
Technical Note

No. TN7

Performance evaluation of Diagnostic Chemicals Limited L3K bicarbonate reagent on the Hitachi 717, Brown, J.A., Walker, M.J., Jewells, T., *Diagnostic Chemicals Limited, Charlottetown, P.E.I., Canada, C1E 1B0.*

For more information, contact DCL Technical Services at 800-565-0265.

Diagnostic Chemicals Limited

800-565-0265 (Canada) • Charlottetown, PE, Canada C1E 2A6

902-566-1396 • Fax 902-566-2498

800-325-2436 (USA) • Oxford, Connecticut 06478, USA

203-881-2020 • Fax 203-888-1143

www.dclchem.com • E-mail - sales@dclchem.com

08/05/99

Performance evaluation of Diagnostic Chemicals Limited L3K bicarbonate reagent on the Hitachi 717.

Brown, J.A., Walker, M.J., Jewells, T., *Diagnostic Chemicals Limited, Charlottetown, P.E.I., Canada, CIE 1B0.*

INTRODUCTION

A single liquid bicarbonate reagent is being developed which uses an NADH analog in an enzymatic assay to provide a single liquid reagent with excellent stability and performance on the Hitachi 717.

Historically, enzymatic bicarbonate (CO₂) reagents have been susceptible to degradation due to the oxidation of NADH cofactor, and this process is greatly accelerated when reagents are exposed to atmospheric CO₂. Synthetic NADH analogs have been produced which are less prone to oxidative degradation and are stable at near-neutral pH¹. These qualities make them ideally suited for use in liquid enzymatic reagent systems. In this study, the performance and stability of a CO₂ reagent under development at DCL using a cofactor analog were evaluated on the Hitachi 717. A method comparison study was carried out against the Reflab® CO₂ reagent produced by Medical Analysis Systems, and interference studies were carried out on both reagents.

METHODS

Reagents

DCL CO₂: A single liquid reagent containing buffer (pH 7.5 at 25°C), 12.5 mmol/L PEP, >320 U/L PEPC (botanical), >4100 U/L malate dehydrogenase (mammalian), 0.88 mmol/L NAD analog, activators, stabilizers, and a preservative.

RefLab® CO₂: (Medical Analysis Systems, Cat No S1223) A single liquid reagent containing 8.5 mmol/L PEP, 400 U/L PEPC (microbial), 1500 U/L malate dehydrogenase (microbial), 1.68 mmol/L NADH, 2.95 mmol/L magnesium, a buffer, surfactant, stabilizer and preservative.

Instrument Parameters

The following instrument parameters were used on the Hitachi 717:

	<u>DCL CO₂</u>	<u>RefLab CO₂</u>
Assay Code:	2 Point (Endpoint)	2 Point (Endpoint)
Read Times:	36 sec. initial 288 sec. final	24 sec. initial 192 sec. final
Sample Vol:	3 uL	3 uL
Reagent Vol:	300 uL	240 uL + 60 uL diluent
Wavelength:	415 primary 505 secondary	376 primary 415 secondary

Calibration

Both chemistries were calibrated with a two point calibration using isotonic saline set at 0.0 mmol/L, and a 30.0 mmol/L standard.

Precision

Within-run and total precision was assessed for the DCL CO₂ by assaying two serum based controls in two runs per day over 20 days.

Linearity

Linearity was evaluated by assaying five levels of Casco CO₂ standard in quadruplicate. Carbon dioxide concentrations in the standards ranged from 5 to 50 mmol/L. The lower limit of detection was assessed by analyzing 10 replicates of a blank sample. LLD was then calculated as the mean result + 3SD's.

Interference

Interferences due to hemolysis, icterus, and lipemia were evaluated in DCL and Reflab® CO₂ reagents following NCCLS EP7-P dose-response guidelines. **Bilirubin** interference from 43-684 μmol/L (2.5-40mg/dL) was studied in a serum pool spiked with a solution of reference grade bilirubin (Pfanstiehl, Waukegan, IL) dissolved in a 0.1 M solution of TRIS in DMSO. **Hemoglobin** interference at levels from 8-155 μmol/L (50-1000mg/dL) was studied in a serum pool spiked with hemolysate produced by osmotic shock of red blood cells. **Lipemia** was simulated by adding 20% Intralipid solution (Kabi Vitrum, Clayton, NC) to pooled human serum to produce lipid levels from 100-1000 mg/dL. A 1000 mg/dL Intralipid level is equivalent to approximately 30 mmol/L (3000mg/dL) triglycerides.

Method comparison

Thirty- seven patient serum samples were analyzed using both DCL and RefLab® CO₂ reagents.

On-board Stability

Reagents were left on-board the Hitachi 717 in open 20 mL reagent containers for 17 days. Assays of serum controls, and linearity standards were performed every 2-5 days. Instrument re-calibration was performed when sample recoveries showed variance from day 0 results exceeding 1.5 mmol/L or 5%.

Statistics

Precision, linearity, and method comparison data were analyzed using EP Evaluator software, Version 3 (David G. Rhodes Associates, Inc., Kennett Square, PA).

RESULTS AND DISCUSSION

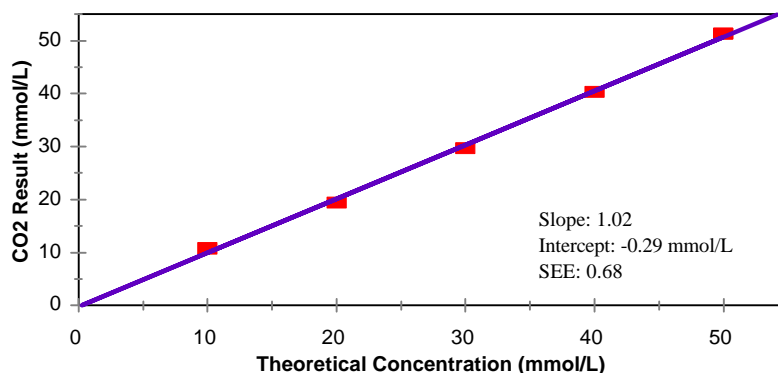
Precision

Within-run and total precision results are presented in Table I.

TABLE I, Precision Results

Sample	Mean mmol/L (mEq/L)	Within-run SD mmol/L (mEq/L)	Within-run CV %	Total SD mmol/L (mEq/L)	Total CV %
Level 1	13.4	0.19	1.4	0.52	3.8
Level 2	23.9	0.32	1.3	0.74	3.1

Figure 1, DCL CO₂ Linearity



Linearity

The reportable range was found to be 2-50 mmol/L with less than 1 mg/dL or 5% deviation from theoretical. Linearity results are presented in Figure 1.

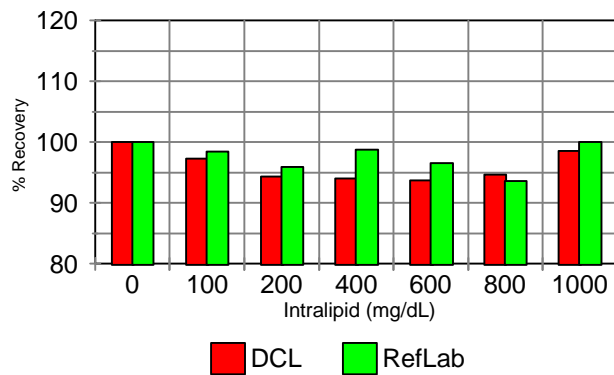
TABLE II, On-board Stability Results

Sample	Results (mmol/L)								Mean Result n = 8	Precision SD (CV%)																								
	Day 0	Day 2	Day 6	Day 8	Day 8	Day 13	Day 15	Day 17																										
DC Trol, Level 1	13.5								13.78	0.462 (3.4%)																								
<p>Fig. 3 % Recovery vs Hemoglobin Concentration</p> <table border="1" style="margin: 10px auto; border-collapse: collapse;"> <caption>Data for Figure 3: % Recovery vs Hemoglobin Concentration</caption> <thead> <tr> <th>Hemoglobin (mg/dL)</th> <th>DCL (% Recovery)</th> <th>RefLab (% Recovery)</th> </tr> </thead> <tbody> <tr><td>0</td><td>100</td><td>100</td></tr> <tr><td>100</td><td>94</td><td>94</td></tr> <tr><td>250</td><td>91</td><td>93</td></tr> <tr><td>375</td><td>95</td><td>97</td></tr> <tr><td>500</td><td>94</td><td>92</td></tr> <tr><td>750</td><td>97</td><td>89</td></tr> <tr><td>1000</td><td>105</td><td>99</td></tr> </tbody> </table>											Hemoglobin (mg/dL)	DCL (% Recovery)	RefLab (% Recovery)	0	100	100	100	94	94	250	91	93	375	95	97	500	94	92	750	97	89	1000	105	99
Hemoglobin (mg/dL)	DCL (% Recovery)	RefLab (% Recovery)																																
0	100	100																																
100	94	94																																
250	91	93																																
375	95	97																																
500	94	92																																
750	97	89																																
1000	105	99																																
		13.6	14.8	14.0	13.4	13.4	13.8	13.7																										
DC Trol, Level 2	24.2	25.1	24.7	26.3	24.6	24.2	24.0	23.3	24.55	0.886 (3.6%)																								
Casco Stds:																																		
10 mmol/L	9.1	9.5	9.8	10.4	9.3	9.1	9.4	9.3	9.49	0.432 (4.6%)																								
20 mmol/L	18.9	19.5	19.8	20.7	19.2	18.9	19.0	19.4	19.43	0.604 (3.1%)																								
30 mmol/L	29.6	30.8	30.9	31.2	29.4	29.5	29.5	29.3	30.03	0.792 (2.6%)																								
40 mmol/L	38.9	41.7	41.8	42.2	39.7	39.0	39.2	39.4	40.24	1.405 (3.5%)																								
Average Var from Day 0 (mmol/L)	-	1.0	1.3	1.8	0.3	0.1	0.2	0.4																										
Calibration	Calibrated	Day 0 Cal	Day 0 Cal	Day 0 Cal	Re-cal'd	Day 8 Cal	Day 8 Cal	Day 8 Cal																										

Interference

Interference results are presented in Figures 3, 4 and 5. Using a significance criterion of >10% variance, the DCL assay showed no significant interference due to hemolysis, icterus, or lipemia. The RefLab® reagent demonstrated significant hemoglobin interference at a level of 800 mg/dL, and no significant icteric or lipemic interference.

Fig. 4
% Recovery vs Intralipid Concentration



CONCLUSIONS

The DCL Bicarbonate reagent demonstrates excellent precision, linearity, and stability on the Hitachi 717. It compares well to the RefLab® reagent on patient samples and shows superior performance on hemoglobin interference samples.

References

- 1) US Patent No 5,801,006

Fig. 5
% Recovery vs Bilirubin Concentration

