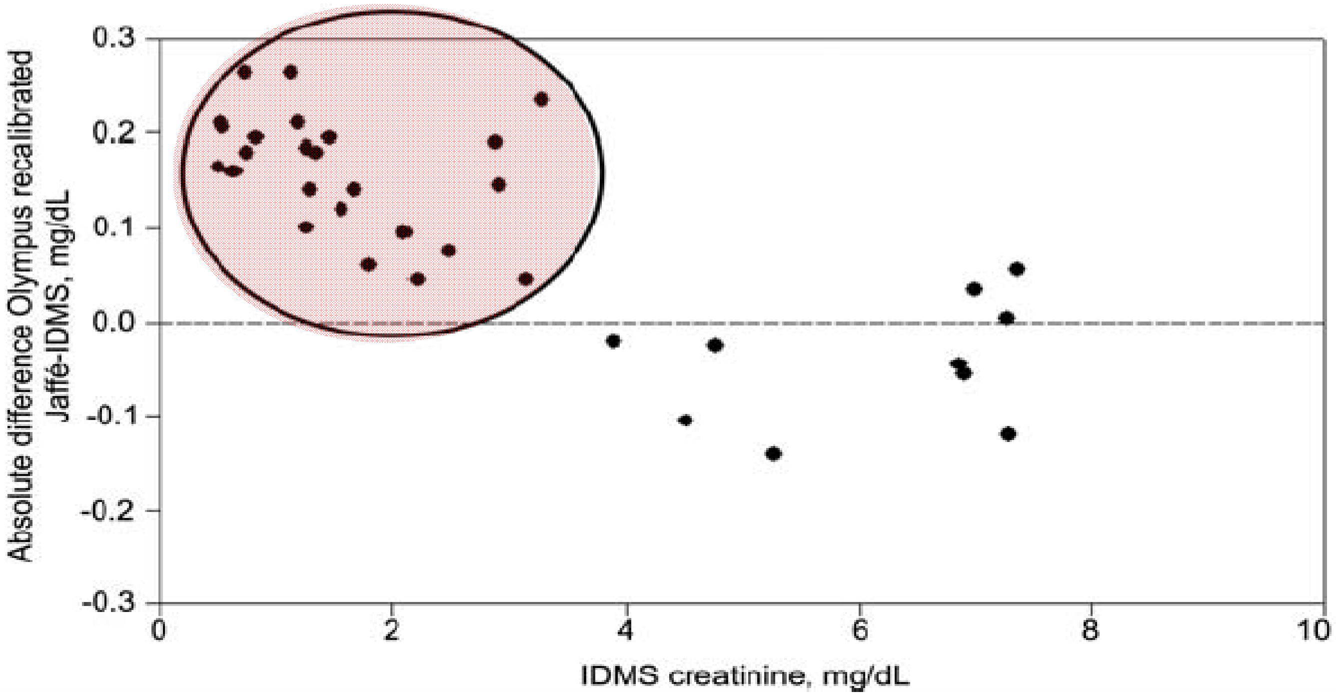
Impact of separation delay on eGFR





John Shepherd. Ann Clin Biochem. 2007 Jul;44(Pt 4):384-7

- The lack of specificity is a major problem for Jaffe methods
- Standardization does not correct for analytical non-specificity problems

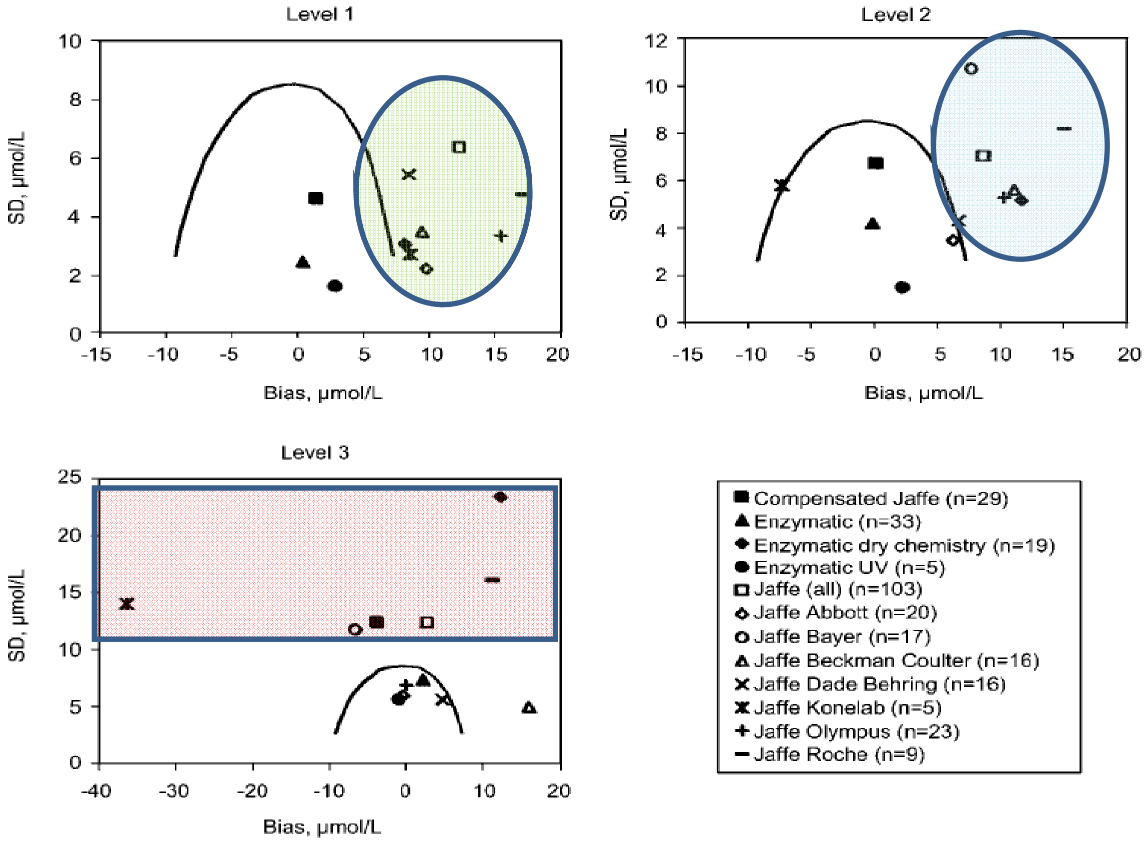


Intercept of 18 µmol/L Or 0.204 mg/dl

Mauro Panteghini*, Enzymatic assays for creatinine: Time for action Clin Chem Lab Med 2008;46(4)

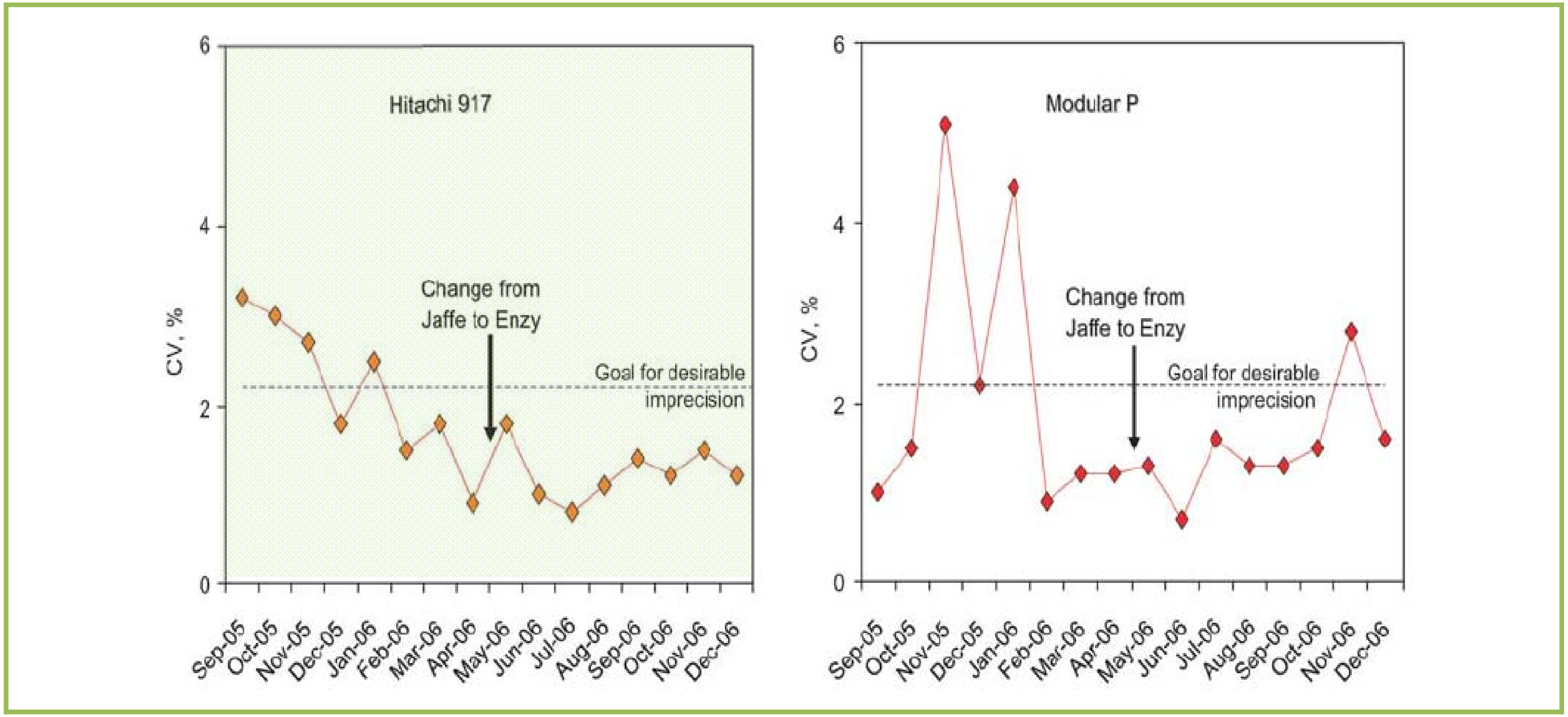
Creatinine analytical performance

Pre-standardisation



Joris R. Delanghe et al. Clin Chem Lab Med 2008;46(9):1319–1325

Imprecision

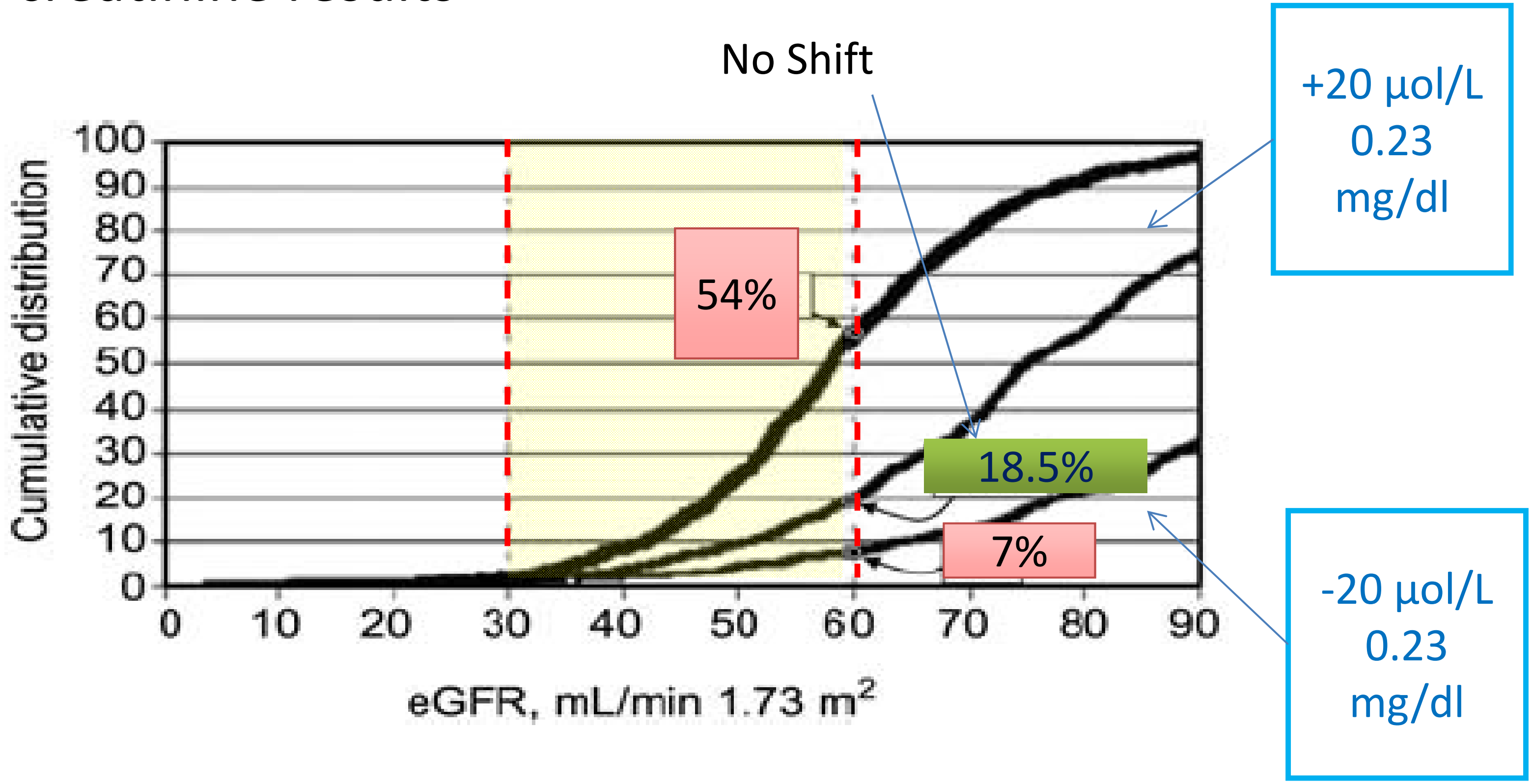


Pantighinin M, Clin Chem Lab Med 2008;46(4)

Jaffe-based methods: Clinical usefulness

- Impact on eGFR

Bias in creatinine results



Klee GG, et al., Clin Chem Lab Med 2007;45: 737

Impact on AKI
The effect of analytical variation on creatinine

Analytical %CV	RCV at 95 % probability	RCV at 99 % probability
1.0	14.1	19.8
1.70 *	14.4	20.4
3 (desirable)	15.2	21.5
4.0 Ŧ	16.7	23.6
5.0	18.1	25.6
6.0	19.7	27.8
7.4**	22.1	31.2

Creatinine Assays Performance

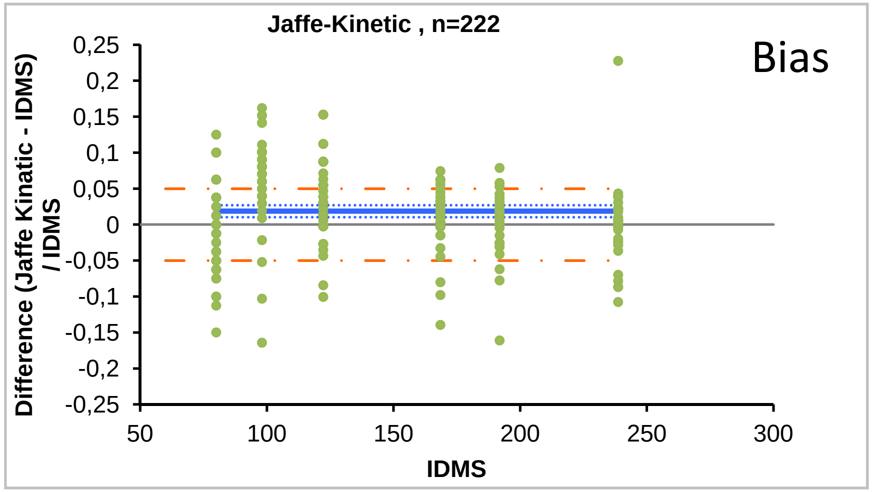
5 years post standardisation

Certification of Creatinine in a Human Serum Reference Material by GC-MS and LC-MS

- SRM 967 - 66.5 umol/L for serum pool 1 (a value close to the diagnostically important concentration of 88.4 umol/L) and 346.2 umol/L for serum pool 2 (a concentration corresponding to that expected in a patient with chronic kidney disease).

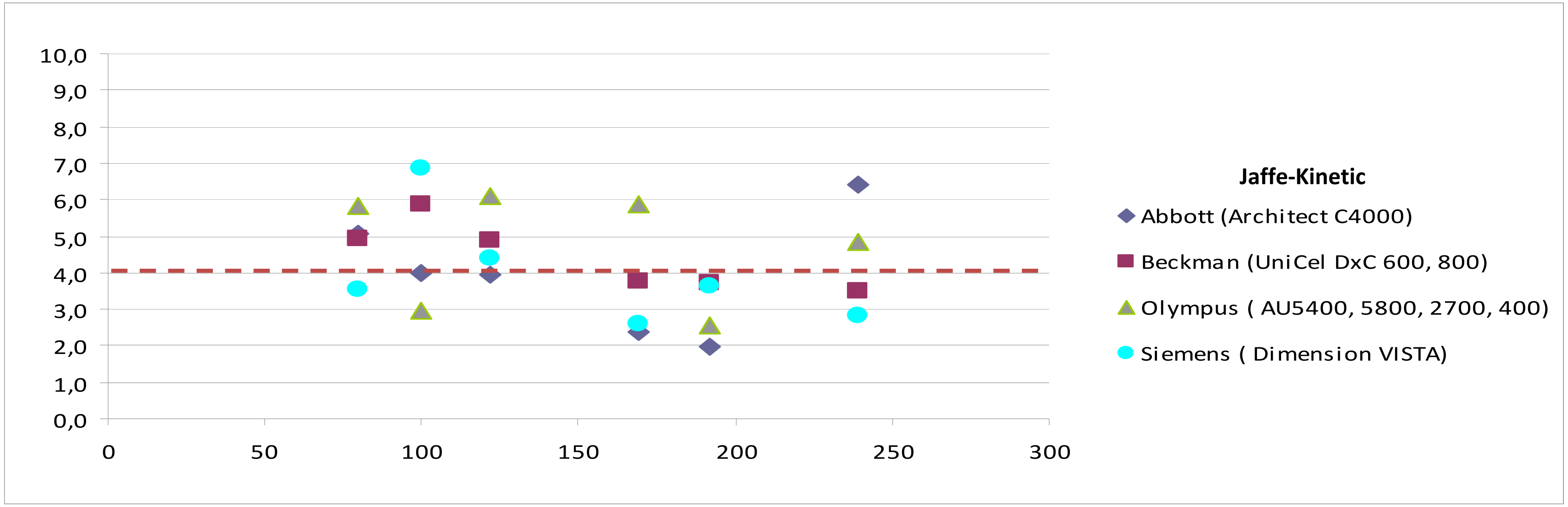
Clin Chem 2007;53:1694-1699

Jaffe Kinetic 2017



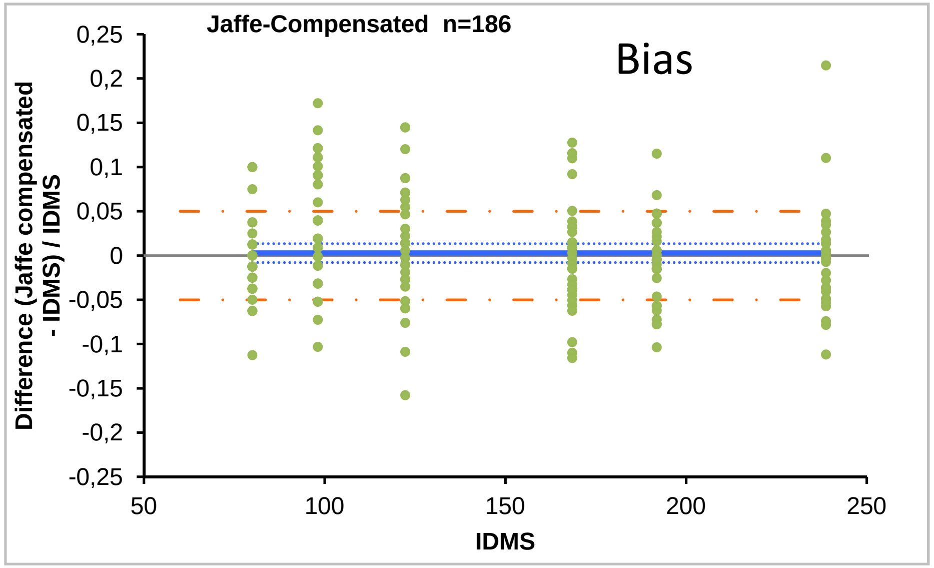
- Major positive bias

At higher concentration , magnitude of bias decreases



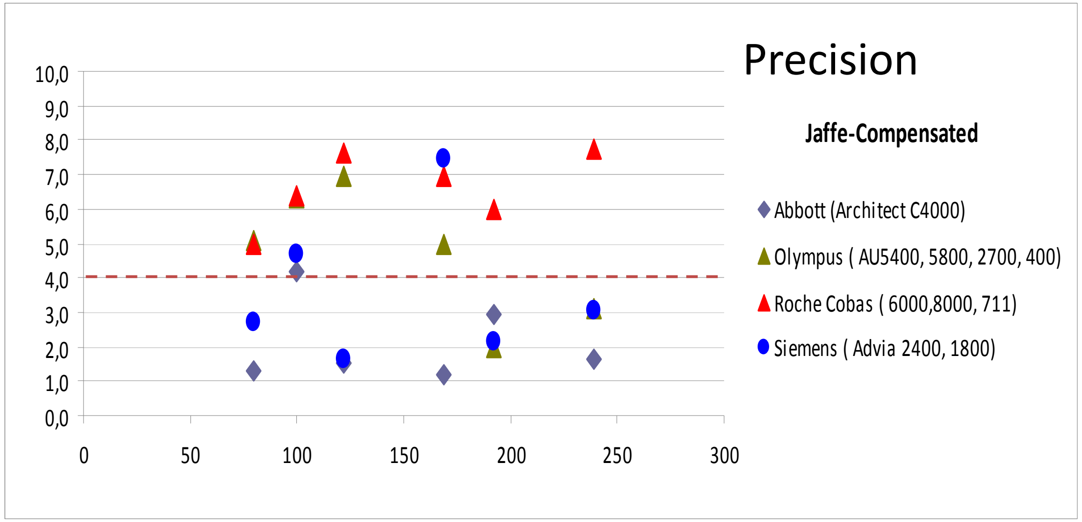
Jassam N et al. Annals of Clinical Biochemistry 2017. 54(3):386-393

Jaffe Compensated



- Significant positive bias
- Bias due to interference (Glu.)
- Minor but significant bias *(CCLM 2008: 46(9) 1319)*

Analytical platform dependent precision



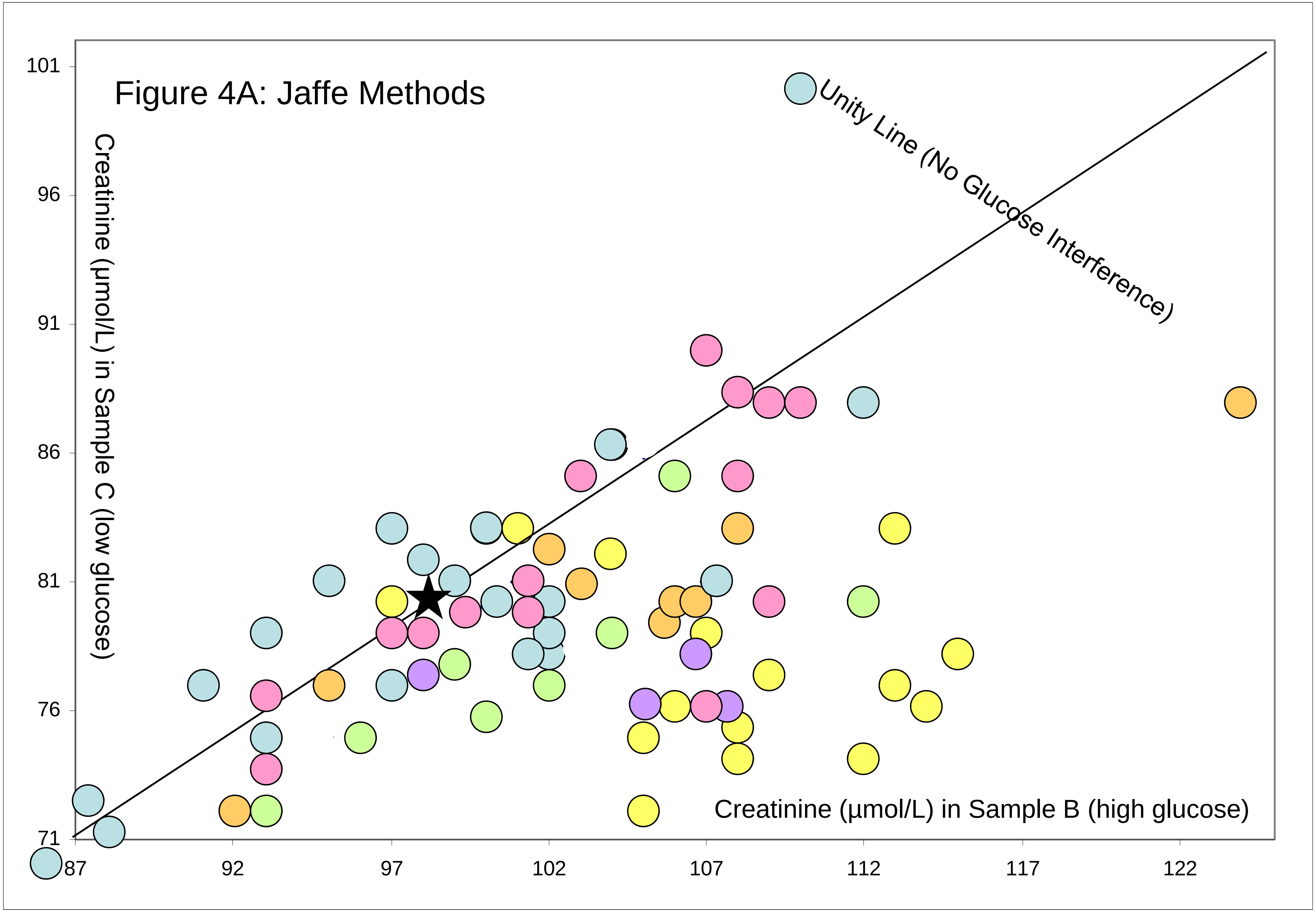


Figure 4A: Jaffe Methods
Unity Line (No Glucose Interference)
Creatinine (µmol/L) in Sample C (low glucose)
Creatinine (µmol/L) in Sample B (high glucose)
101
96
91
86
81
76
71
87
92
97
102
107
112
117
122

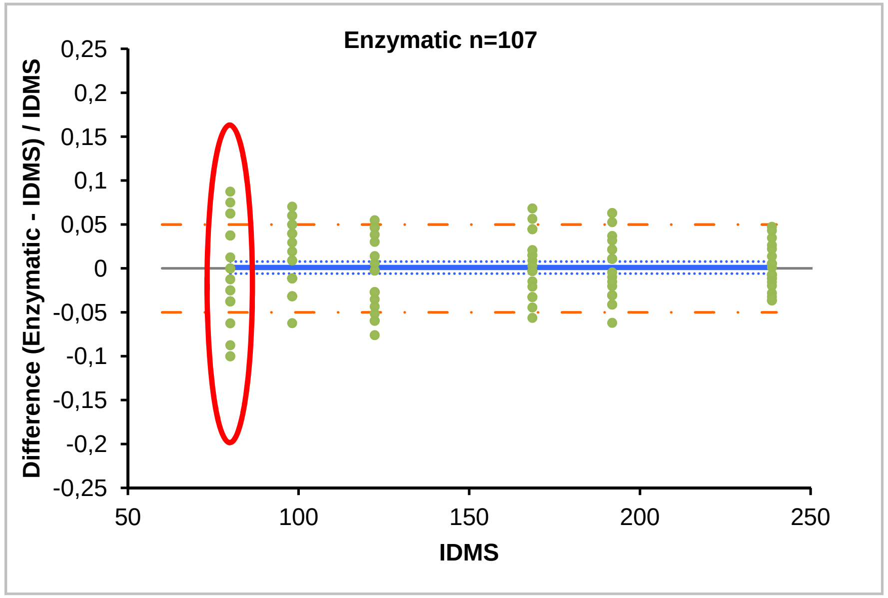
Letter to the Editor

Cas Weykamp*, Aldy Kuypers, Dirk Bakkeren, Paul Franck, Douwe van Loon, Jacqueline Klein Gunnewiek, Robert de Jonge, Herman Steigstra and Christa Cobbaert

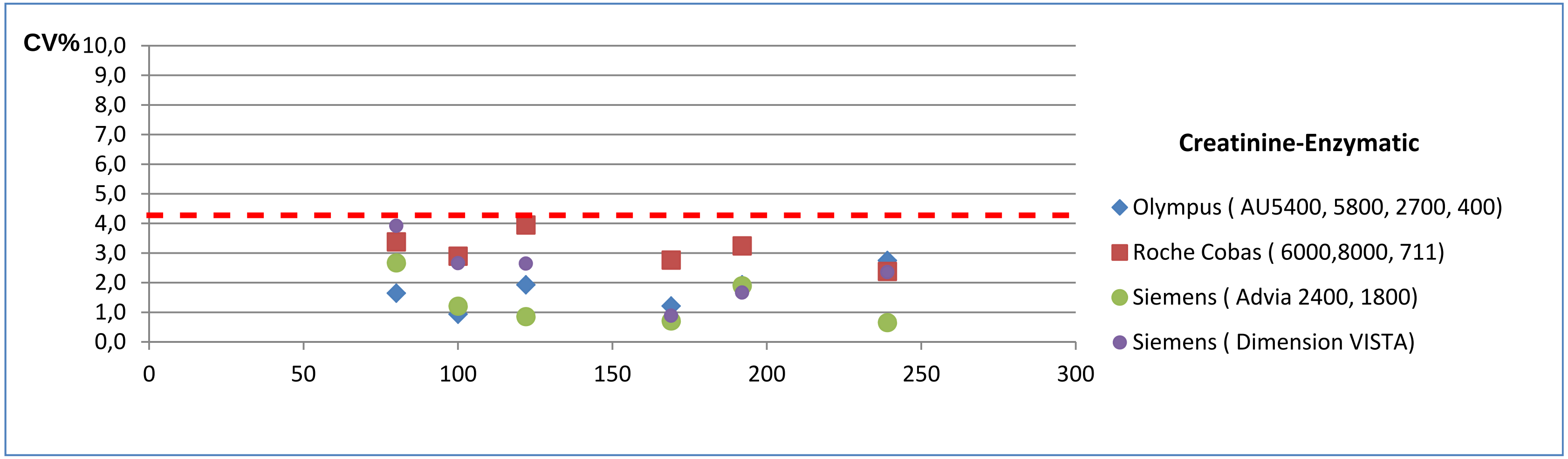
Creatinine, Jaffe, and glucose: another inconvenient truth

Measurement procedure	EQA/PT samples					
	n	Creatinine, µmol/L		Bias to RMP, µmol/L		ΔBias, µmol/L
		S1[a] Gluc 29.5 Protein 48 g/L	S2[b] Gluc 5.1 Protein 66 g/L	S1	S2	S1–S2
RMP	1	54	72	0	0	0
Jaffe procedures						
Abbott Architect	8	77	71	+23[d]	−1	+24
Beckman UniCel DxC	29	59	69	+5[d]	−3	+8
Roche Cobas 6000	17	56	71	+2	−1	+3
Siemens VISTA	4	59	70	+5	−2	+7
Siemens VISTA NR						
Enzymatic procedures						
Abbott Architect	11	56	72	+2	0	+2
Beckman Unicel DxC	1	51	70	−3	−2	−1
Roche Cobas 6000	111	55	71	+1	−1	+2
Siemens VISTA	7	53	71	−1	−1	0

Enzymatic methods



- Evidence of a concentration dependent performance ?
- Critical for those of eGFR of 60 ml/min 1.73^2
- Paediatrics creatinine



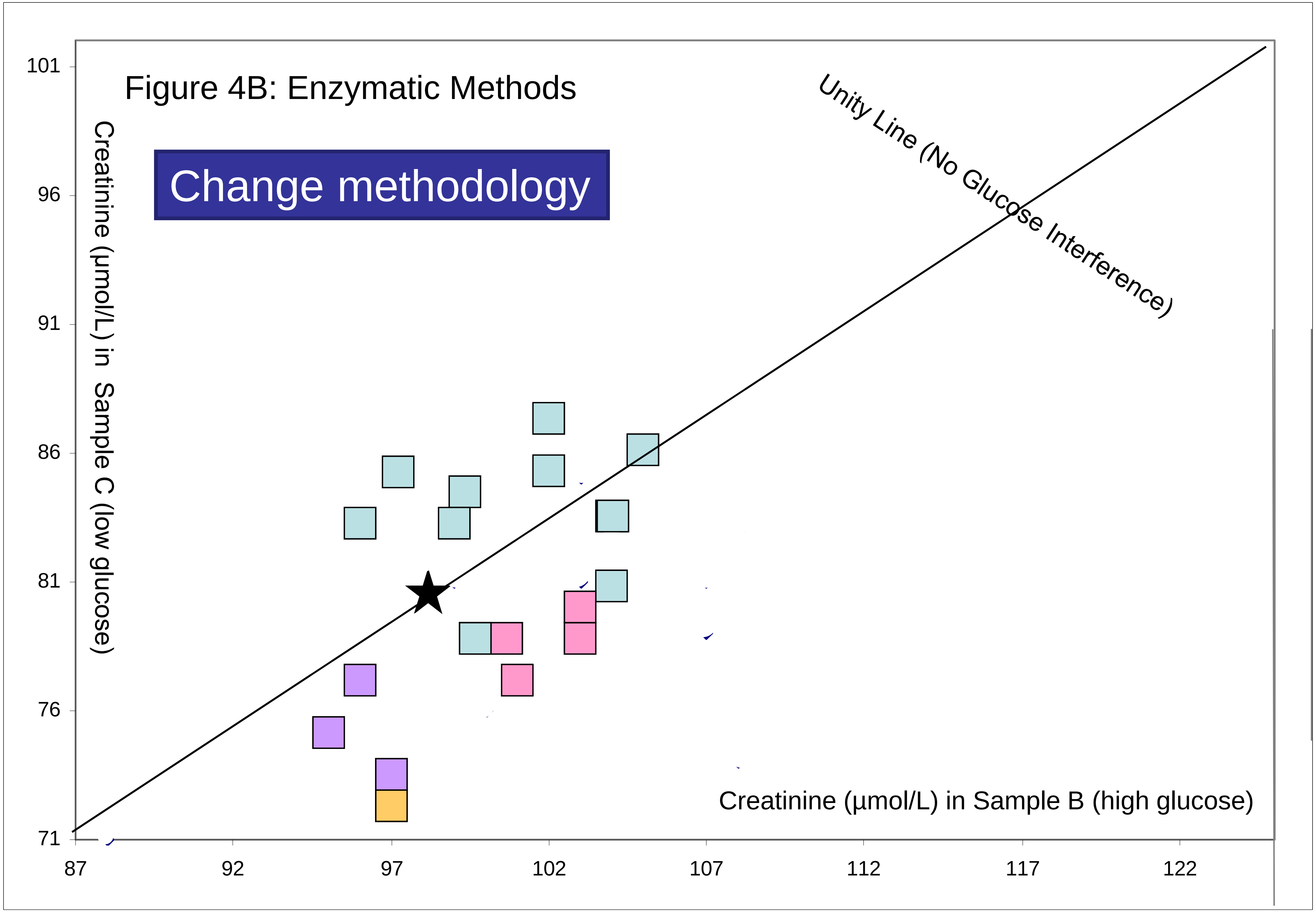


Figure 4B: Enzymatic Methods
Change methodology
Unity Line (No Glucose Interference)
Creatinine (µmol/L) in Sample C (low glucose)
Creatinine (µmol/L) in Sample B (high glucose)
101
96
91
86
81
76
71
87
92
97
102
107
112
117
122

Eltrombopag interference in creatinine assays

GOV.UK

Home > Drug Safety Update

Eltrombopag (Revolade): reports of interference with bilirubin and creatinine test results

Eltrombopag: liver toxicity, kidney injury or assay interference?

KW Choy. 2016Volume 48, Issue 7, Pages 754–756

- Creatinine 4 Jaffe kinetic, 3 enzymatic = 30-64 , IDMS result is 56 umol/L

- Eltrombopag inference appears to be analyzer specific.
- No interference in Roche Cobas (enzymatic)
- Creatine supplements interfere with the enzymatic assay

N.Jassam, Ann Cli Biochem, Supp 2019

Verena Gounden and Zhen Zhao, Ann Clin Biochem. 2016 Sep;53(Pt 5):611-4

Thank you for listening

ANY QUESTION