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DEVELOPMENT OF AN IMMUNOTURBIDIMETRIC ASSAY FOR THE DETERMINATION OF HAPTOGLOBIN INCORPORATING A NEW READY TO USE REAGENT

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INTRODUCTION

The primary function of the plasma protein haptoglobin is to bind to free haemoglobin thereby preventing haemoglobin-driven oxidative tissue damage,[1] the renal excretion of iron and the subsequent kidney damage following intravascular hemolysis. The plasma levels of this protein are reduced during episodes of hemolysis and the measurements are used in the diagnosis of haemolytic anaemia.[2] Haptoglobin is also a positive acute-phase protein with immunomodulatory properties, the levels of this protein are elevated in inflammatory, infectious processes and in malignancies.[3]

This study reports the development of an immunoturbidimetric assay for the determination of haptoglobin in serum samples, which incorporates a new ready to use reagent leading to a simplified procedure and a reduction of handling errors prior to analysis. The assay is applicable to a variety of automated analysers. This is of value for application in clinical laboratories.

METHODOLOGY

- The assay is immunoturbidimetric, the sample containing haptoglobin reacts with anti (human) haptoglobin antibody; insoluble complexes are formed allowing quantitative measurement at 340 nm. The amount of complex formed is proportional to the concentration of haptoglobin in the sample.
- The assay kit includes the liquid reagent ready to use. The assay is traceable to ERM-DA470k/IFCC Sample No. 09648.
- On board stability was assessed by storing three lots of reagent uncapped on the analyser for a minimum period of 28 days. The performance was compared to fresh material.
- Within-run precision was assessed by testing serum samples at defined medical decision levels, 3 replicates of each sample were assayed 3 times, twice a day, for 20 days.
- Correlation studies were conducted using a commercially available assay system.

RESULTS

Sensitivity and linearity

Haptoglobin assay (ready to use reagent)		
Sensitivity (mg/dl)	Linearity (mg/dl)	Sample type
15	410	Serum

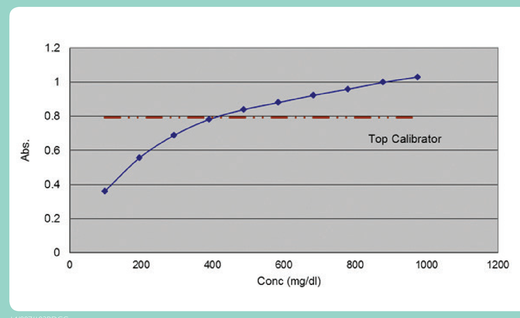
14/003, 007/103RDCCC

Haptoglobin assay: traceability to ERM-DA470k/IFCC sample No. 09648				
Sample	Target (mg/dl)	Lot	Measured (mg/dl)	% Recovery
ERM-DA470k/ IFCC sample No. 09648	88.9	Lot 1	88.8	99.9
		Lot 2	90.8	102.1
		Lot 3	90.2	101.5

14/010/103RDCC

Prozone

Prozone was not observed up to 764 mg/dl.



14/007/103RDCC

Precision

Haptoglobin Assay (ready to use reagent): within-run precision (n=120)					
Lot 1		Lot 2		Lot 3	
Haptoglobin Mean concentration (mg/dl)	%CV	Haptoglobin Mean concentration (mg/dl)	%CV	Haptoglobin Mean concentration (mg/dl)	%CV
18.63	2.1	17.88	2.3	18.13	2.5
49.78	0.7	50.48	0.8	50.80	0.7
129.65	0.6	130.95	0.5	131.03	0.6
403.87	2.2	399.56	2.5	396.58	2.8

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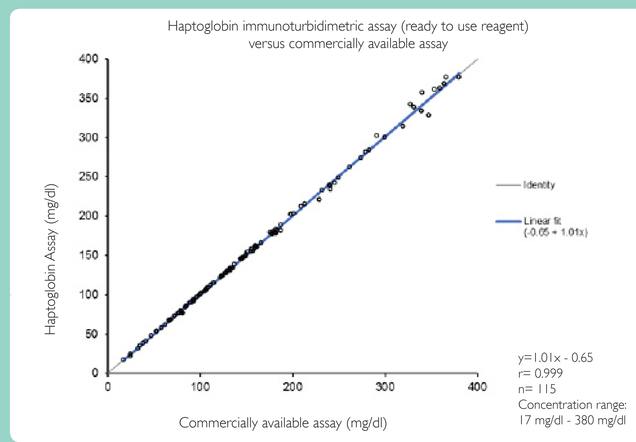
Correlation



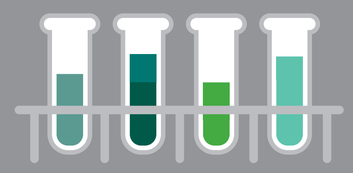
Reagent on-board stability

Sample	Day 0			Day 7			Day 14			Day 22			Day 29		
	On-board Reagent	Fresh Reagent	% Difference on-board reagent versus fresh	On-board Reagent	Fresh Reagent	% Difference on-board reagent versus fresh	On-board Reagent	Fresh Reagent	% Difference on-board reagent versus oresh	On-board Reagent	Fresh Reagent	% Difference on-board reagent versus fresh	On-board Reagent	Fresh Reagent	% Difference on-board reagent versus fresh
	Mean Concentration (mg/dl)			Mean Concentration (mg/dl)			Mean Concentration (mg/dl)			Mean Concentration (mg/dl)			Mean Concentration (mg/dl)		
Serum Control 1	50	50	0.0	50	51	-2.0	51	50	2.0	51	50	2.0	51	50	2.0
Serum Control 2	130	129	0.8	131	131	0.0	129	130	-0.8	133	132	0.8	130	131	-0.8
Serum Control 3	200	200	0.0	202	201	0.5	199	201	-1.0	204	201	1.5	200	200	0.0
Calibrator	392	400	-2.0	399	385	3.6	414	408	1.5	399	405	-1.5	397	391	1.5

14/008/103RDCC

CONCLUSION

The results indicate applicability of this immunoturbidimetric assay to the determination of haptoglobin in serum samples. The inclusion of a new ready to use reagent leads to a simplified procedure and a reduction of handling errors prior to analysis. This is of value for application in clinical laboratories.



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