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Evaluation of Five New Liquid Stable Applications on the Roche Cobas Integra

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Summary: In the present study the analytical performances of five new liquid applications on the Roche Cobas Integra were evaluated: urea and high density lipoprotein (HDL) cholesterol in serum and glucose, creatinine and inorganic phosphorus in urine.

The analytical evaluation consisted of imprecision, linearity and method comparison performed against either the actual Cobas Integra granulate applications or the corresponding methods on a Hitachi 704, according to the National Committee for Clinical Laboratory Standards protocols. Over 3700 results were obtained within 3 months. Average values of within-run and between-day coefficients of variation (CVs) were 1.15% and 1.48%, respectively, holding to a mean total CV of 2.17%. The linearity was excellent for all the five applications evaluated as the relative non-linearity was always within 1.53%, thus completely fulfilling the 2.5% upper limit. A strict correlation was observed by comparing results of 120 samples with either the corresponding granulate applications on Cobas Integra or the Hitachi reagents. Linear regression analysis of the results yielded correlation coefficients always above 0.987 and the slopes of the *Passing & Bablok* regression lines did not deviate by more than 7% from unity. No drift was observed over 4 hours of operations. In conclusion, the performance of these new Cobas Integra liquid applications, as demonstrated by the present study, proved them to be highly suitable for routine use in clinical laboratories.

Introduction

Productivity, quality, rapidity and reduction of the costs are essential factors in reaching efficiency in modern clinical laboratories. The first step for the fulfilment of this objective is the reorganization of the laboratories and the rationalisation of the work flow, purposes that can be fairly achieved by the introduction of new, modern and fast multifunctional analysers able to mate various analytical techniques (1). Several clinical chemistry "consolidated" analysers fulfilling these important requirements have been presented over the last decade.

The Roche Cobas Integra is a multifunctional random- and continuous-access clinical chemistry analyser with a total capacity of 68 tests maintained on board along with multiple ion-selective electrodes. Eighty-five reagents, contained in cassettes homogeneous in shape and dimensions, allow the performance of over 111 different applications in human body fluids. The analyser is provided by multiple analytical techniques (photometry, turbidimetry, ion selective electrodes and fluorescence polarization) that allow simultaneous measurements of disparate common clinical chemistry analytes, enzymes, proteins, electrolytes, drugs and hormones.

Previous reports have established the analytical performances of the instrument equipped with granulate reagents (2–5). The purpose of the present study was the evaluation of the analytical performances of five new Cobas Integra liquid reagents designed to replace the current granulate ones: urea and high-density lipoprotein (HDL) cholesterol assayed in serum and glucose, inorganic phosphorus and creatinine assayed in urine. Imprecision, linearity and the method comparison study were assessed according to the NCCLS protocols (9–11).

Materials and Methods

Materials

The Cobas Integra was purchased from Roche Diagnostics, a Division of F. Hoffmann-La Roche Ltd, Basel, Switzerland. The instrument used for the comparison study was a Hitachi 704 (Boehringer Mannheim, Mannheim, Germany) (complete technical descriptions of the instruments are given in l.c. (2, 6)) Original reagents, calibrators and controls were used strictly following the instructions of the manufacturers. Lot numbers were unchanged from the beginning of the evaluation in both analysers.

Imprecision

The imprecision was evaluated according to the NCCLS EP5-T2 protocol (9) and results were expressed in terms of within-run,

between-day and total imprecision. The study consisted of the evaluation of two urine or two serum patient pools (low and high) and two urine or two serum lyophilized human control materials: Baxter Dade Urine level 1 and 2 for urine (Baxter) or Roche control N and P for serum (Roche) measurements. The urine and serum pools were prepared by mixing patients' urine or sera in order to achieve final concentrations in the lower and in the upper range of linearity. Aliquots of these pools were stored at −20 °C. Two runs were performed each day separated by a minimum interval of 4 h for 20 operating days (6 days a week). After the first run of each day the samples were tightly closed and stored at 4 °C for further analysis. The time between the runs was dedicated to daily routine tests. Within each run the analyte concentration of the samples was determined in duplicate. Final results were given in terms of CV.

Linearity

The linearity was evaluated according to the NCCLS EP6-P protocol (10). A volume of at least 3 ml of patient urine or serum sample with an analyte concentration close to or slightly exceeding the claimed upper linear range was used as primary sample and dilutions were performed in saline according to the NCCLS protocol. A total of twelve concentrations were used, ten obtained by mixing the starting samples with saline. Starting concentrations and sample dilutions were assayed in duplicate and average results were used for the statistical evaluation. The linearity of the assays was evaluated using common linear regression and *Pearson*'s correlation coefficient (r); the relative non-linearity was measured employing the algorithm described by *Emancipator* et al. (results below 2.5% indicate a good linear fit) (12).

Method comparison

The method comparison for the new liquid applications was performed against the current Cobas Integra granulate applications and with the respective methods available on the Hitachi 704 according to the NCCLS EP9-T protocol (11). A hundred and twenty randomly selected samples with analyte concentrations evenly distributed over the assay range of the application were measured in duplicate for each application. The experiment was spread over a period of eight days by testing 15 patient samples each day. Only fresh urine or serum samples were employed. For serum samples, blood was drawn daily from volunteers in vacuum tubes containing no additive. Serum was obtained after 10 min centrifugation at 3000 *g* and tested with each method within 2 h from venipuncture. Two controls were analysed daily with the respective method. The calculation of the parameters was performed according to the *Passing & Bablok* non-parametric regression procedure (13); relative correlation coefficient (r) and standard error of estimated y ($S_{y,x}$) were obtained by linear standard regression.

Results and Discussion

Imprecision

The results of the within-run, between-day and total imprecision are reported in table 1. Ninety-five percent of the results were below 2%, either for the within-run or the between-day imprecision. The mean within-run and between-day imprecision averaged 1.15% and 1.48%, respectively, far below the previously proposed acceptance criteria (14) and yielding a total mean imprecision of 2.17%. The acceptance criteria could not be achieved in only two out of twenty samples. Higher CVs were observed assaying glucose and phosphorus in urine pool low (tab. 1). To assess drift of the results, the mean values obtained early in the morning were compared with those obtained after the daily routine workload of the instrument (nearby 4 h). After statistical elaboration of the data by *Student*'s t-test, a significant difference was observed only for inorganic phosphorus assayed in urine pool low ($p < 0.01$). Although no deeper analyses were conducted, the absence of significant drift in three out of four samples assayed for inorganic phosphorus and the concomitant elevated between-run CV (3.86%) (tab. 1) rule out a defective performance of the instrument, suggesting faulty behaviour of the urine pool. This finding should not be surprising as the random acquisition of elevated within-run and between-day CVs for phosphorus in both urine and serum is fairly common (6−8).

Tab. 1 Imprecision of serum (HDL-cholesterol and urea) and urine (inorganic phosphorus, glucose and creatinine) assays evaluated on the Cobas Integra according to the NCCLS EP5-T2 protocol (9).

Sample	Mean concentration	CV (%)		
		Within-run	Between-run	Total
HDL-cholesterol (mmol/l)				
1	0.20	1.47	2.65	3.03
2	0.73	0.59	1.71	2.49
3	1.93	0.39	2.74	2.95
4	1.91	0.26	1.55	1.60
Urea (mmol/l)				
1	4.08	2.27	1.02	3.90
2	5.57	1.97	1.67	3.20
3	18.91	0.82	1.69	2.02
4	30.97	0.89	2.46	2.76
Inorganic phosphorus (mmol/l)				
1	13.09	1.31	3.86	4.63
2	11.38	1.21	0.79	1.45
3	27.07	1.19	0.87	1.47
4	27.00	0.94	0.70	1.40
Glucose (mmol/l)				
1	0.54	3.40	1.25	3.81
2	1.16	1.36	1.13	1.83
3	16.88	0.71	0.99	1.26
4	27.45	0.63	0.77	0.99
Creatinine (mmol/l)				
1	4.43	0.88	0.62	1.08
2	6.44	1.2	0.75	1.42
3	18.15	0.66	0.95	1.15
4	15.03	0.81	0.19	0.90

Linearity

The results of the linearity studies are given in table 2. All tests evaluated completely fulfilled the major requisites of linearity required for modern clinical chemistry analysers. The range of linearity met or exceeded the upper limit claimed by the manufacturer. The evaluation of the relative non-linearity according to the method of *Emancipator* et al. (12) confirmed the excellent results for each application, ranging from a minimum of 0.19%

Tab. 2 Linearity of serum (HDL-cholesterol and urea) and urine (inorganic phosphorus, glucose and creatinine) assays evaluated on the Cobas Integra according to the NCCLS EP6-P protocol (10). The linearity of the assays was evaluated by common linear regression and *Pearson's* correlation coefficient; the relative non-linearity was measured employing the algorithm described by *Emancipator* et al. (12).

	Upper limit (mmol/l)	Slope	Inter-cept	$S_{y,x}$	Non-linearity (%)
HDL-cholesterol	5.18	1.00	0.03	0.02	0.78
Urea	34.03	1.01	0.25	0.4	1.53
Inorganic phosphorus	53.12	0.99	0.08	0.23	0.28
Glucose	117.54	1.00	−0.22	0.47	0.19
Creatinine	99.15	1.00	0.16	0.47	0.64

for glucose to a maximum of 1.53% for urea and hence constantly lower than the 2.5% limit.

Method comparison

Results of the method comparison between Cobas Integra liquid applications and both Cobas Integra granulate applications and Hitachi corresponding methods are shown in table 3. No significant deviations from the linearity were observed. The comparison between Cobas Integra liquid and granulate applications yielded *Passing & Bablok* regression slopes not deviating by more than 6% from unity and correlation coefficients not beyond 0.998. The results obtained with the Cobas Integra liquid applications also showed good agreement with the corresponding Hitachi methods. The regression coefficients were always higher than 0.987, reaching correlation coefficients of 0.999 or higher for urea, glucose and phosphorus. The deviation of the slopes was within a 3% upper limit from unity, the only exception being phosphorus (7%).

Conclusions

In the present study the analytical performance of five new liquid applications dedicated to the new Roche Cobas Integra chemistry analyser were evaluated. The entire evaluation of the instrument was performed under routine conditions as Cobas Integra is the principal analyser in our laboratory. The study for imprecision, linearity and method comparison was carried out according to the NCCLS protocols (9−11).

The acceptance criteria for the imprecision study could not be matched only in two out of twenty samples evaluated and thus the final results of the three-months evaluation reflected good global analytical performances of the new reagents and were highly comparable to those previously reported for either the current Cobas Integra granulate reagents (2−5) or other similar reagents on other instruments. In summary, the new Cobas Integra liquid stable applications offer a great advantage over the current granulate reagents, providing highly comparable analytical performances without requiring reconstitution of the granulate reagents and hence ruling out a potential source of pre-analytical variability.

Tab. 3 Method comparison between analytes measured on the Cobas Integra by liquid stable reagents (y) and comparison methods (x), according to the NCCLS EP9-P protocol (11). Regression analyses were performed according to the *Passing & Bablok* non-parametric regression procedure (13) and relative correlation coefficients (r) were obtained by linear standard regression.

	Roche Cobas granulate reagent			Hitachi reagent		
	Regression	$S_{y,x}$	r	Regression	$S_{y,x}$	r
HDL-cholesterol	y = 1.06x+0.02	0.01	1.000	y = 0.97x−0.12	0.03	0.987
Urea	y = 1.00x−0.10	0.14	1.000	y = 1.01x−0.44	0.25	1.000
Inorganic phosphorus	y = 0.98x−0.20	0.68	0.999	y = 1.07x−1.24	0.59	0.999
Glucose	y = 1.00x	0.02	1.000	y = 1.02−0.03	0.07	1.000
Creatinine	y = 0.98x−0.17	0.11	0.998	y = 1.03x−0.97	0.60	0.993

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