A Multicentre Evaluation of the Boehringer Mannheim/Hitachi 704 Analysis System

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Summary: The selective multitest Boehringer Mannheim/Hitachi 704 analysis system was examined according to the ECCLS guidelines in a multicentre evaluation involving four laboratories. Ten routine parameters, covering most of the application settings of the instrument, were measured in the respective laboratory at temperatures 25, 30 or 37 °C. The trial lasted four months and gave more than 40 000 data. It yielded the following results:

1. Within the four laboratories the mean coefficients of variation for three control sera at different concentrations were found to be equal to or better than 1.6% for the within-run imprecision and 2.8% or better for the between-day imprecision.

2. No drift was observed during eight hours.

3. Because of the high linear measuring range a re-run analysis was seldom necessary.

4. Sample-related carry-over was not seen. Reagent-dependent carry-over was measured from cholesterol to uric acid and from triacylglycerols to lipase. Through modification of the cholesterol and triacylglycerol reagents, the carry-over effect was practically eliminated.

5. The recovery of the assigned values of control sera showed average values between 99 and 104%. For bilirubin, creatinine, creatine kinase and alanine aminotransferase some control sera showed deviations greater than 10%.

6. In all cases, regression analysis of the results obtained in comparisons of the present instrument with the Hitachi 705 or 737 yielded slopes close to unity with extreme values of 0.95 and 1.06.

7. During the entire evaluation period there was no malfunction or breakdown of the instruments.

The evaluators came to the conclusion that the analytical performance as well as the reliability and practicability of the Hitachi 704 can be rated as excellent.

Introduction

To establish reliable performance criteria, the Boehringer Mannheim/Hitachi 704 analysis system[1]) was evaluated in a multicentre study. Four clinical laboratories participated in the evaluation. The evaluation protocol mainly follows the ECCLS guidelines for the evaluation of analysers in clinical chemistry (1). These guidelines have already been applied successfully to the multicentre evaluation of the Hitachi 737 (2).

This is the first time that a multicentre trial has been undertaken at three different measuring temperatures. This entailed a certain risk with regard to the comparability of the data, especially the enzyme activities.

To ensure uniformity between the different laboratories, similar instruments (Hitachi 705 and 737 resp.) were used for comparison.

These instruments were chosen because of their wide distribution together with their good performance and acceptance. Moreover, these instruments are already installed for routine analysis in the four laboratories.

Within four months, more than 40 000 data had been collected in the four centres and statistically evaluated at Boehringer Mannheim.

Data were evaluated in four stages:

Familiarization period

The instrument users were trained by experts of the company. An acceptance test was carried out covering imprecision and inaccuracy with 3 control sera and a minimum of 4 parameters.

Initial trial

An imprecision study was carried out for 10 days on the Hitachi 704 and the comparison instrument, using the same 10 analytes as in the main trial.

Main trial

Extensive performance data were obtained from the main trial, which involved imprecision studies, recovery studies, method comparisons, linearity studies, carry-over and drift experiments for 10 analytes. In contrast to the ECCLS-protocol, the within-run im-

precision was determined only on one day instead of three, and the between day imprecision data from both the initial and main trial were used.

Description of the Hitachi 704

The Hitachi 704 is a discrete, selectively operated multitest analyser, which can handle up to 23 chemical analytes including the electrolytes sodium, potassium and chloride; these are determined with an optional ion-selective electrode module. The Hitachi 704 was developed as a logical successor of the Hitachi 705, offering additional special features: an alphanumeric keyboard and improved printout, a real time quality control program, data monitoring and special software for immunological tests (non-linear curve fitting, prozone check). A bidirectional link with a host computer is possible.

The Hitachi 704 is available in 4 versions: a basic model with 37 °C measuring temperature only, a model with cooling unit and three measuring temperatures (25, 30, 37 °C) and both of these versions with an ISE-unit. For the multicentre evaluation the model with cooling unit was used. ISEs were tested in a different laboratory; the results will be published elsewhere. The instrument specificiations are listed in table 1; figure 1 shows the analysis modes of the Hitachi 704. For easy reagent handling special system packs for the Hitachi 704 are available (listed in tab. 2).

Methods

Materials and general procedure

The reagents used on the Hitachi 704 and the comparison analysers are listed in table 3; table 4 shows the most important application settings. The control sera used in the respective trials are shown in table 5.

The four participants of the multicentre evalutation used the same lot of reagent for each of the ten methods.

Predetermined factors were used in the estimation of enzyme activities; a reagent blank was run daily. The factors were the mean of 5 calibration runs using the calibrator for automated systems (Lot. No. 151 266[2])). With the exception of bilirubin, all other substrates were calibrated with a daily freshly prepared calibrator. For the bilirubin assay a fixed factor was determined by 5 calibration runs with the Precibil® control serum (tab. 5).

Measurements were performed at 37 °C in laboratories 1 and 3, at 30 °C in laboratory 4 and at 25 °C in laboratory 2.

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[1]) Abbreviated to Hitachi 704 in the following text.

[2]) Cat. No. 608 432, Boehringer Mannheim GmbH.

Tab. 1. Hitachi 704 instrument specifications   921

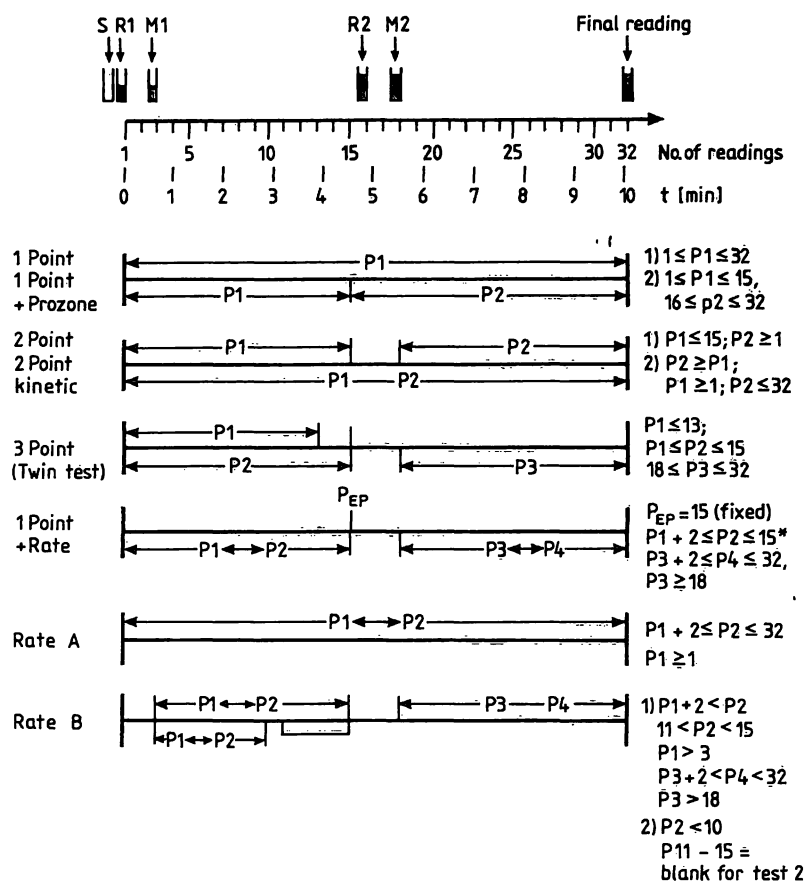| 1	Type of instrument	Discrete selective multianalyser.
2	Test channels	20; with optional ISE-module 23.
3	Test procedures	Endpoint, endpoint with sample blank, kinetic with serum or substrate start, fixed-time kinetic, combination of two endpoint tests, endpoint and kinetic tests, two kinetic tests, performing two tests in one cuvette, two prozone check procedures, ISE, linear and non-linear calibration, isoenzyme calibration, serum-indices (indicating haemolytic, lipaemic and icteric specimens).
4	Throughput	180 tests, with ISE 360 tests, additionally 60 tests with a TWIN-test reagent (two tests in one cuvette).
5	Specimen disk	Turntable with 90 positions in total — 40 in the outer ring for routine samples — 30 in the middle ring for standards/calibrators — 20 in the inner ring for control material, ISE calibrator, stat samples and washing solution Sample cups with 2 ml maximum volume can be used. The minimum sample volume depends on the number of requested tests, on average 50 μl.
6	Sample pipettor	3—20 μl per test, 20 μl for ISE. Imprecision < 1%.
7	Reagent cooling	Optional. Cold water circuit, refrigerator temperature 5—15 °C.
8	Reagent bottles	23 and 50 ml sizes.
9	Reagent dispenser	1 reagent pipettor for dispensing reagents 1 and 2: 50—500 μl.
10	Mixing procedure	1 stirrer mixes the reaction solution after addition of reagents 1 and 2.
11	Reaction rotor	Turntable; one rotation (49 cuvettes + 1) in 20 seconds = 1 working cycle.
12	Reaction cuvettes	48 polyacryl cuvettes, disposable. Volume required: minimum 350 μl, maximum 700 μl. Optical path length: 6 mm.
13	Reaction cycle	10 minutes; addition of reagent 2: 5 minutes after reagent 1.
14	Temperature control	Water bath 37 ± 0.1 °C. With cooling unit 25, 30 and 37 °C.
15	Photometer	o Single beam photometer with 11 available wavelengths: 340, 376, 415, 450, 480, 505, 546, 570, 600, 660 and 700 nm; mono- or bichromatic measurement with freely selectable wavelength combination. o Light source: halogen lamp. o Wavelength adjustment: fixed, via a grating chromator, inaccuracy < 2 nm. o Half band width: 4 nm in the UV range 10 nm in the visible range. o Photometric range of linearity: $A = 0 - 2.5$. o Photometric resolution: $A = 0.0002$. o Photometric inaccuracy: max. 1%.

15	Ion-selective electrodes	o Indirect potentiometry; flow-through electrode with liquid membrane.
		o Reference electrode: Ag/AgCl.
		o Dilution ratio 1 : 31.
		o Incubation temperature 37 ± 0.1 °C, with cooling unit 25, 30, 37 °C.
		o Calibration: automatic 2-point.
		o Measuring range: Na^+ 80—180 mmol/l,
		                K^+ 1.5—10 mmol/l,
		                Cl^- 60—120 mmol/l.

16	Data processing	o Interface: RS 232 C. Bidirectional link with a host computer is possible. o Data input: via alphanumeric keyboard and item select keys. o Data control: CRT-display, 12 inches. o Data output: printer, 80 characters/line. o 2 Floppy disks, 5 1/4 inches, 1.6 M bytes, 1 system disk and 1 data disk for 400 samples and 40 stats. o Reaction monitor for 50 tests.
17.	Water supply	Internal reservoir with an external supply, Quality: ≤ 1 μS, Consumption during operation: 10 l/h.
18	Ambient temperature	15—27 °C.
19	Relative humidity	40—80% at reaction temperature of 37 °C 40—75% at 25 °C or 30 °C.
20	Physical dimensions	Width 1.06 m, Depth 0.78 m, Height 1.03 m.
21	Weight	approx. 300 kg.

Fig. 1. Analysis modes of the Hitachi 704
s = sample
R1, R2: reagent 1, 2
M1, M2: mixing 1, 2
Pi = reading points
*) correction for test 2

Tab. 2. Available Hitachi 704 system packs

Enzymes	Substrates
Alanine aminotransferase	Albumin
Alkaline phosphatase	Bilirubin
α-Amylase	Bicarbonate
Aspartate aminotransferase	Calcium
Cholinesterase	Cholesterol
Creatinine kinase	Creatinine
γ-Glutamyltransferase	Glucose
Lactate dehydrogenase	Iron
Lactate dehydrogenase-1-	Total protein
isoenzyme (α-HBDH)	Triacylglycerols
Lipase	Urea
	Uric acid

Proteins	TWIN-Tests
Immunoglobulins G, A, M	Aspartate/Alanine
Transferrin	aminotransferase
Complement factors 3, 4	Triacylglycerols/Cholesterol
C-reactive protein	Urea/Glucose

For some analytes, several methods are available.

Imprecision

For the within-run imprecision study three different control sera containing three different levels of the corresponding analyte were used (tab. 6). Each participant carried out 21 determinations with each of the analytes. Additionally two fresh human serum pools with different levels of the analytes were measured.

The same control sera were used to establish the between-day imprecision with the Hitachi 704 and the comparison methods. As the between-day imprecision study of the main trial suc-

ceeded directly the initial trial, and both studies were performed under the same conditions, the data of both trials were combined to obtain 21 single data[3]. Only the second value of the duplicate measurements was taken for the statistical calculation.

Drift

Drift effects were studied for all the ten analytes with the same three control sera used in the imprecision trial. Several vials from the same lot were pooled and kept in closed containers in a refrigerator. Small portions were transferred into the sample cups of the analyser 15 minutes before the measurements which were taken hourly over a period of 8 hours. For the bilirubin assay the control material was reconstituted every 2 hours to avoid stability problems of the analyte.

Measuring range and linerarity

The measuring range and the linearity of the methods were checked by mixing a high level serum — which in some cases had to be spiked — with a low level serum to obtain a series of 10 different concentrations. Fresh reagents were used. The measurements were made on two different days.

Carry-over

The carry-over caused by the sample probe was studied in detail according to a model of Broughton (18). Five successive portions of a specimen with a high value (h_1 ..., h_5) followed by 5 successive portions of one with a low value (l_1 ..., l_5) were determined ten times for each of the analytes.

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[3] Twenty days in laboratory 1, and eleven days in laboratory 4 for the comparison instrument Hitachi 705.

Tab. 3. Reagents used during the multicentre evaluation.

Analyte	Method	References	Cat-No., Boehringer Mannheim GmbH		
			Hitachi 704	Hitachi 705	Hitachi 737
Alanine aminotransferase EC 2.6.1.2	"opt. stand. method"*)	3, 4	816 442	620 076	791 628 791 636
α-Amylase EC 3.2.1.1	Substrate= p-nitrophenyl- α-D-maltoheptaoside	5, 6	704 016	704 016	791 385 791 393
Creatine kinase EC 2.7.3.2	"opt. stand. method"*)	3, 4, 7, 8	763 870	763 870	791 474 791 482
γ-Glutamyltransferase new EC 2.3.2.2	Szasz	9, 10	816 345	620 157	791 644 791 652
Bilirubin	2,5-Dichlorophenyl- diazonium salt (DPD)	11	816 299	704 148	791 407 791 415
Cholesterol	Cholesterol oxidase/ peroxidase	12	816 302	704 121	791 440
Creatinine	Jaffé without deprotein- ization	13	816 426	704 130	791 504 781 512
Triacylglycerols	Glycerol-3-phosphate oxidase/peroxidase	14, 15	816 370	704 113	791 768
Urea	Urease/glutamate dehydrogenase	16	816 361	773 484	791 695 791 709
Uric acid	Uricase/peroxidase	17	816 353	704 156	791 679 791 687

*) "optimized standard method" conforming to the recommendations of the Deutsche Gesellschaft für Klinische Chemie.

Tab. 4. Application settings of the selected methods.

Method	Wavelengths		Mea- suring points	Measuring mode and time[1] (min)	Reaction course incr./ decr.[2]	Sample blank	Sample volume (µl)	Reagent volume	
	main (nm)	side (nm)						R1 (µl)	R2 (µl)
Alanine aminotransferase	340	376	19—32	con 5.9—10	decr.		20	350	70
α-Amylase	415	660	25—32	con 7.8—10	incr.		20/10[3])	350	70
Creatine kinase	340	376	22—32	con 6.8—10	incr.		10	350	70
γ-Glutamyltransferase	415	660	19—32	con 5.9—10	incr.		10	350	70
Bilirubin	546	600	15/32	EP 10	incr.	+	10	350	350
Cholesterol	505	700	32	EP 10	incr.	—	5	500	—
Creatinine	505	570	19—23	kin 5.9—7.2	incr.		20	350	70
Triacylglycerols	505	700	32	EP 10	incr.	—	5	500	—
Urea	340	376	19—22	kin 5.9—6.8	decr.		5	350	100
Uric acid	570	660	32	EP 10	incr.	—	5	100	350

[1]) kin = kinetic mode, EP = Endpoint, con = continuous mode
[2]) decr. = decreasing, incr. = increasing
[3]) 10 µl at 37 °C

A carry-over constant can be calculated according to the following relation:

$$K = \frac{\text{mean } (l_1 - l_s)}{\text{mean } (h_s - l_s)} \ (n = 10)$$

Reagent-related carry-over can be caused by the reagent pipetting system, the stirrer and the cuvettes. Carry-over from the lactate dehydrogenase of the alanine aminotransferase assay to the lactate dehydrogenase assay caused by reagent sampling was checked in one centre. Reagents A and B were pipetted alternately, where A represents the alanine aminotransferase and B the lactate dehydrogenase. For this experiment only, the reagent containing the lactate dehydrogenase (about 3000 U/l; A 1) was pipetted before reagents 1 and 2 of B.

Similar studies with other reagent combinations such as cholesterol-uric acid and triacylglycerols-lipase were carried out by the evaluation group of Boehringer Mannheim, and are discussed in the Results section.

Comparison of the analytical procedures

Accuracy was examined by 3 experiments:

Mean values obtained in the between-day imprecision study were compared:

a) with the assigned values of the three control sera and
b) with the mean value of the comparison method.

Tab. 5. Control sera chosen for the Hitachi 704 evaluation.

Control serum*)	Lot.-No.
Precinorm® U	151 407, 151 621 1-595, 1-599
Precipath® U	1-528, 150 801 151 464, 150 456 150 457, EV 704
Precinorm® E	3-372, 151 935
Precipath® E	3-344, 152 147
Precilip®	1-376, 150 817 151 661
Precilip® EL	1-308, 153 302
Precibil	1-210

(Boehringer Mannheim GmbH, Mannheim, FRG)

M + D Moni-trol I	204
M + D Moni-trol II	104

(AHS/Deutschland GmbH, Bereich Merz + Dade, München, FRG)

Validate® N	5 334 113
Validate® A	5 345 113

(Gödecke AG, Freiburg, FRG)

Kontrollogen®-L	623 124 C
Kontrollogen®-LP	623 210 A

(Behringwerke AG, Marburg/Lahn, FRG)

Seronorm	162
Pathonorm L	20
Pathonorm H	20

(E. Merck AG, Darmstadt, FRG)

*) The Precinorm® U 151 621, Precipath® EV 704 and Precipath® U 151 464 were used as control sera 1, 2 and 3 in the imprecision and recovery studies.

In a second experiment, up to 25 different control sera (dependent on the analyte) were measured in duplicate assays with the Hitachi 704 and the comparison method. The recoveries of the assigned value were calculated and compared with those for the comparison method.

Each of the 4 laboratories analysed 100 fresh human sera (10 sera per day for 10 days), covering the entire analytical range for each of the 10 analytes with the Hitachi 704 and the comparison instrument. Both methods were measured in parallel.

Results

The ten methods were not chosen arbitrarily but were selected with a view to checking as many application settings of the instrument as possible (e. g., wavelength, measuring points, sample and reagent volumes and reaction course). Nearly all performance criteria of the instrument are covered by these ten analytical methods.

Tab. 6. Assigned values of the control sera 1—3 at 25, 30 and 37 °C.

Analyte	Temp. (°C)	CS 1 Precinorm® U 151 621	CS 2 Precipath® EV 704*)	CS 3 Precipath® U 151 464
Alanine amino-transferase (U/l)	25	30.5	66.0	79.5
	30	40.0	—	107
	37	55.5	121	146
α-Amylase (U/l)	25	214	300	391
	30	281	—	523
	37	398	568	739
Creatine kinase (U/l)	25	111	180	212
	30	168	—	319
	37	258	414	489
γ-Glutamyl-transferase (U/l)	25	34.0	63.0	116
	30	43.0	—	148
	37	57.0	103	192
Bilirubin (μmol/l)	37	39.2	75.3	105
Cholesterol (mmol/l)	37	2.98	5.10	2.80
Creatinine (μmol/l)	37	210	407	345
Triacylglycerols (mmol/l)	37	1.13	1.76	1.16
Urea (mmol/l)	37	8.82	18.3	24.8
Uric acid (μmol/l)	37	306	381	538

*) enzyme activities for 30 °C were not declared.

Imprecision

Figure 2 shows the condensed data for the within-run imprecision of all participants and the three control sera.
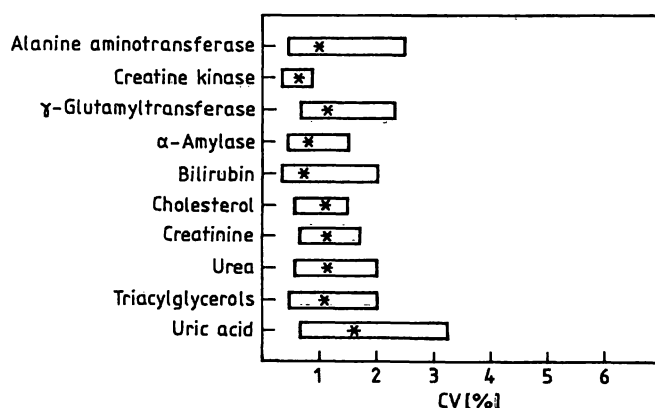

Fig. 2. Within-run imprecision of 4 laboratories and 3 control sera.
For assigned values of the control sera see table 6.
CV = coefficient of variation
* = median of the CV's
□ = range of the CV's

The asterisks respresent the median and the blocks the range of the coefficients of variation[4]) for all laboratories and control sera. The median of the CV for all 10 analytes is equal to or better than 1.6%. Only for alanine aminotransferase, γ-glutamyltransferase and uric acid does the CV exceed 2% (2.5%, 2.3% and 3.2% respectively).

Similar results were obtained for the within-run imprecision in human sera. Table 7 shows the data for a normal and pathological serum obtained by the laboratories measuring at 37 °C.

Tab. 7. Within-run imprecision in human sera with the Hitachi 704 at 37 °C (n = 21).

Analyte (unit)	Mean	Coefficient of variation (%)
Alanine aminotransferase (U/l)	20	2.3
	82	0.6
α-Amylase (U/l)	97	1.5
	143	0.8
Creatine kinase (U/l)	47	1.6
	902	0.4
γ-Glutamyltransferase (U/l)	62	0.7
	246	0.4
Bilirubin (μmol/l)	13.7	2.4
	35.9	0.8
Cholesterol (mmol/l)	4.77	0.6
	11.9	0.4
Creatinine (μmol/l)	70.7	2.2
	178	1.1
Triacylglycerols (mmol/l)	1.64	1.8
	4.08	0.5
Urea (mmol/l)	5.89	2.1
	24.3	0.9
Uric acid (μmol/l)	168	2.5
	492	0.6

For the same control sera, figure 3 shows the CV of the between-day imprecision study for the Hitachi 704 and the comparison instrument. The CV is below 3% except for alanine-aminotransferase (3.1%), bilirubin (6.5%), urea (3.5%) and uric acid (3.9%). The median of the CV is around 2% (2.8% for bilirubin and 0.7% for amylase).

Figure 4 gives a more detailed presentation of the between-day imprecision data for the control sera 1 and 3, for one laboratory in each case. The imprecision of the Hitachi 704 is remarkably better than that of the comparison instruments. This may be the case if a new instrument is compared with one that has been in routine use for years.

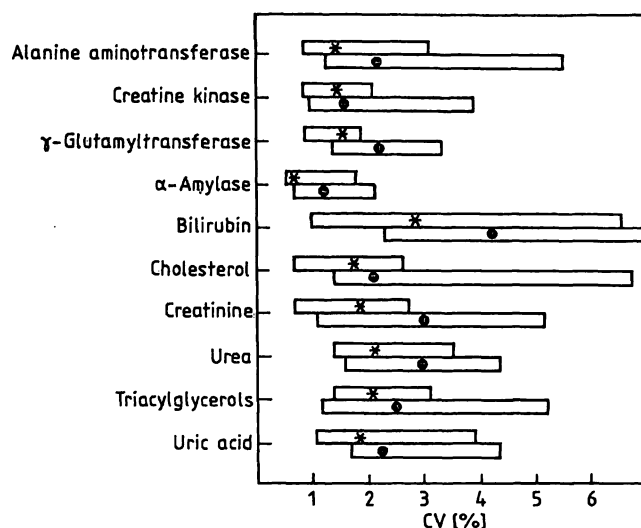[4]) Abbreviated to CV in the following text.

Fig. 3. Between-day imprecision of 4 laboratories and 3 control sera for the Hitachi 704 and the comparison instrument. For assigned values of the control sera see table 6.
CV = coefficient of variation
* = median of the CV's for the Hitachi 704
o = median of the CV's for the comparison instrument
□ = range of the CV's, upper block for the Hitachi 704, lower block for the comparison instrument

The relatively high CV (maximum 6.5%) of bilirubin reflects the instability of this analyte: the bilirubin value is dependent on the influence of light and the reconstitution time of the control serum. Therefore it lends itself poorly to instrument testing unless pre-analytical conditions (mainly reconstitution and storage) are standardized.

Drift

All data have been represented graphically. Only bilirubin in one laboratory shows a severe drift because the pre-analytical conditions were not maintained. For all other methods no drift was found. Figure 5 illustrates the excellent reproducibility for amylase and cholesterol over 8 hours.

Measuring range and linearity

The linearity study for alanine aminotransferase at 37 °C is shown in figure 6. In the left-hand diagram the measured activity is plotted against the target activity, which is obtained by regression calculation of the first 4 points, this being the segment more likely to be linear. The higher values tend to deviate more and more from the identity y = x. A better critical examination is possible for the right-hand diagram which shows the relative deviation of measured and expected values. The acceptance criteria for linearity was set at a limit of ± 5% from the expected value.
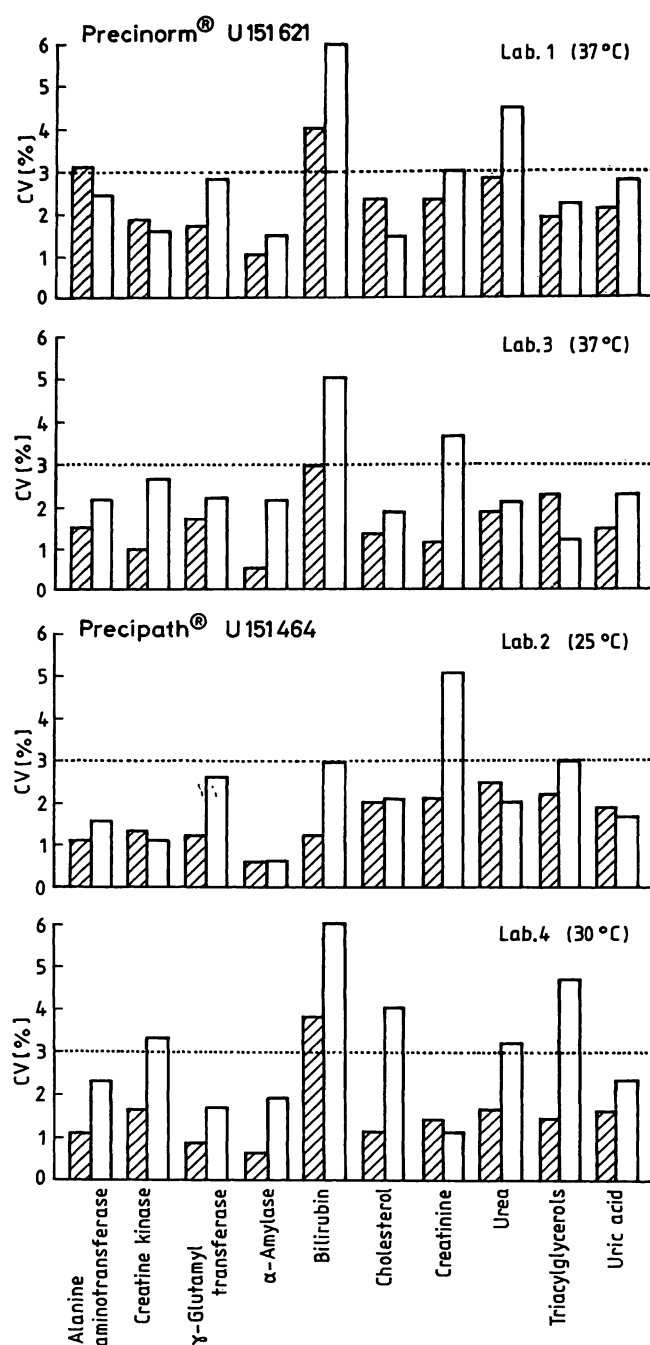
Fig. 4. Between-day imprecision in each laboratory (Hitachi 704 and comparison instrument).
For assigned values of the control sera see table 6.
▨ = Hitachi 704
☐ = Hitachi 705 (Lab. 2: Hitachi 737)

In the given example the 5% deviation is attained at 700 U/l, i.e. the method is linear up to 700 U/l.

Table 8 summarizes the results of the linearity experiment. The measuring range is shown in the last column while the preceding column represents the values up to which the linearity has been checked. A wide linear measuring range was found, seldom requiring a sample predilution.

Because the linearity was checked with fresh reagents, the linearity limits were even higher than those claimed by the manufacturer.
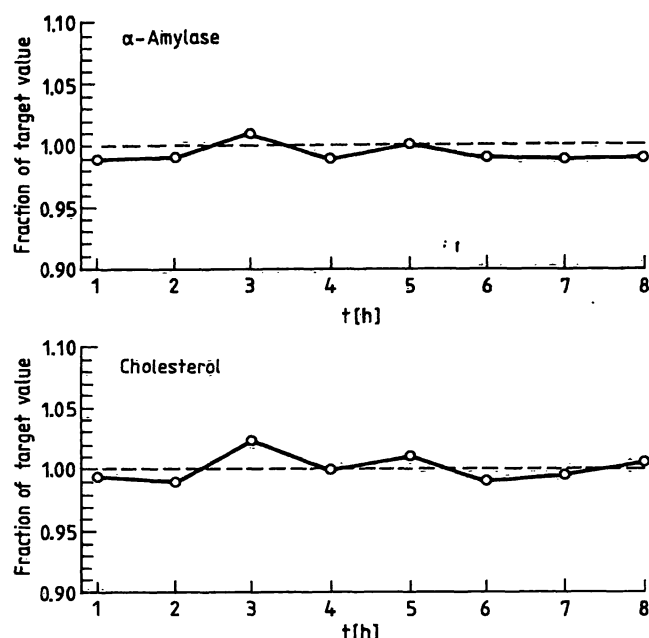
Fig. 5. Eight-hour drift-experiment; recovery of α-amylase and cholesterol in control serum 2.

Tab. 8. Linearity on the Hitachi 704*).

Analyte	Temp. °C	Unit	Linearity	
			tested up to	found
Alanine amino-transferase	25	U/l	1350	1100
	30		1850	950
	37		1200	700
α-Amylase	25	U/l	1650	≥ 1650
	30		5200	3000
	37		1700	≥ 1700
Creatine kinase	25	U/l	1250	≥ 1250
	30		2000	1500
	37		1200	≥ 1200
γ-Glutamyl-transferase	25	U/l	1500	≥ 1500
	30		2000	≥ 2000
Bilirubin	30	μmol/l	360	≥ 360
Cholesterol	25	mmol/l	25	≥ 25
Creatinine	25	μmol/l	2100	≥ 2100
Triacylglycerols	25	mmol/l	13	≥ 13
Urea	37	mmol/l	57	47
Uric acid	25	μmol/l	1370	1000

*) freshly prepared reagents. For the substrates only the highest tested linearity is presented.

Carry-over

Table 9 shows results from the investigation of sample-related carry-over. Only the highest carry-over recorded by any laboratory for an analyte is reported in this table. The minimal and maximal values of the
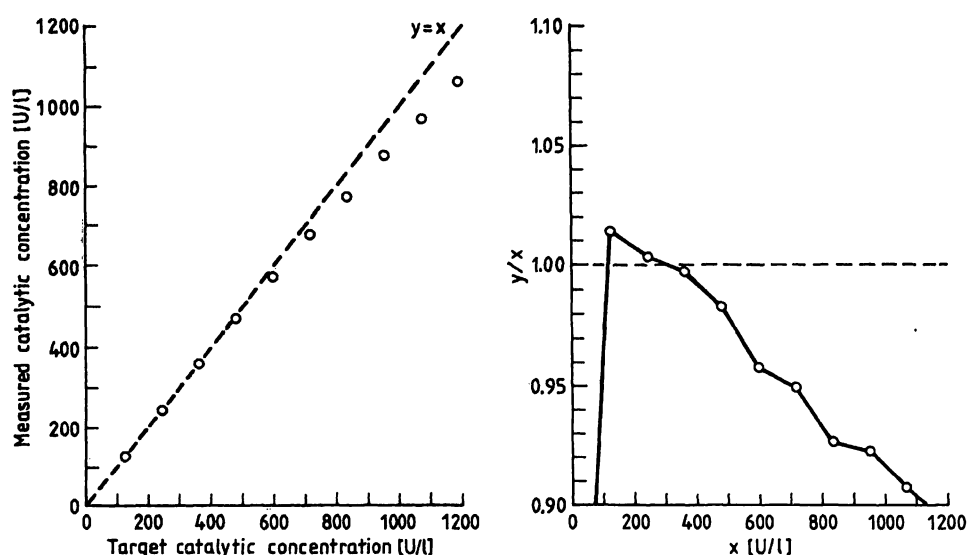
Fig. 6. Linearity of alanine aminotransferase at 37 °C (lab. 1).

Tab. 9. Sample-related carry-over on the Hitachi 704.

Analyte	Unit	High concen-tration h	Low concen-tration l[1])	$l_1 - l_5$ minimum	median	maxi-mum	Standard devia-tion[2]) of l_3, l_4, l_5	90% range[2]) of l_3, l_4, l_5	Carry-over constant $K \cdot 10^4$
Alanine aminotransferase	U/l	> 4000	25.1	− 1.7	0.3	1.5	0.59	1.95	0.35
α-Amylase	U/l	2350	66.0	− 4.0	1.0	4.0	1.98	5.5	0.88
Creatine kinase	U/l	2100	33.0	− 1.0	0.0	1.0	0.52	2.0	− 0.48
γ-Glutamyltransferase	U/l	170	24.0	− 2.4	0.0	2.4	0.95	2.7	12.1
Bilirubin	μmol/l	650	10.1	− 0.86	−0.26	0.86	0.80	2.14	− 2.1
Cholesterol	mmol/l	11.9	2.25	− 0.03	0.0	0.05	0.02	0.05	0
Creatinine	μmol/l	1079	76.9	− 2.7	0.0	2.7	1.59	6.2	0
Triacylglycerols	mmol/l	12.0	0.62	− 0.01	0.0	0.29	0.05	0.14	26.0
Urea	mmol/l	35.0	2.83	− 0.17	0.0	0.50	0.20	0.58	15.4
Uric acid	μmol/l	803	334	−14.3	−1.8	9.5	8.7	24.1	−13.0

[1]) median of the ten values of l_3, l_4 and l_5
[2]) n = 30

difference $l_1 - l_5$ as well as the median of the ten series are reported. In all cases the median is less than the standard deviation of the low concentration. If the maximal value of the difference $l_1 - l_5$ is compared with the 90% range of the low concentration, the difference is lower than the range, except for triacylglycerols (twice the range). But this poor result for triacylglycerols was found in only one of the ten series, so that the occurrence of systematic carry-over cannot be claimed.

We think that the comparison of the interference effect with the imprecision of the uninfluenced low values is more illustrative than the carry-over constant. As the carry-over effect is within the noise level of the determination of the respective analyte, the difference often becomes negative.

Reagent-dependent carry-over for the combination alanine aminotransferase to lactate dehydrogenase is negligible, values showing less than 10 U/l at 37 °C, i. e. less than 3% of the reference limit.

A carry-over caused by both the reagent pipettor and the cuvettes was found with the system cholesterol to uric acid. The uric acid results are increased by about 7.8 μmol/l at 37 °C, if this assay is performed after a cholesterol assay, or in a cuvette which has previously been used for a cholesterol test. The cholesterol oxidase is responsible because it affects the colorimetric reagent of the uric acid test. Boehringer Mannheim has modified this enzyme, so that the carry-over is now negligible.

The lipase result is increased about 600 U/l if a lipase assay is performed after a triacylglycerol test. This is due to the lipase activity of the triacylglycerol reagent. In the past it was recommended that the lipase assay should be run in batch-mode and with a separate cuvette ring. The latter is not necessary if the triacyl-glycerol test is programmed so that the reaction takes place in the first 5 minutes of the reaction cycle and then hydrochloric acid is added to deactivate the lipase in the reaction mixture. The sample carry-over

can be avoided, if both channels are separated from each other and a lipase assay is never requested after a triacylglycerol test. Taking these points into consideration the lipase assay can then be performed in the usual selective mode.

Comparison of analytical procedures

Recovery of the assigned value in control samples

Figure 7 shows the recovery of assigned values for control serum 1. These results were obtained from the mean of the between-day imprecision (n = 20 or 21; 2nd of the duplicate measurements). Only the urea and uric acid recoveries in laboratories 2 and 3, respectively, are clearly outside the ± 5% limit, although they are within the ± 10% range. Similar results were found with the other two control sera.

Table 10 summarizes the results of the recovery studies in 25 different control sera. The mean recovery (median) is very close to 100% for all analytes with extreme values of 99 and 104%. Hence the majority of deviations from the assigned value is within ± 5%. A more marked deviation can be seen in the case of creatine kinase, with 7% of the values (4 labs with 20 to 25 sera) deviating more than 20% from the assigned value. This is possibly due to the specific composition of control sera from different sources, and the dependency on the reconstitution time. The deviations (more than 10%) in the case of bilirubin can be explained by the instability of the analyte. The target value of creatinine is dependent on the method. For both analytes the agreement with the comparison instrument is good.

It should be mentioned that these results were established by a single experiment, whereas the mean of several independent series should be chosen for the examination of the accuracy.
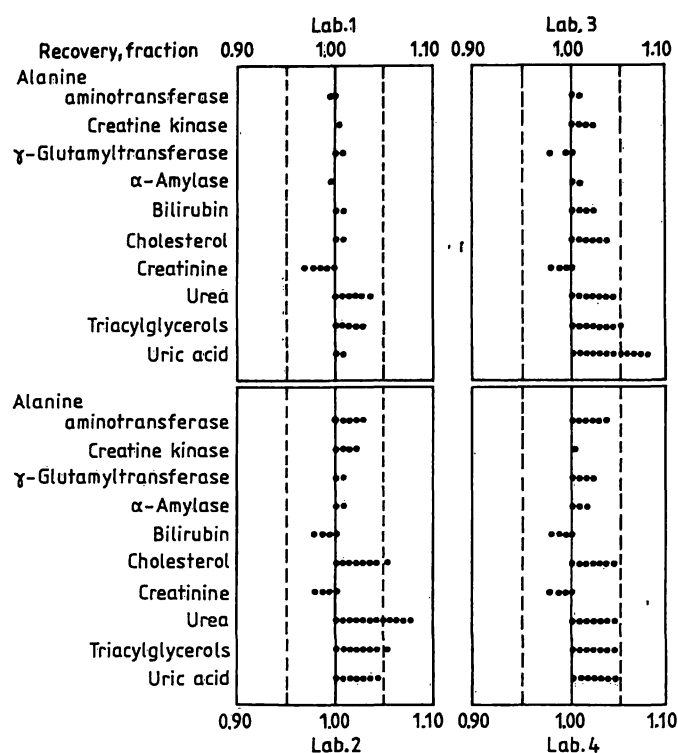

Fig. 7. Recovery of assigned values for control serum 1 (lab. 1 to 4).

Method comparison with patient specimens

The statistical evaluation by a non-parametric method of *Passing & Bablok* (19, 20) yielded slopes for the regression line which are close to unity in all cases with extreme values of 0.95 and 1.06. The intercepts are negligible (see tab. 11). The medians of the relative differences do not show any significant deviations from the comparison method. The results reflect close agreement with the comparison instrument, i.e. the established laboratory method.

Plots for the comparison of methods are shown in figure 8. To improve the graphical presentation, not all figures show the whole measuring range.

Tab. 10. Recovery in 25 different control sera.
Distribution of the recoveries (in %) as a function of the % deviation from assigned value.

Analyte	Mean recovery (median) of assigned values in %	% Deviation from assigned value				
		0 − <5	5 − <10	10 − <15	15 − <20	≥20
		Distribution of the recoveries in %				
Alanine aminotransferase	103	51	36	9	3	1
α-Amylase	101	89	9	1	0	0
Creatine kinase	101	81	12	0	0	7
γ-Glutamyltransferase	101	88	9	3	0	0 ·
Bilirubin	102	58	23	15	4	0
Cholesterol	104	66	31	3	0	·· 0
Creatinine	99	69	19·	10	2	0
Triacylglycerols	103	55	35	7	3	0
Urea	101	81	16	2	1	0
Uric acid	104	69	27	2	2	0

Tab. 11. Method comparison with human sera on the Hitachi 704 (y) and comparison instrument (x) (Hitachi 705, Lab. 1, 3, 4 and Hitachi 737, Lab. 2; n = 100).

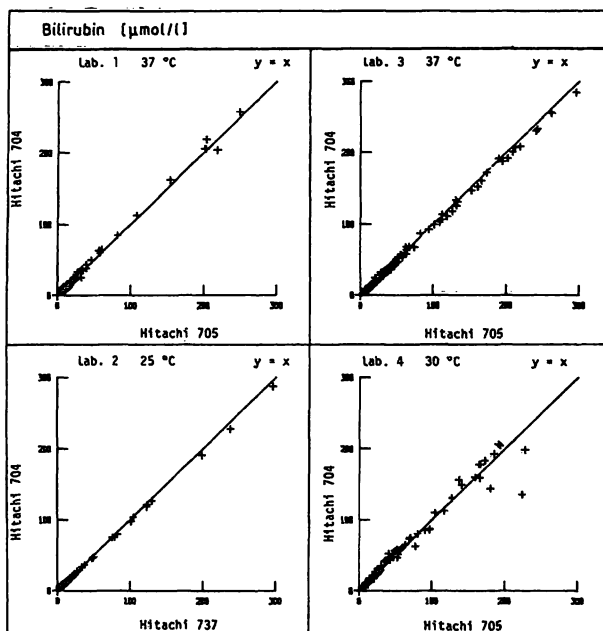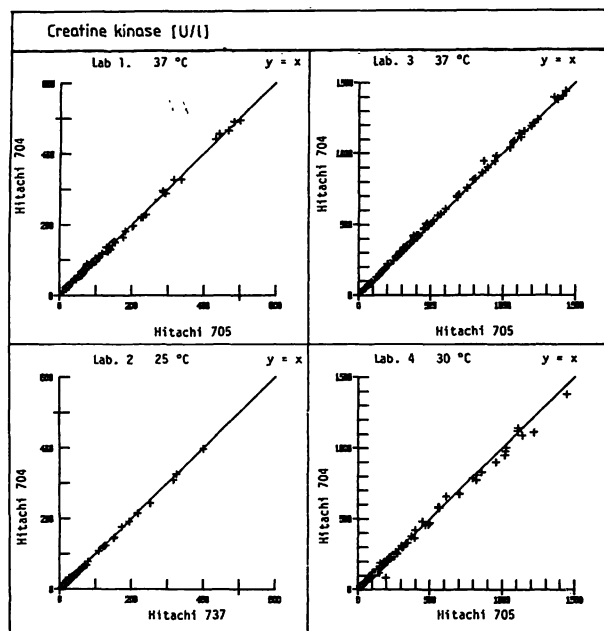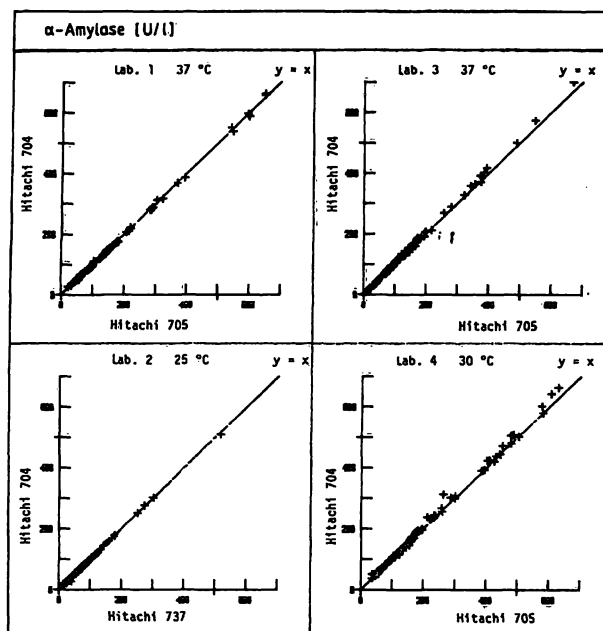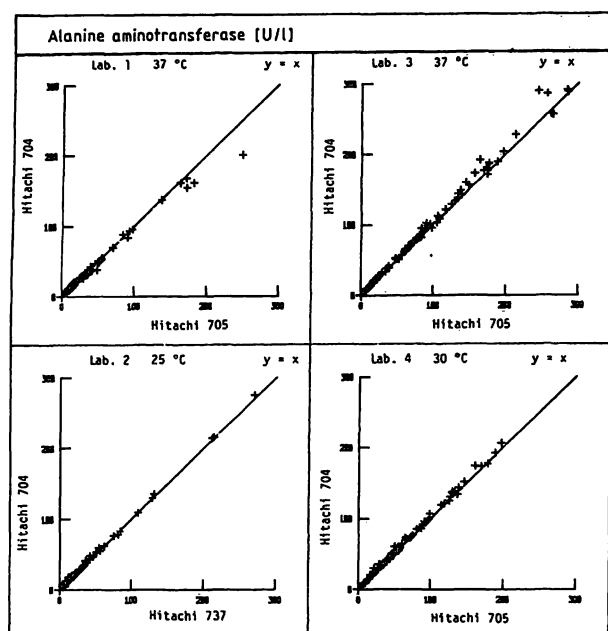| Analyte (Unit)	Lab.	Temp. (°C)	Range (from x)	Regression analysis y = bx + a		Median rel. diff. (%)
				b	a	
Alanine aminotransferase	1	37	4 — 465	0.97	0.44	−0.47
(U/l)	3	37	7 — 900	1.03	0.47	3.86
	2	25	3 −1929	1.00	0.00	0.00
	4	30	5 — 427	1.03	0.32	3.29
α-Amylase	1	37	23 −1235	0.98	1.00	−0.82
(U/l)	3	37	9 −2206	1.04	−1.25	2.74
	2	25	13 −3906	1.00	0.00	0.00
	4	30	36 — 630	1.03	−0.97	2.52
Creatine kinase	1	37	4 −1101	0.99	1.77	1.47
(U/l)	3	37	27 −1859	1.02	0.53	2.15
	2	25	4 −1037	0.99	−1.82	−6.25
	4	30	8 −3200	0.96	2.79	−2.22
γ-Glutamyltransferase	1	37	7 — 728	1.02	0.92	2.76
(U/l)	3	37	6 −1872	1.01	0.80	2.01
	2	25	3 — 951	0.95	0.16	−5.17
	4	30	6 −1900	1.00	0.00	0.00
Bilirubin	1	37	6 — 775	1.06	−0.68	2.11
(μmol/l)	3	37	1,5 — 451	0.97	−0.34	−3.47
	2	25	1,7 — 296	0.96	0.68	1.00
	4	30	3 — 397	1.04	−0.51	1.92
Cholesterol	1	37	0.93— 10.0	1.00	0.00	0.00
(mmol/l)	3	37	3.26— 15.0	1.00	0.03	0.32
	2	25	1.40— 24.4	0.98	0.03	−1.35
	4	30	1.26— 14.3	1.00	0.03	0.48
Creatinine	1	37	24.8 −1048	1.02	−0.88	0.40
(μmol/l)	3	37	61.9 −1676	1.02	−1.77	0.81
	2	25	44.2 −1087	1.02	−0.88	1.28
	4	30	26 — 674	1.04	−2.65	2.85
Triacylglycerols	1	37	0.43— 9.1	1.01	0.00	1.28
(mmol/l)	3	37	0.40— 15.5	1.00	0.04	1.59
	2	25	0.49— 29.1	1.02	0.02	2.87
	4	30	0.35— 9.67	1.02	−0.01	1.48
Urea	1	37	2.50— 45.0	1.00	−0.17	−2.49
(mmol/l)	3	37	3.50— 55.8	1.03	−0.21	1.37
	2	25	2.50— 25.0	1.00	0.00	0.00
	4	30	1.50— 43.2	1.04	0.06	4.62
Uric acid	1	37	79 — 767	1.05	−11.30	1.39
(μmol/l)	3	37	175 −1122	1.00	7.14	1.39
	2	25	119 — 660	1.00	0.00	0.00
	4	30	31 — 861	1.01	−0.59	0.43

Interferences

Since the multicentre evaluation was primarily concerned with the Hitachi 704 instrument and not with the reagents applied to it, interference studies were not carried out.

Assessment of practicability

In addition to the analytical performance, characteristics such as reliability, practicability and integration into the laboratory organisation are important for the evaluation of an analytical instrument. These attri-

butes cannot be measured accurately and no general evaluation protocol can be applied. Nevertheless, these points should be observed during the evaluation period. Only a brief summary of the impressions will be presented in the following.

No problems were associated with the installation of the Hitachi 704. The laboratory staff was well acquainted with the instrument within two days, which is normal for an operator with experience on other Hitachi analysers. The instrument can be operated easily, and the data input is very simple. However, the characters of the keyboard are arranged alpha-

Alanine aminotransferase [U/l]

α-Amylase [U/l]

Creatine kinase [U/l]

Bilirubin [µmol/l]

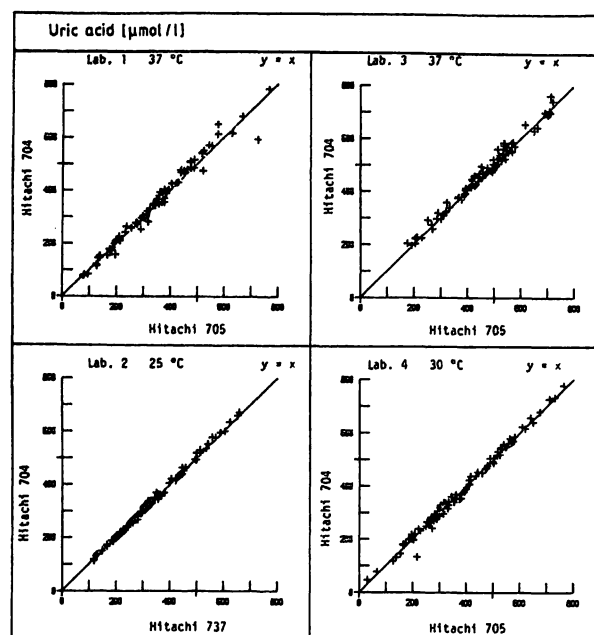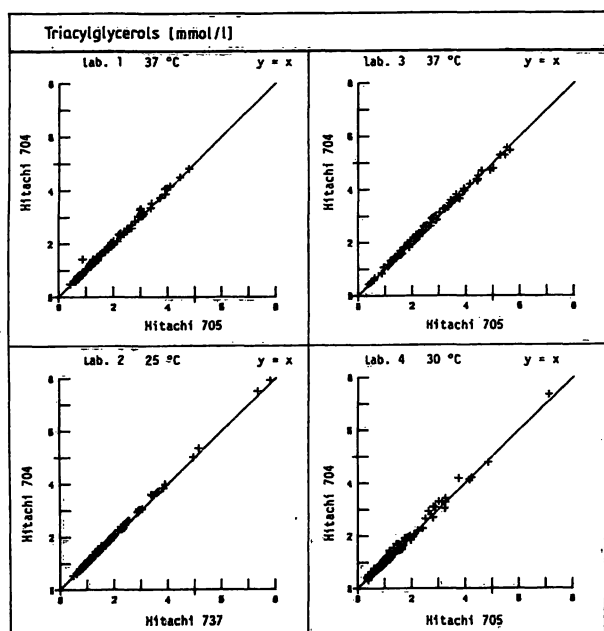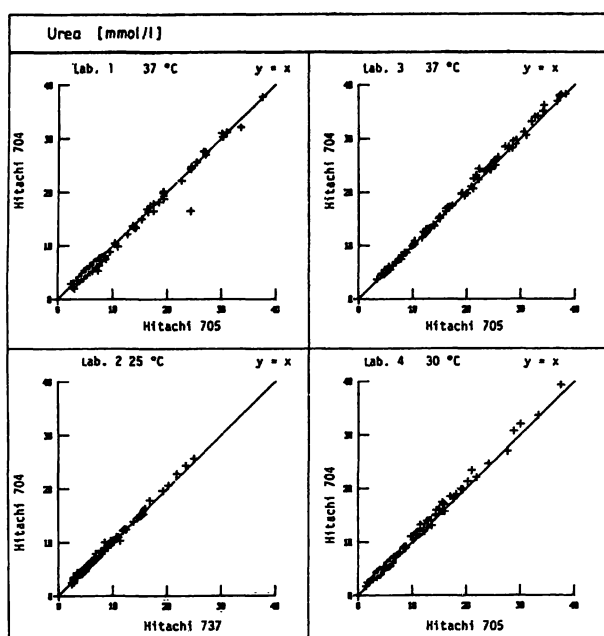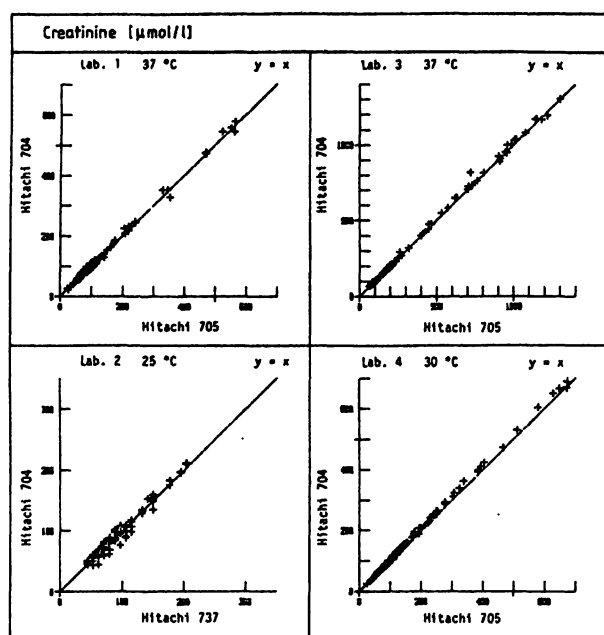γ-Glutamyltransferase [U/l]

Cholesterol [mmol/l]
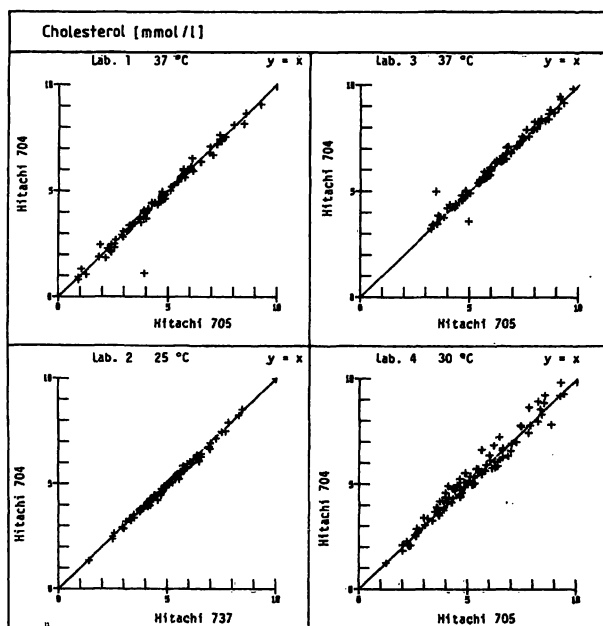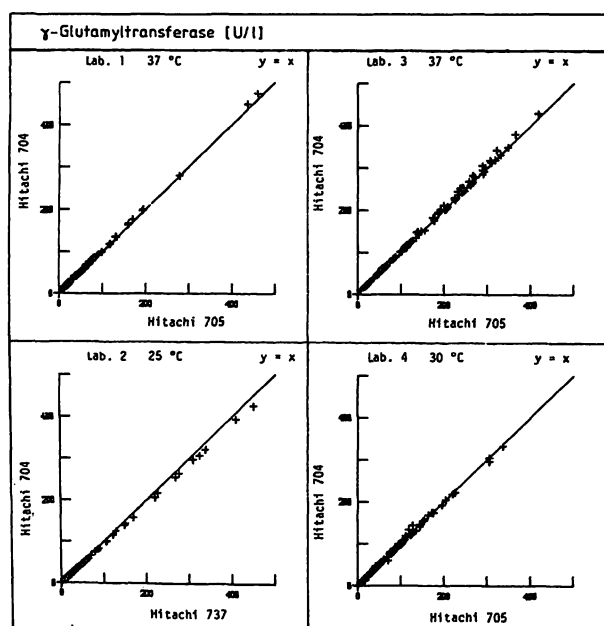
Fig. 8. Method comparison from 4 laboratories.

betically, which is unusual for users who are familiar with the conventional typewriter keyboard. On the other hand, one can easily get accustomed to this arrangement.

For the frequently requested analytes the reagent bottles are too small for longer series. Boehringer Mannheim will introduce concentrated reagents for more than ten methods so that at least twice the number of determinations per bottle can be made.

The ECCLS-protocol recommends a logbook in which any breakdown or defect of the instrument is recorded. In our case, keeping this logbook was not actually necessary. There were no breakdowns in any of the laboratories at any time during the entire evaluation period. Only during the initial trial in laboratory 4, was there a problem with the light source of the photometer. This was recognized from the results of the day-to-day imprecision run. After exchange of the lamp, this instrument ran without any fault.

Reliability and practicability, as well as the analytical performance of the Hitachi 704, can be rated as excellent. The Hitachi 704 is well suited for routine operation and emergency analysis in small to medium-sized laboratories, and as a back-up system for large laboratories.

Acknowledgement

References

1. Haeckel, R., Busch, E. W., Jennings, R. D., Kohholm, G. & Truchaud, A. (1985) ECCLS Document 5, No. 6 (3rd draft).
2. Knedel, M., Haeckel, R., Seidel, D., Thiery, J., Vonderschmitt, D. J., Haenseler, E., Hubbuch, A., Stockmann, W. & Völkert, E. (1986) J. Clin. Chem. Clin. Biochem. 24, 409–432.
3. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie (1970) J. Clin. Chem. Clin. Biochem. 8, 182–192.
4. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie (1972) J. Clin. Chem. Clin. Biochem. 10, 182–192.
5. Rauscher, E., Bülow, S. v., Neumann, U. & Schaich, E. (1981) Ber. Österr. Ges. Klin. Chem. 4, 150.
6. Scholer, A. & Hohenwallner, W. (1984) J. Clin. Chem. Clin. Biochem. 22, 677–684.
7. Szasz, G., Gruber, W. & Bernt, E. (1976) Clin. Chem. 22, 650–656.
8. Gruber, W. (1978) Clin. Chem. 24, 177–178.
9. Persijn, J. P. & Van der Slik, W. (1976) J. Clin. Chem. Clin. Biochem. 14, 421–427.
10. Szasz, G. & Persijn, J. P. (1974) J. Clin. Chem. Clin. Biochem. 12, 228.
11. Wahlefeld, A. W., Herz, G. & Bernt, E. (1972) Scand. J. Clin. Lab. Invest. 29, Suppl. 126, Abstract 11.12.
12. Seidel, J. Hägele, E. O., Ziegenhorn, J. & Wahlefeld, A. W. (1983) Clin. Chem. 29, 1075–1080.
13. Bartels, H. (1972) Clin. Chem. Acta 37, 193–197.
14. Wahlefeld, A. W. (1969) Ann. Clin. Biochem. 6, 24.
15. Nägele, U., Hägele, E. O., Sauer, G., Wiedemann, E., Lehmann, P., Wahlefeld, A. W. & Gruber, W. (1984) J. Clin. Chem. Clin. Biochem. 22, 165–174.
16. Neumann, U. & Ziegenhorn, J. (1977) XVI. Nordiska kongressen for klinisk kemi och klinisk fysiologi Oulu, Finland.
17. Klose, S. (1981) Boehringer Mannheim GmbH, pers. communication.
18. Broughton, P. M. G., Gowenlock, A. H., McCormack, J. J. & Neill, D. W. (1984) Ann. Clin. Biochem. 11, 207–218.
19. Passing, H. & Bablok, W. (1983) J. Clin. Chem. Clin. Biochem. 21, 709–720.
20. Passing, H. & Bablok, W. (1984) J. Clin. Chem. Clin. Biochem. 22, 431–445.

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