The Effect of Hemolysis and Storage Conditions on Insulin Stability

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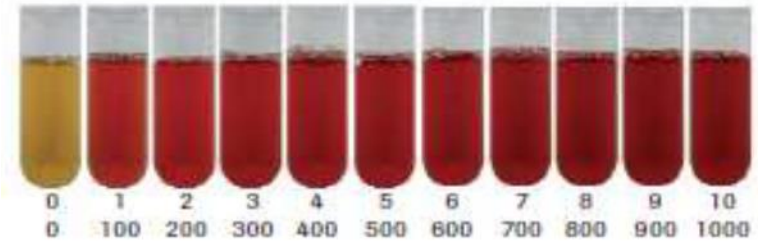
INTRODUCTION

Evaluation of insulin levels is a part of the clinical diagnosis tools useful for several clinical conditions including

Hypoglycemia → **classification of diabetes mellitus** → **insulin resistance**

Sample hemolysis may exert a strong influence on result reliability.



CLSI C56-A

Biochemical or spectrophotometric measurements are known to be more affected by hemolysis when compared to immunochemical analysis. This situation can often lead to less consideration on immunoassays.

Laboratory parameters affected by haemolysis and/or blood cell lysis in the specimen.

Parameter	Bias	Cause	Reference
Adrenocorticotropic hormone	Negative	Proteolysis	36
Activated partial thromboplastin time	Negative	Release of thromboplastic substances	39
Antithrombin	Negative	Analytical interference	41
Aspartate aminotransferase	Positive	Cellular release	27
Alanine aminotransferase	Positive	Cellular release	27
Albumin	Negative	Dilution	27
Alkaline phosphatase	Negative	Analytical interference	27
Bilirubin (neonatal)	Variable	Analytical interference	29
Bilirubin (total)	Negative	Analytical interference	23
Calcitonine	Positive	Proteolysis	36
Chloride	Negative	Dilution	27
Cortisol	Negative	Analytical interference	31
Glucagon	Negative	Proteolysis	36
Glucose	Negative	Dilution	27
Haptoglobin	Negative	Analytical interference	38
Homocysteine	Negative	Analytical interference	37
Insulin	Negative	Proteolysis	36
Iron	Positive	Analytical interference	27
Lactate dehydrogenase	Positive	Cellular release	27
Lipase	Positive	Analytical interference	27
Magnesium	Positive	Cellular release	27
Parathormon	Negative	Proteolysis	36
Phosphorus	Positive	Cellular release	27
Potassium	Positive	Cellular release	27
Prostate specific antigen	Positive	Analytical interference	31
Prothrombin time	Positive	Release of thromboplastic substances	39
Sodium	Negative	Dilution	27
Urea	Positive	Cellular release	27
Testosterone	Negative	Analytical interference	31
Troponin I	Positive	Analytical interference	31
Troponin T	Negative	Analytical interference	33
Vitamin B12	Negative	Analytical interference	31

A significant negative bias in the measurement of insulin was observed due to hemolysis. The most likely cause of the negative bias is insulin degradation. The degradation process is initiated by the insulin degrading enzyme (IDE) released from erythrocytes.

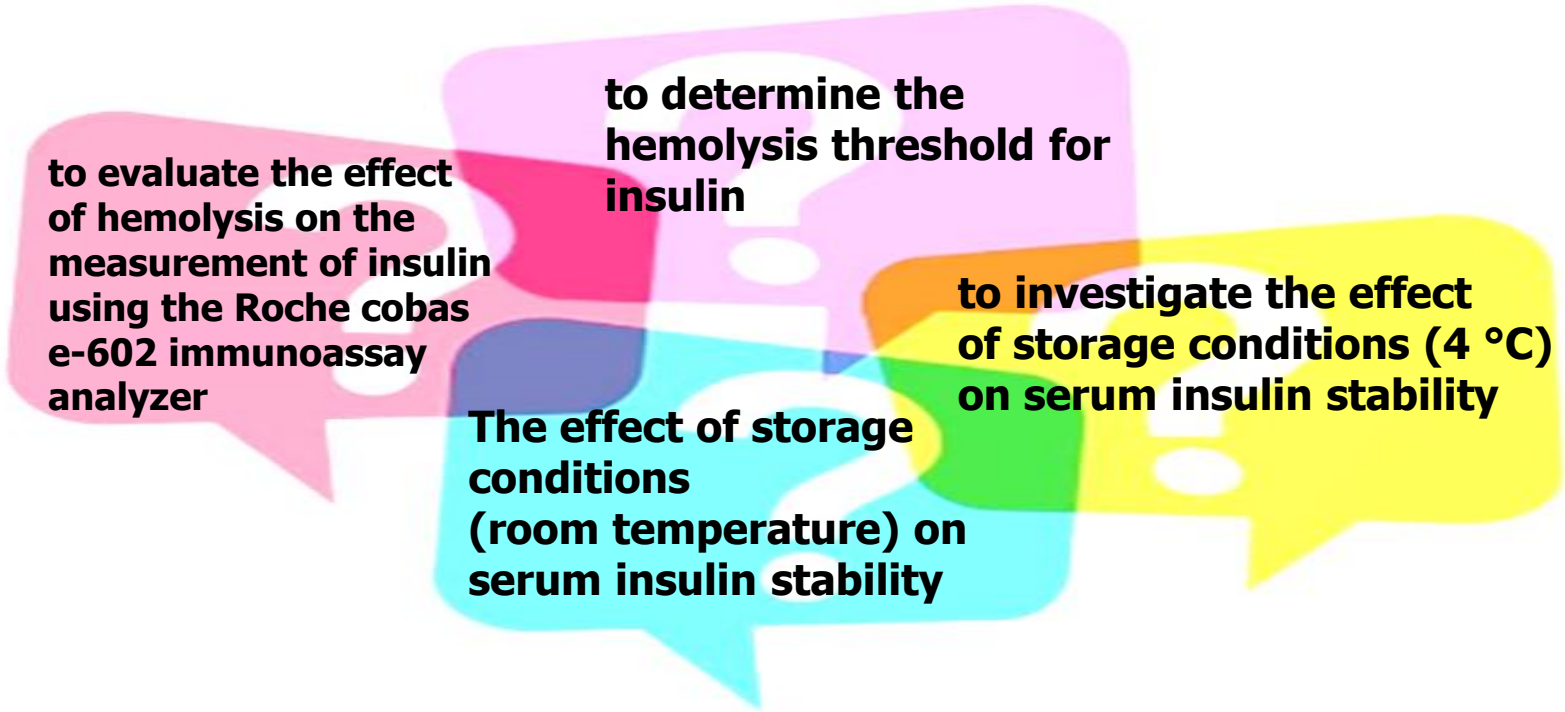
Clin Chem Lab Med 2008

INTRODUCTION

Only a few studies have been done to evaluate the effect of hemolysis on the measurement of insulin using the Beckman Coulter Unicell DXI 800 or the Architect®-Ci8200 immunoassay analyzer. These studies have demonstrated that hemolysis can cause negative interference with insulin assays.

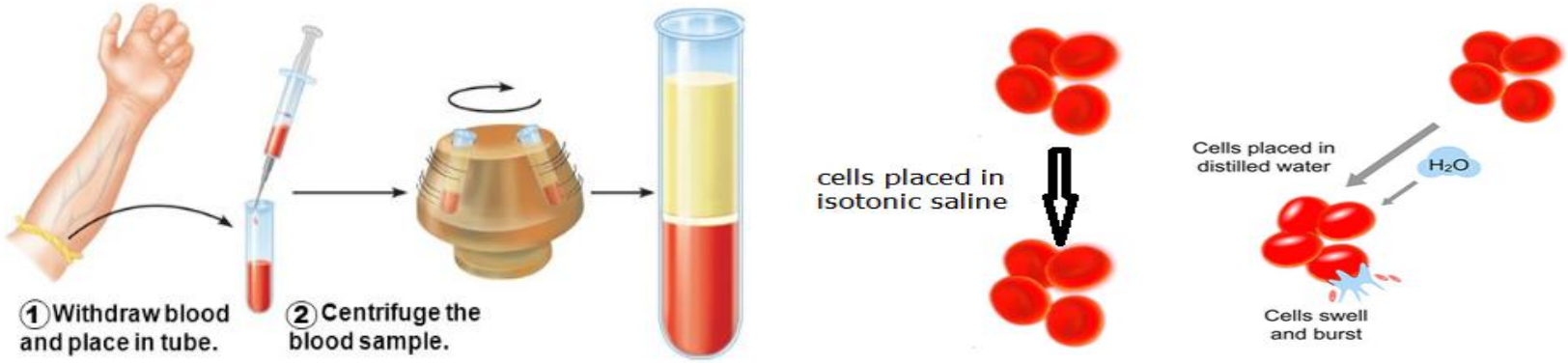
However, it is important to evaluate interference for insulin on all analytical platforms as the degree and direction of interference may not be the same.

OBJECTIVES: The aims of this study were



DESIGN AND METHODS:

Hemolysates were prepared from blood sample collected on lithium heparin.



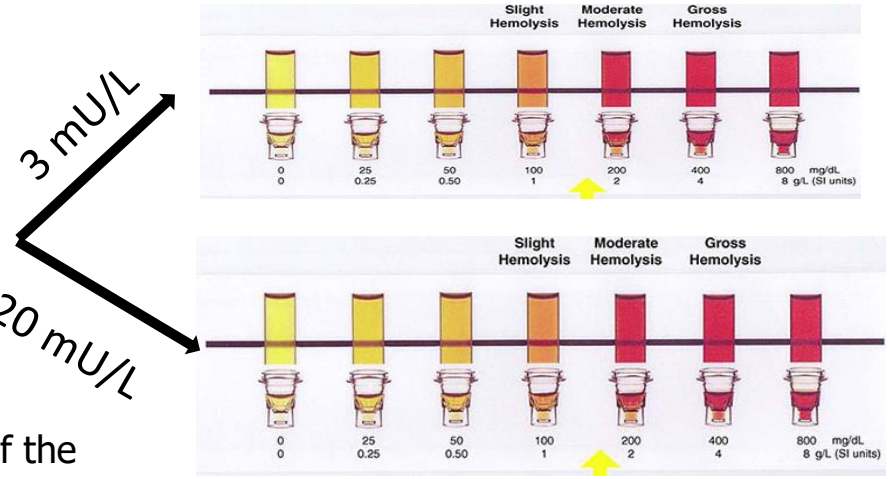
After centrifugation, the plasma was replaced with an equal volume of isotonic saline and the cells re-suspended. The sample was centrifuged and the saline wash repeated five times. After the final wash, the saline was replaced with distilled water.

Hemolysis was induced by using the osmotic shock method (recommended by the CLSI.78). After freezing at −20 °C, the sample was thawed, mixed and centrifuged. The hemolysate was transferred to a clean tube and the Hb content measured by spectrophotometry. Stock solutions of hemolysate were prepared in isotonic saline with hemoglobin concentrations ranging from 0–800 mg/dL.

DESIGN AND METHODS:

Interference studies

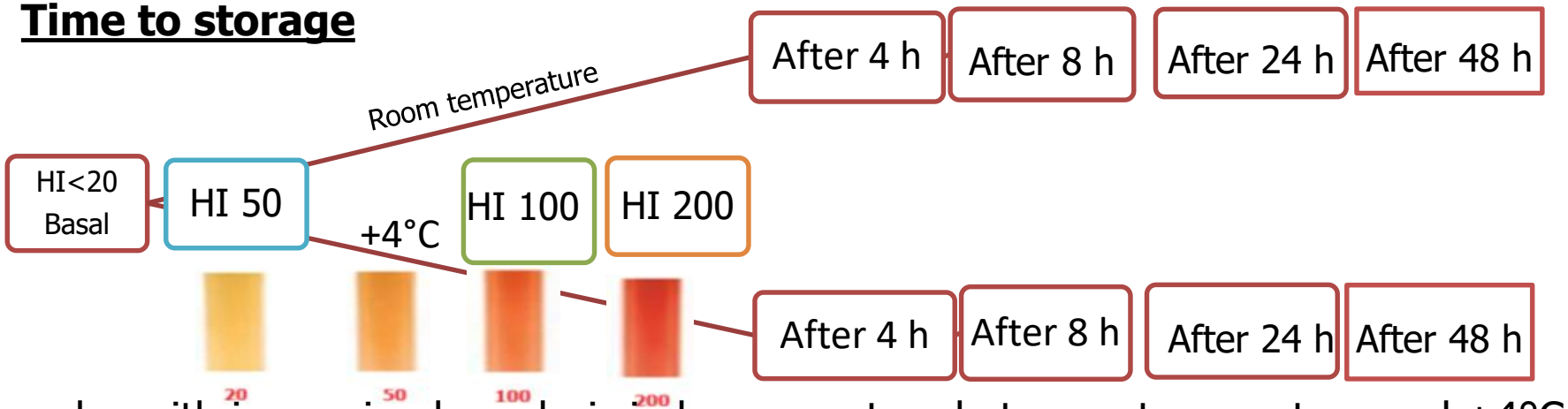
Interference studies were conducted by adding hemolysates with increasing hemoglobin concentration to serum pools of known insulin levels.



Two pooled serum samples (free of hemolysis) with low and high insulin concentrations were used for interference studies.

To 900 µL of each of the pooled serum samples, 100 µL of each of the hemolysate stock solutions was added.

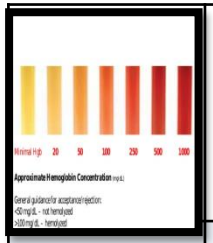
Time to storage

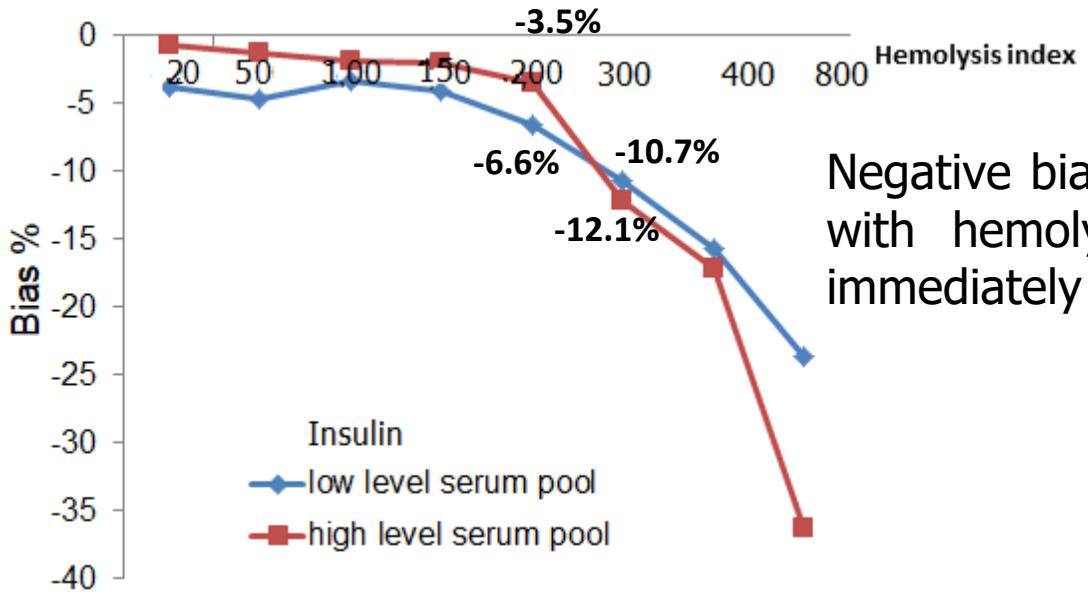


Samples with increasing hemolysis index were stored at room temperature and +4°C.
The insulin levels of these samples were measured using the cobas e-602 at 0, 4, 8, 24 and 48 hours.

A change of >10% from baseline results (non-hemolyzed sample) was taken as evidence of significant interference.

RESULTS

	Serum pool + saline	HEMOLYSIS INDEX							
		20	**50**	**100**	**150**	**200**	**300**	**400**	**800**
Insulin	**7.75**	7.46	7.39	7.49	7.44	7.24	6.92	6.54	6.06
Insulin	**24.94**	24.79	24.63	24.48	24.43	24.06	21.9	20.67	15.87

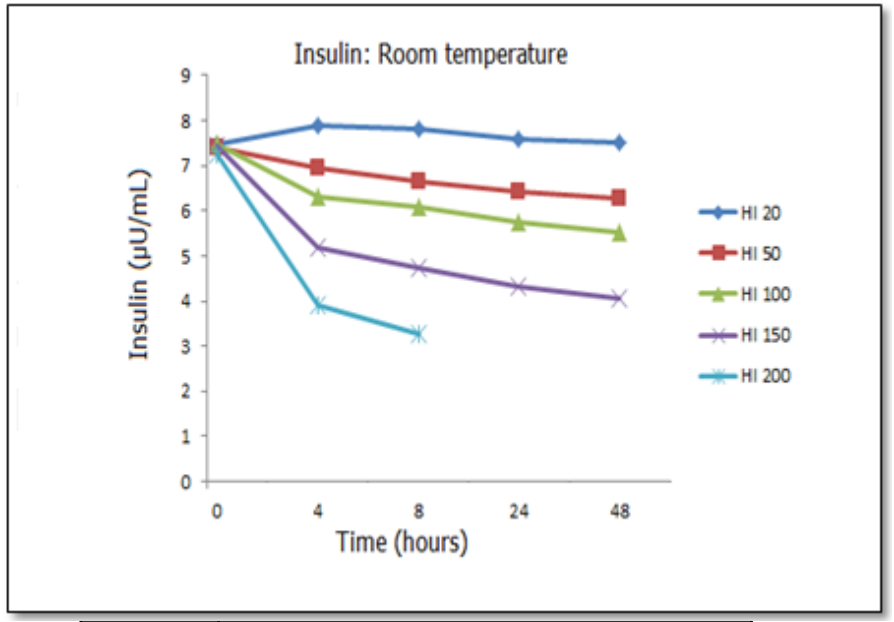


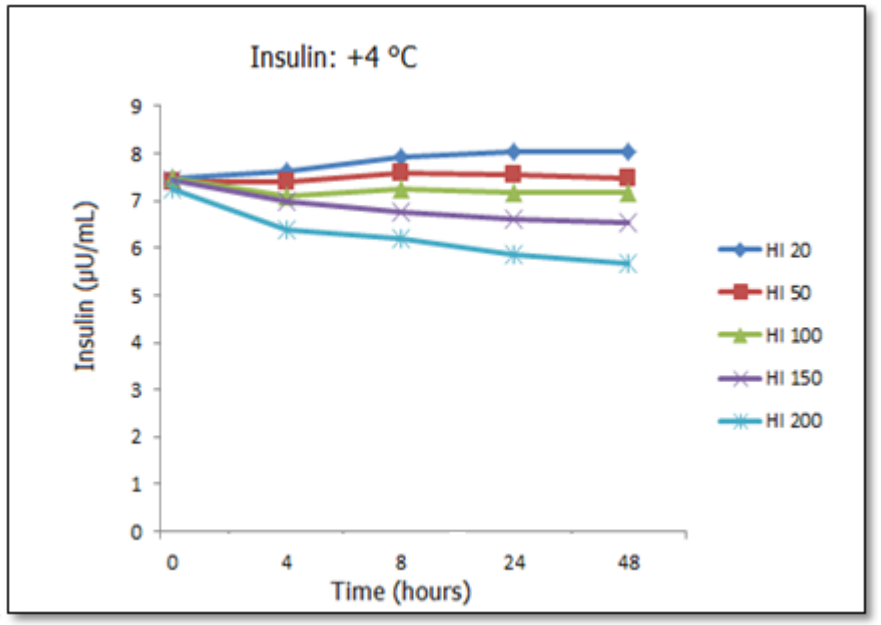
Negative bias was detected as <10% in the samples with hemolysis index of 200 mg/dL which were immediately analysed after centrifugation.

The hemolysis threshold for insülin was determined as 200 mg/dL.

RESULTS

Influence of the storage condition on the insulin assay in samples with low insulin concentration





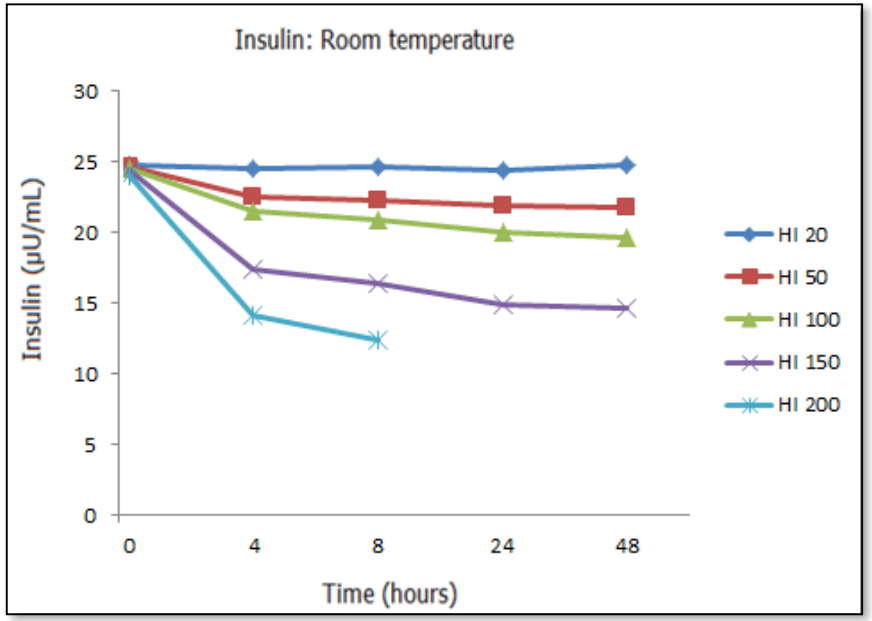
	Bias %			
	4 hours	**8 hours**	**24 hours**	**48 saat**
HI 20	5,8	4,6	1.8	0.5
HI 50	-6	-10	-12.85	-15
HI 100	-15,88	-18,7	-23,23	-26,3
HI 150	-30,37	-36,15	-42	-45
HI 200	-45,85	-54,83	-	-

	Bias %			
	4 hours	**8 hours**	**24 hours**	**48 saat**
HI 20	2	6	7.5	7.6
HI 50	- 0.1	2.8	2	1.2
HI 100	-5	-3	-4	-4.1
HI 150	-5.9	-9.1	-11	-11.9
HI 200	-11,7	-14,5	-19	-21.5

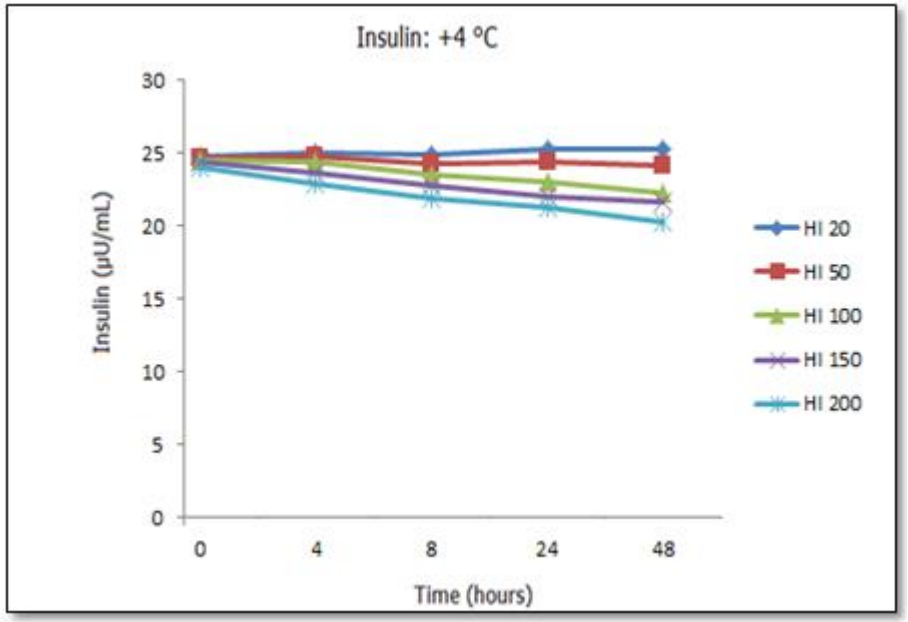
Negative bias was determined as ≤ 10% in the samples with hemolysis index of 50, which stayed for 8 hours at room temperature.

RESULTS

Influence of the storage condition on the insulin assay in samples with high insulin concentration



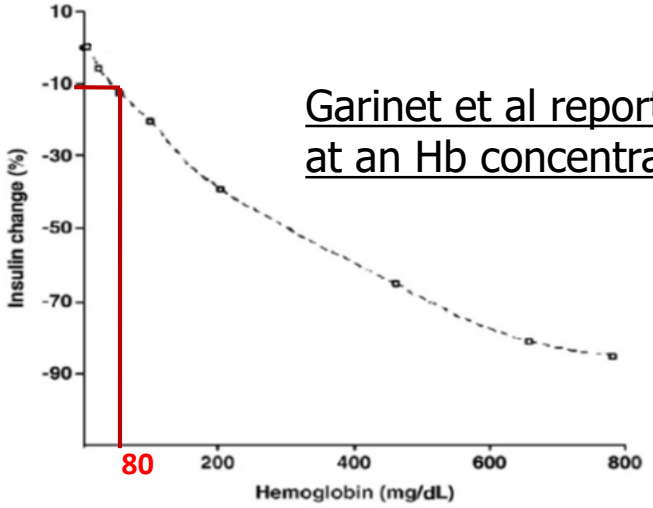
	Bias %			
	4 hours	8 hours	24 hours	48 saat
HI 20	-1.4	-1.3	-2.2	-0.5
HI 50	-8.3	-9.5	-11.2	-11.6
HI 100	-11.8	-14.74	-19,73	-18,25
HI 150	-28.61	-32.66	-39	-39.8
HI 200	-41	-48.21	-	-



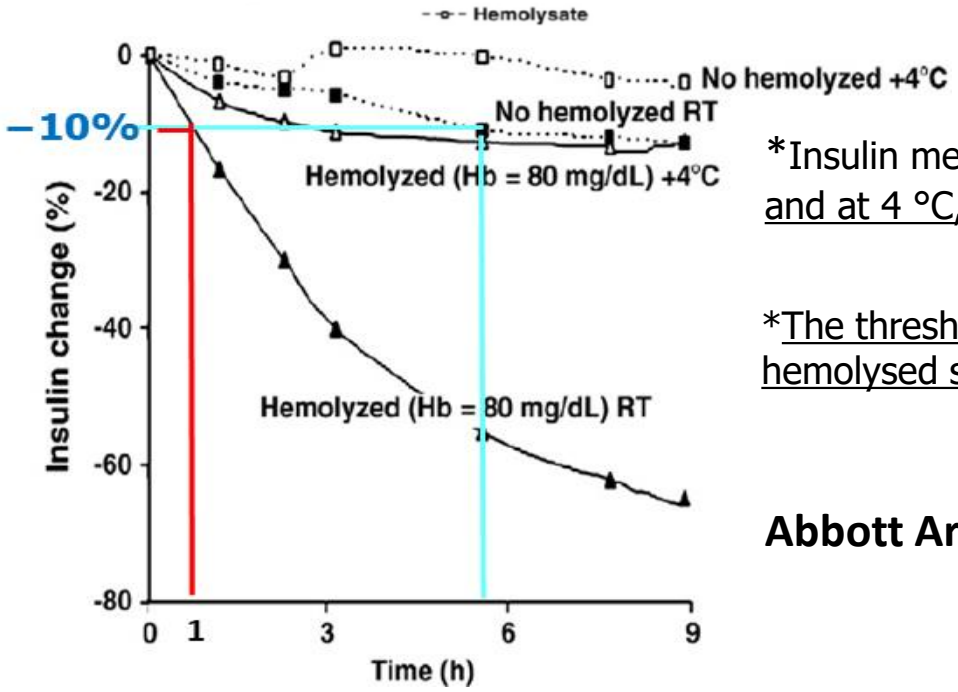
	Bias %			
	4 hours	8 hours	24 hours	48 hours
HI 20	0.1	0.1	1.2	1.5
HI 50	0.5	-1.5	-1.1	-1.6
HI 100	-0.2	-3.9	-6	-8.9
HI 150	-3.15	-6.5	-11.2	-15.3
HI 200	-4.9	-8.9	-11.6	-15.8

Serum samples with hemolysis index of 100 were stable for 48 hours at the +4°C.

DISCUSSION



Garinet et al reported that 10% decrease from control value was observed at an Hb concentration of 80 mg/dL using the Architect immunoassay analyzer.



*Insulin measurement remained stable up to 5 and 9 hours at room temperature and at 4 °C, respectively in the non-hemolysed samples

*The threshold interference (−10%) was reached more rapidly (50 min) in hemolysed samples (80 mg/dL) at room temperature.

Abbott Architect®-Ci8200

S. Garinet et al. / Clinical Biochemistry 47 (2014) 445–447

DISCUSSION

The effect of hemolysis on the insulin assay at two different initial concentrations of insulin is shown in Table 1 and Fig. 1.

Table 1
Influence of hemolysis on insulin analysis.

Part A			Part B		
Hb (g/L)	Insulin (mU/L)	% change from baseline value	Hb (g/L)	Insulin (mU/L)	% change from baseline value
0	2.49		0	17.41	
2	1.99	20	2	13.68	21
4	1.56	37	4	10.40	40
6	1.27	49	6	8.05	54
8	1.03	59	8	6.45	63
10	0.78	69	10	5.29	70

200 mg/dL

Cook et al reported that 20% decrease from baseline value was observed at an Hb concentration of 200 mg/dL at two different concentrations of insulin using Beckman Coulter immunoassay analyzer.

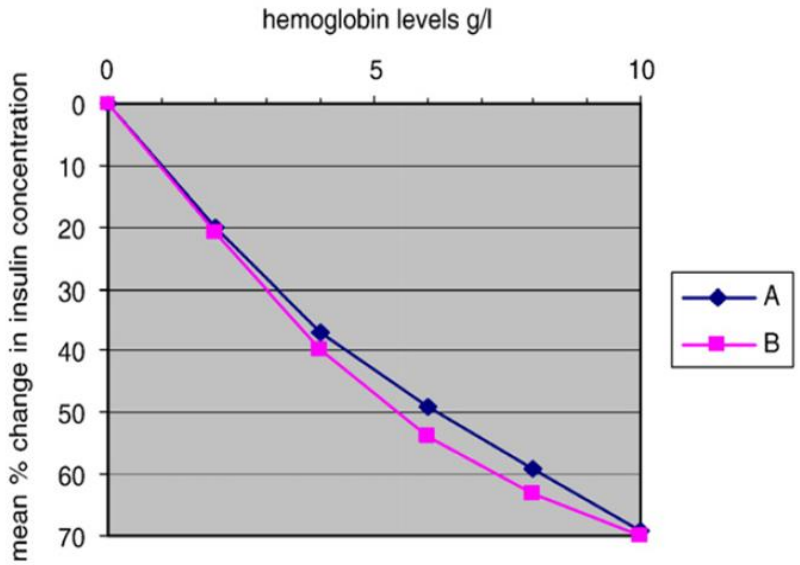
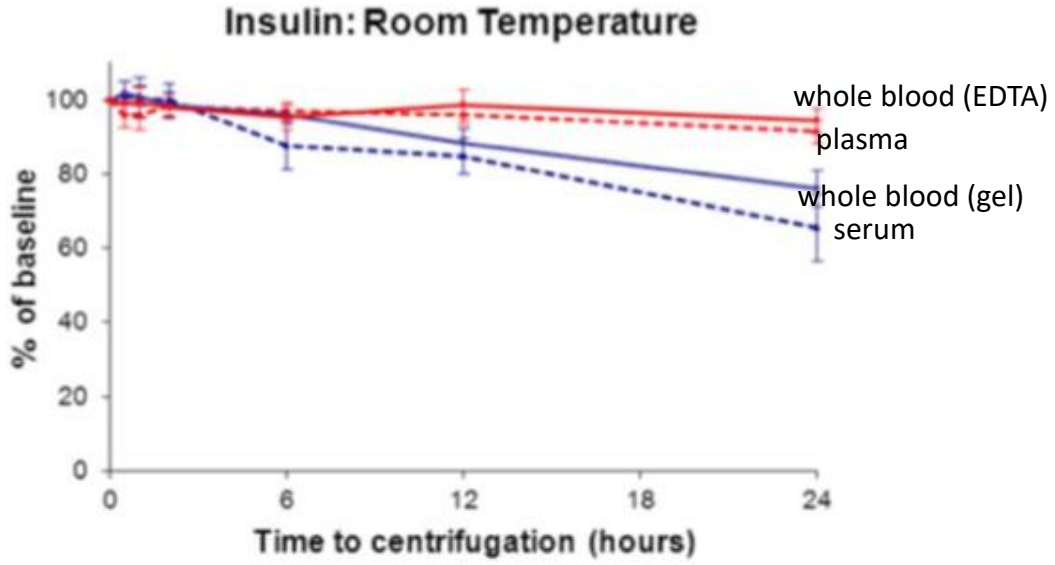


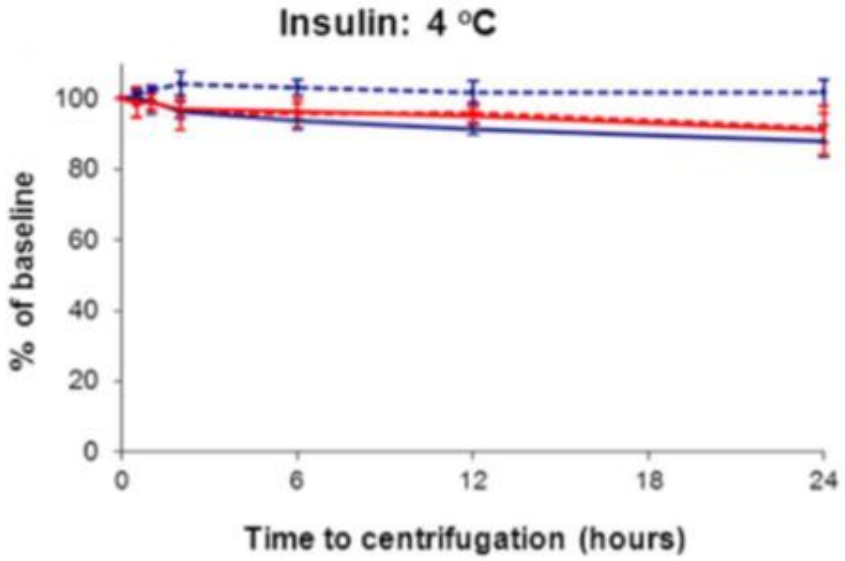
Fig. Interferogram showing the magnitude of the effect of hemolysis on the insulin assay. Line A represents insulin at a mean baseline concentration of 2.49 mU/L and Line B represents insulin at a mean baseline concentration of 17.41 mU/L.

The difference between the two methods may be explained by the lack of specificity of polyclonal antibodies (the immunoassay also cross-reacts with the degradation products).

P.R. Cook et al. / Clinical Biochemistry 43 (2010) 621–622

DISCUSSION





In another study,

insulin concentration decreased 66% from baseline value in serum samples stored for 24 hours at room temperature.

Insulin concentration was stable in serum samples stored for 24 hours at 4°C.

Our study clearly showed the inhibitory effect of low temperature on IDE activity. Low temperature significantly reduces insulin losses

Stability of insulin in plasma and serum at room temperature and 4°C. The samples were stored at room temperature and in the fridge at 4°C at 0.5, 1.0, 2.0, 6.0, 12 and 24 hours. Red lines indicates samples collected K-EDTA, blue lines indicate blood collected into serum gel, solid lines indicate whole blood and dashed lines indicate sample centrifuge at baseline.

CONCLUSIONS

Our data underline the importance of controlling the three main pre-analytic critical points in insulin assays: hemolysis, storage temperature and delay between centrifugation and analysis.

- Threshold for hemolysis index is 200 mg/dL
- Insulin analysis is not suitable for even slight hemolysed serum samples (hemolysis index of 50) that stayed more than 8 hours at room temperature.
- Insulin concentrations in serum samples with hemolysis index of 100 or below were stable for 48 hours at +4 °C.

Thank you