Journal of Automatic Chemistry Vol. 11, No. 5 (September–October 1989), pp. 206–211

Multicentre evaluation of the Monarch (IL) clinical chemistry analyser

Ferruccio Ceriotti, Emma Guagnellini†, Francesco Zoppi‡, Norberto Montalbetti‡, Carlo Okely† and Pierangelo Bonini

Istituto Scientifico H. S. Raffaele, Via Olgettina 60, 20132 Milano (Italy)
† Ospedale Bassini, Cinisello Balsamo ‡ Ospedale Niguarda Ca' Granda

A multicentre evaluation of the Monarch centrifugal analyser is reported. Precision, linearity and accuracy were assessed by comparison with routine methods. Calibration stability, photometric and dispensing accuracy, and carry-over related to samples and reagents were also evaluated. The overall performance of the instrument was good, showing an excellent photometric and dispensing accuracy, absence of sample-dependent carry-over, and almost negligible reagent carry-over. Good precision, linearity and correlation with routine methods were found for the parameters tested. The instrument is reliable and is now used as the routine clinical chemistry analyser in two of the three laboratories taking part in the evaluation.

Keywords: Instrument evaluation; centrifugal analyser.

Introduction

The Monarch is an automatic random access centrifugal analyser for clinical chemistry determinations. Random analysis of samples is possible using a robotic system to

Table 1. Methods used on the Monarch and on the comparison instrument.

	Monarch					*Comparison methods*		
Parameters tested	Principles	Wavelength (nm)	Volumes (µl) Sample	Reagent(s)	Company	Principles	Company	Analyser
Glucose	hexokinase/G6PDH (EP)	340/380	3	200	IL	hexokinase/ G6PDH	BM†	Hitachi 737
Cholesterol	chol. oxid./trinder (EP)	500/690	2	200	IL	chol. oxid./trinder	Miles	Hitachi 737
Total bilirubin	sulphanilic ac. SDS (EP)	550/620	8	50/72	IL (Italy)	Jendrassik	BM	Hitachi 737
Urate	uricase/trinder (EP)	550/690	4	100	IL (Italy)	—		
Total protein	biuret (EP)	550/620	5	200	IL	—		
Calcium	cresolphtalein complexone (EP)	570/650	4	180	IL	—		
Phosphate	unreduced phosmolibdate (EP)	340/520	4	150	IL (Italy)	—		
Triglyceride	lipase/glycerol kinase/UV (FX)	340	3	150	IL	lipase/glycerol trinder	Miles	Hitachi 737
Creatinine	picric acid (FX)	520	9	150	IL	picric acid kinetic	BM	Hitachi 737
Urea	urease/GLDH (FX)	340	2	200	IL	urease/GLDH	BM	Hitachi 737
AST	Scandinavian Soc. 37 °C (K)	340	16	100/37	IL (Italy)	IFCC 37 °C without P5P	BM	Hitachi 737
ALT	Scandinavian Soc. 37 °C (K)	340	16	100/37	IL (Italy)	IFCC 37 °C without P5P	BM	Hitachi 737
ALP	Scandinavian Soc. 37 °C (K)	405	2	150/50	IL (Italy)	diethanolamine buffer 37 °C	BM	Hitachi 737
Sodium	I.S.E.	—	30	1080	IL	flame photometry	IL	IL 943
Potassium	I.S.E.	—	30	1080	IL	flame photometry	IL	IL 943
Chloride	I.S.E.	—	30	1080	IL	coulometry	Eppendorf	6610
Magnesium	calmagite (EP)	650/570	2	200	Lancer	atomic absorption	Pie Unicam	SP9000
Iron	ferene-S (EP)	600/690	25	60/60	Sentinel	ferrozine	Merck	ERIS

Notes: EP = end-point; FX = fixed time; and K = kinetic. † Boehringer Mannheim.

change the disposable cuvette rotors automatically. The disposable reaction rotors, made of UV-transmitting plastic material, contain 39 usable optical cuvettes, all with a pathlength of 0·744 ± 0·013 cm (and a reference cuvette). The instrument can deliver sample volumes between 2 and 89 µl with a minimum reaction volume of 150 µl and a maximum of 260 µl. It is able to perform colorimetric, nephelometric and fluorimetric analyses, and, in a separate module of the instrument, potentiometric tests. The optical unit consists of a monochromator (wavelength between 300 and 800 nm), and 12 interference filters (from 340 to 690 nm) with a bandwidth of about ±1·5 nm. An optional ISE module determines sodium, potassium and chloride using the indirect potentiometric technique.

The Monarch has a refrigerated reagent tray (15 °C) that can hold up to 20 wedge-shaped reagent boats (maximum capacity 16 ml); the sample ring holds up to 44 cups, 38 sample or control positions and six calibrator positions. The instrument is programmed via a keyboard and a video display unit with a user-friendly menu.

The theoretical throughput of the instrument is about 400 results/h when performing photometric analyses, and about 600 results/h when the ISE module is included. The Monarch automatically organizes the analytical cycle to maximize throughput, which depends upon the number of samples, the number of analyses per sample and whether one or two reagents are being used.

The evaluation reported here took photometric analyses (biochromatic equilibrium, fixed time, kinetic) and the ISE module into account. Additionally, general features, such as photometric performance and dispensing and diluting accuracy using a bichromate solution, were considered.

The work was done on three different instruments installed in three laboratories. For practical and organizational reasons it was not possible to perform every experiment in each laboratory, as defined in the ECCLS multicentre evaluation protocol [1]; therefore each participating laboratory performed a different part of the evaluation, but some critical tests were repeated in more than one laboratory.

Materials and methods

Reagents

Table 1 shows the reagent and methods used both on Monarch and the comparison instrument.

The Monarch was calibrated with four different calibrators:

(1) ReferrIL A (lot No. 6071) for glucose, urea, creatinine, phosphate, sodium, potassium and chloride (lab 1).

(2) ReferrIL B (lot No. 6051) for cholesterol, total protein, sodium, potassium and chloride (lab 1).

(3) ReferrIL 3 C (lot No. 6071) for bilirubin (lab 1) and urate.

(4) Ultimate (Beckman lot No. M 791935) for bilirubin (lab 2).

The within- and between-batch imprecision was evaluated using the following materials:

(1) Pool of normal sera, subdivided in aliquots and stored at −20 °C.

(2) Precinorm U lot No. 153148 (Boehringer Mannheim).

(3) Precipath U lot No. 152794 (Boehringer Mannheim).

(4) Serachem Lipid lot No. 221085 (Fisher Scientific Orangeburg, New York 10962, USA) (only for cholesterol and triglycerides – high level).

Table 2. Imprecision of the Monarch.

	Level I				*Level II*				*Level III*			
	Mean	CVw (%)	CVb (%)	CVo (%)	Mean	CVw (%)	CVb (%)	CVo (%)	Mean	CVw (%)	CVb (%)	CVo (%)
Glucose (mmol/l)	5·026	1·73	1·47	2·27	6·602	1·60	1·29	2·06	14·848	0·94	1·02	1·38
Cholesterol (mmol/l)	2·886	3·21	4·09	5·20	5·191	2·21	2·96	3·70	8·023	1·53	2·20	2·68
Total bilirubin (µmol/l)	10·76	1·85	2·28	2·93	38·39	1·18	2·03	2·35	98·15	0·59	2·41	2·48
Triglyceride (mmol/l)	0·982	1·95	2·50	3·17	1·149	2·42	2·36	3·38	3·392	1·37	1·95	2·38
Urea (mmol/l)	6·173	2·01	3·99	4·47	10·106	2·39	2·44	3·42	22·67	1·40	2·19	2·60
Creatinine (µmol/l)	86·45	2·84	4·29	5·20	183·87	2·03	2·61	3·30	296·67	1·48	1·83	2·35
AST (U/l)	17·50	2·86	2·13	3·57	59·00	1·35	2·08	2·48	130·40	0·96	1·66	1·92
ALT (U/l)	11·87	4·35	4·89	6·55	48·00	1·35	0·39	1·41	112·42	0·75	0·95	1·21
ALP (U/l)	174·91	1·28	1·18	1·74	333·93	2·80	2·53	3·77	529·40	1·74	1·34	2·20
Na (mmol/l)	119·43	0·47	0·74	0·87	136·69	0·52	0·70	0·88	143·14	0·51	0·69	0·86
K (mmol/l)	4·308	0·61	1·01	1·18	4·437	0·47	0·82	0·94	6·320	0·54	1·12	1·25
Cl (mmol/l)	101·08	0·50	0·88	1·01	106·52	0·44	0·90	1·01	121·40	0·51	1·08	1·19

Notes: w, within batch; b, between batch; o, overall.

Table 3. Calibration stability.

	Period				Trend
	No. days	No. results	Slope	r	probability p†
Glucose	68	37	−0·425	−0·0699	0·677
Urea	69	38	3·093	0·4308	0·007‡
Creatinine	57	30	0·251	0·3265	0·078
Cholesterol	67	41	−0·928	−0·1303	0·417
Urate	71	38	0·107	0·0852	0·610
Total bilirubin	54	36	0·173	0·1688	0·320
Total protein	71	41	0·120	0·1020	0·526
Calcium	69	36	4·221	0·3913	0·018‡
Phosphate	64	40	2·268	0·2994	0·060
Magnesium	36	32	−0·397	−0·4435	0·011‡
Iron	30	25	0·052	0·3178	0·121

† Probability that the slope significantly differs from 0, calculated by Student's t-test.
‡ Significant trend probability.

Table 4. Photometric accuracy; bichromate solution analysed with the pre-load mode.

	Theoretical	Instrument 1			Instrument 2		
Dilution	values Abs†	Abs	(CV%)	Δ%	Abs	(CV%)	Δ %
1	0·667	0·690	(0·4)	+3·45	—		
2	0·995	1·010	(0·6)	+1·51	—		
3	1·320	1·310	(0·5)	−0·76	—		
4	1·6413	1·650	(0·5)	+0·53	1·620	(0·3)	−1·3

† Obtained on Uvikon 860 spectrophotometer.

Quality control materials were reconstituted at the beginning of each working day.

Bichromate stock solution: 20 mmol/l of potassium bichromate in H_2SO_4 0·01 N.

Experimental design

Imprecision: materials at three different levels of concentration were analysed five times per day for 10 days.

Calibration stability: the absorbances of the calibrators were recorded during a period of 30 to 71 days, and a regression analysis was performed to evaluate the possible presence of any significant trends.

Photometric and dispensing accuracy: the rotor was loaded manually, independently of the instrument, with several dilutions of the bichromate stock solution and then analysed with the 'pre-load' mode (this option enables the instrument to be used just as a photometer) using a wavelength of 340/690 nm. The theoretical absorbance of the bichromate solutions were obtained on an Uvikon 860 spectrophotometer (Kontron Instruments). The same solution as above, and a dilution of 1 : 5 of it, were assayed as samples using increasing sample volumes (from 2 to 20 µl), diluted with a constant volume of 180 µl of distilled water; a whole rotor was run for each tested volume. Ten to 100 µl of a 1 : 37 dilution of the same solution were then dispensed with the reagent pipette (setting the sample volume to zero) and diluted with a constant volume of 100 µl of distilled water.

Table 5. Sample dispensing accuracy: bichromate solution analysed as sample.

	Reference	Instrument 1			Instrument 2		
Dilution	values Abs†	Abs	(CV%)	Δ%	Abs	(CV%)	Δ %
2	0·690†	0·680	(1·8)	−1·47	0·710	(0·7)	+2·90
3	1·010†	1·010	(1·4)	0·00	1·010	(0·4)	0·00
4	1·310†	1·310	(1·0)	0·00	1·310	(0·6)	0·00
5	1·650†	1·630	(0·9)	−1·21	1·620	(0·6)	−1·82
5‡	0·3273	0·320	(2·2)	−2·20	0·303	(1·5)	−7·42
10‡	0·6395	0·630	(0·8)	−1·49	0·624	(1·1)	−2·42
15‡	0·9341	0·920	(0·7)	−1·51	0·927	(0·9)	−0·76
20‡	1·2110	1·210	(0·8)	−0·08	1·214	(0·9)	+0·25

† Obtained on the Monarch n.1 with the 'pre-load' mode (see text).
‡ Bichromate solution diluted 1 : 5, theoretical absorbances calculated from the Uvikon values.

Table 6. Dispensing accuracy: bichromate as reagent.

µl	Theoretical values Abs	Instrument 1 Abs	(CV%)	Δ%	Instrument 2 Abs	(CV%)	Δ %
10	0·1492	0·150	(1·2)	+0·54	0·143	(4·2)	−4·16
20	0·2735	0·260	(0·8)	−4·92	0·265	(4·2)	−3·11
30	0·3793	0·360	(0·4)	−5·09	0·362	(1·3)	−4·56
50	0·5471	0·525	(0·5)	−4·04	0·512	(0·5)	−6·42
80	0·7290	0·710	(0·3)	−2·61	0·686	(0·6)	−5·90
100	0·8200	0·790	(0·4)	−3·66	0·770	(0·6)	−6·10

Table 7. Method linearity.

Analyte	Range tested	Claimed limits	r^2	Curvilinearity probability†
Glucose	4·4–38·8 mmol/l	27·7	0·99953	0·1221
Triglyceride	0·9–12·2 mmol/l	11·3	0·99897	0·0346
Total bilirubin	5·1–431 µmol/l	340	0·99995	0·1346
Urea	4·8–30·8 mmol/l	33·3	0·99963	0·0868
Creatinine	97·2–1485 µmol/l	1326	0·99204	0·3504
AST	23–191 U/l	300	0·99979	0·0825
ALT	27–393 U/l	300	0·99990	0·0500
ALP	123–1350 U/l	1000	0·99916	0·1941
Sodium	99–158 mmol/l	120–160	0·99981	0·6810
Potassium	1·6–15·2 mmol/l	2·0–8·0	0·99900	0·8000
Chloride	80–173 mmol/l	75–120	0·99900	0·1000

† According to Burnett and Martin [3, 4].

Method linearity: samples containing high levels of analyte were diluted, in varying proportions, with sera with low levels of analyte. Each dilution was then measured in duplicate.

Method comparison: 60–120 patient samples were analysed on the Monarch, in five–10 runs, over three weeks for each analyte. Results were compared with those obtained by methods and instruments in routine use in the evaluators' laboratories (see tables 1 and 8).

Specimen-dependent carry-over: this was assessed by running six sequences of three high samples (H) followed by three low ones (L). The carry-over was calculated using the formula:

$$\frac{L1 - (L2 + L3)/2}{H3 - (L2 + L3)/2}$$

Method-to-method carry-over: this was evaluated by analysing a mid-level human serum pool, in triplicate, so that

Table 8. Method comparison.

Analyte		N	$\bar{x}$	$\bar{y}$	Slope	Intercept	r	Sxy
Glucose	mmol/l	116	6·199	6·066	0·9782	0·014	0·9965	0·2342
Cholesterol	mmol/l	129	4·431	4·377	1·0000	−0·078	0·9876	0·2125
Total bilirubin	µmol/l	60	60·14	59·05	0·9670	−0·735	0·9988	5·2670
Triglyceride	mmol/l	129	1·678	1·485	0·9024	−0·046	0·9952	0·1110
Urea	mmol/l	124	7·349	7·680	1·0263	0·138	0·9938	0·3940
Creatinine	µmol/l	125	99·0	106·1	1·0796	−0·619	0·9933	4·9504
AST	U/l	122	34·65	33·34	0·8727	3·06	0·9991	1·5482
ALT	U/l	123	36·63	39·33	1·0156	2·66	0·9987	1·9926
ALP	U/l	120	278·5	245·1	0·8258	14·59	0·9996	6·1573
Sodium	mmol/l	84	141·7	140·1	0·9375	7·20	0·9346	1·1942
Potassium	mmol/l	84	4·34	4·21	0·9732	−0·006	0·9970	0·0443
Chloride	mmol/l	115	104·3	104·2	1·0000	0·000	0·9730	1·2314
Magnesium	mmol/l	80	0·85	0·885	0·9800	0·045	0·9790	0·0505
Iron	µmol/l	150	13·80	13·87	0·9870	0·234	0·9880	1·2351

each chemistry was preceded and followed by the other. It was considered that no carry-over had occurred if the variations in a certain sequence were within twice the CV of the method obtained in the within-batch precision.

Results and discussions

Imprecision

The different components of the imprecision were calculated according to the analysis of variance [2]; the results are shown in table 2. The within-run precision was acceptable in the majority of cases. The overall precision of the electrolyte determinations was excellent (CVs always lower than 1% in the case of sodium, and lower than 1·25 for potassium and chloride) and was good for every other analyte tested, with the exception of cholesterol and urea at low concentrations.

Calibration stability

Results are shown in table 3. The stability of the calibration seemed to be good, and a significant trend exists only in the case of urea, calcium and magnesium. For these analytes a daily calibration is advised.

Photometric and dispensing accuracy

As shown in table 4, the accuracy of the photometric system seemed acceptable. Also when bichromate was dispensed as a sample, the results were good (table 5); however, when it was used as a reagent, both instruments showed a consistent negative bias (table 6). This is not very important if the instrument is calibrated with external calibrators. When using the diluted bichromate solution (see table 5 – second half, and table 6), the experimental design does not allow the operator to distinguish between imprecision and inaccuracy due to the photometric system or to the dispensing device. The absorbances obtained were therefore compared with the theoretical value for a similar dilution of the solution. So it is possible to evaluate the overall accuracy of the system, but the components of any imprecision cannot be identified. The linearity of response was also calculated using the formulas proposed by Burnett and Martin [3, 4]. In each of the three cases, the curvilinear probability was not significant (pre-load mode $p = 0{\cdot}5110$ $r^2 = 0{\cdot}9996$, bichromate as sample $p = 0{\cdot}11418$ $r^2 = 0{\cdot}9996$, bichromate as reagent $p = 0{\cdot}3371$ $r^2 = 0{\cdot}9998$.

Linearity

As shown in table 7, the linearity of the IL methods was very good and almost always higher than that claimed by the manufacturer.

Carry-over

The specimen-dependent carry-over was found to be negligible for all the methods studied (table 9). A significant method-to-method carry-over was found only when total protein determination was followed by urate measurement. In this case, the urate value was reduced.

Table 9. Sample-dependent carry-over.

Analyte	High pool	Low pool	% carry-over
Glucose	64·93 mmol/l	1·28 mmol/l	0·03
Cholesterol	6·73 mmol/l	1·66 mmol/l	0·30
Total bilirubin	241 µmol/l	6·5 µmol/l	0·07
Triglyceride	8·25 mmol/l	0·95 mmol/l	0·06
Urea	40·0 mmol/l	3·83 mmol/l	0·05
Creatinine	1200 µmol/l	79·6 µmol/l	0·24
AST	269 U/l	18 U/l	0·02
ALT	381 U/l	23 U/l	0·10
ALP	845 U/l	69 U/l	0·01
Sodium	162 mmol/l	110 mmol/l	0·04
Potassium	5·0 mmol/l	3·8 mmol/l	0·60
Chloride	120 mmol/l	84 mmol/l	0·00

This is caused by a falsely elevated reagent blank. Should such a combination occur during calibration, an important increase of all the urate values would be observed. The absorbance increase of an urate reagent blank after a total protein assay (0·046 Abs) was similar to that found by adding one part of biuret reagent to 500 parts of urate reagent and reading the absorbance after an incubation of 5 min at 37 °C (0·052 Abs). Therefore, a reagent carry-over of about 1/500 can be assumed; this is evident only when particular reagent combinations occur. No carry-over was found when an ALT was followed by an LDH assay (a combination that is highly critical in other random access analysers [5]), nor was there any carry-over with any other combination of the analytes tested.

Method comparison

Data and correlation parameters are presented in table 3. The regression line was computed with the non-parametric linear regression model of Passing and Bablock [6]. The correlation coefficient (r) was good, or very good, in every case (only for sodium was it somewhat lower, but the range tested was very narrow). The slope of the regression line shows that, in the cases of triglyceride, AST and ALP, there was an important negative proportional bias. This bias could be explained for triglyceride because of the differnt kinds of method and calibration material used and for AST and ALP by the different optimization of the methods, and the use (by the Hitachi 737) of a calibrator for enzymatic analyses.

Conclusions

The overall performance of the apparatus seemed to be satisfactory. The instrument is extremely flexible and suitable for development and application of new reagents and research methods, and, moreover, showed a good reliability. It is now used as the routine clinical chemistry analyser in two of the three laboratories who took part in the evaluation.

Acknowledgements

The authors wish to thank Dr P. Iandolo and Dr G. Luise (IL Company) for their help and fruitful discussion. The research reported here was carried out with the support of CNR (National Council for Research).

References

1. *Guidelines for the Evaluation of Analysers in Clinical Chemistry* (ECCLS Document, Volume 3, Number 2, June 1986).
2. KROUWER, J. S. and RABINOWITZ, R., *Clinical Chemistry,* **30** (1984), 290.
3. BURNETT, R. W., *Clinical Chemistry,* **26** (1980), 644.
4. MARTIN, R. F., *Clinical Chemistry,* **26** (1980), 1509.
5. DEGENAAR, C. P., *Clinical Chemistry,* **29** (1983), 1649
6. PASSING, H. and BABLOCK, W., *Journal of Clinical Chemistry and Clinical Biochemistry,* **21** (1983), 709.