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Investigation of the Performance of the ES 600 Enzymun-Test® System
A Multicentre Study

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Summary: The ES 600 sample-selective multibatch analyser was subjected to a multicentre evaluation in six laboratories in accordance with ECCLS guide-lines. During the 3-month trial, five Enzymun-Test® diagnostics (T_4, TBG, Digoxin, CEA and TSH)[1]) were measured at 25 °C.

The study yielded the following results:

1. The within-series and between-series precision were very good, with mean CV's of approx. 3% and 7% respectively.
2. Recovery of the target values for three control sera was in the range ± 5%.
3. A trend in measurements did not occur in any of the methods investigated (for series of over 240 determinations).

[1]) T_4 = thyroxine, TBG = thyroxine binding globulin, CEA = carcinoembryonic antigen, TSH = thyrotropin.

4. Comparison of the results with those obtained on the ES 22 Enzymun-Test® system showed good agreement.
5. Within the measuring range defined by the standards, no deviations could be ascertained upon dilution of the samples.
6. Total carry-over in the instrument was below 0.05%.

From studies with instruments from the first production series, it became evident that modifications were necessary to improve the reliability. A follow-up measuring programme confirmed a clear improvement in reliability and a reduction in the imprecision, particularly for results from series to series.

Introduction

The proportion of determinations in the clinical chemistry laboratory based on immunochemical methods is continually increasing. Because of the problems associated with radioimmunoassays (special equipment, disposal of waste), the various enzyme-immunoassays are becoming more important. In addition to the rising number of determinations, however, increasingly strict requirements are being placed on the reproducibility of the results in order to meet the increasing demands, for example in following the course of tumours via long-term monitoring with appropriate tumour markers. This makes mechanisation of such procedures necessary.

The first step towards a partially mechanized instrument was made with the ES 22 (1). The ES 600 now available is a fully mechanized instrument which meets all requirements.

The ES 600 system, the instrument together with the corresponding enzyme-immunoassays, was subjected to a multicentre trial in six laboratories in accordance with the recommendations of the ECCLS (2). The goal of the study was the verification of the function of the instrument by comparison with another mechanized procedure for the performance of Enzymun-Test® methods, which has already been investigated against manual techniques.

Description of the ES 600

Enzymun-Test® System ES 600 (ES 600) is a fully mechanized instrument for carrying out, measuring, evaluating and documenting heterogeneous enzyme-immunoassays, in particular those methods based on coated tube technology (ELISA). In addition, turbidometric assays can also be carried out on the instrument.

The ES 600 is a sample-selective multibatch analyser with a modular construction. Within a single run, the instrument carries out up to 15 tests (freely selectable by the user) on up to 150 samples, using up to 600 coated tubes without involvement of the user.

Details of the instrument specifications based on the IFCC guidelines (3, 4) are given in table 1.

Tab. 1. Specification of the ES 600 Enzymun-Test® System according to the guide-lines of the IFCC (3, 4).

1. Type	Sample-selective multibatch analyser.
2. Testing procedure	Enzymun-immunoassay, turbidimetric immunoassays. Evaluation via non-linear calibration curves. A choice of 4 curve functions is available for making the evaluation.
3. Throughput	Work cycle 11 s. Up to 650 working steps per hour (via parallel procedures). The throughput is dependent upon the number of samples, incubation times, methods used and length of series.
4. Samples	*Sample transport:* sample rotor with 150 positions for samples and standards together with an additional 10 positions for control sera. *Sample vessels.* graduated plastic vessels, utilizable volume 2.0 ml. *Dispensing:* dispensing syringe driven by stepping motor. *Sample volumes:* 5—200 µl; selectable in 1 µl increments by the user.
5. Reagents	*Transport:* reagent rotor with 15 positions; up to 4 reagents per method can be used. *Reagent vessels:* 150 ml plastic vessels with "chicken-feed" delivery system. *Dispensing:* 2 diluters driven by stepping motors. *Reagent volumes:* 400—1000 µl; selectable in 1 µl increments by the user.
6. Procedure	*Incubation:* Time-controlled incubation; duration between 5 min and 18 hours selectable in 1 min increments by the user. *Temperature control:* Temperature of liquid flow in incubator and cuvette holder regulated by Peltier elements; temperature selectable between 25 and 30 °C by the user. *Temperature constancy:* ± 0.3 °C *Reaction vessels:* one coated tube for each test. *Mixing:* non-invasive mixing after every dispensing operation and prior to photometric measurements. *Washing unit:* for bound/free separation by rinsing out the tubes with the rinsing solution. The intensity of the washing step is test-specifically selectable. *Measurement:* end-point measurement against blank.

Tab. 1. Continued

7. Photometer	Single beam photometer with reference detector for stabilizing the light intensity. *Light source:* Halogen lamp *Wavelength range:* 360—750 nm Selection via IR-blocked interference filter in a filter wheel with 4 positions + 1 dark current position. Standard equipment: filter 422 ± 1 nm. Half-band width < 4 nm. Linearity −0.1 — 1 A (with reference to a path length of 3 mm. *Imprecision:* within-series CV < 0.5%. *Cuvette:* flow-through cuvette made of fused silica. *Path length:* 3 ± 0.01 mm *Volume:* 16 µl
8. Data processing	Computer (Professional 350 from Digital Equipment) for instrument control and data processing. Interface V24 Input via keyboard and VDU Output via LA 50 Printer from Digital Equipment.
9. Storage of data	Diskette with storage possibilities for a complete run together with data on long-term quality control.
10. Services	Only a power supply is required (instruments can be switched from 110 V/60 Hz to 220 V/50 Hz).
11. Dimensions	ES 600: height: 110 cm width: 78 cm length: 118 cm weight: 250 kg
12. Ambient conditions	*Temperature:* 15—35 °C (temperature drift < 3 °C/h) Relative humidity: up to 70% (condensation must not occur).

Methods

Participants and reagents

Six clinical chemistry laboratories took part in the multicentre evaluation of the ES 600. Five enzyme-immunoassays were employed in the investigations. The test-specific properties of Enzymun-Test® T_4 (5, 11), TBG (6, 7), Digoxin (8), TSH (9) and CEA (10) have already been determined in separate evaluations. Data on the instrument settings for measurements carried out at 25 °C are given in table 2.

Investigations

The testing of the instrument was based on the ECCLS recommendations for the evaluation of clinico-chemical analysers (2).

Within-series precision

In order to determine the within-series precision, three control sera containing the corresponding analytes in differing concentrations were used. All six laboratories worked with the same control materials. In addition, each laboratory prepared three human serum pools having differing analyte concentrations; these were also used for the determination of within-series precision. Each measurement of precision with 20 duplicate determinations was carried out twice.

Between-series precision

In order to determine the between-series precision, duplicate determinations were carried out twice daily on three control sera for 10 days. The same procedure was carried out with two human serum pools prepared in the respective laboratories. Evaluation was always made using the second value obtained.

Trend in measurements

In at least 5 series, the complete group of standards was again determined at the end of the series, and the absorbances compared with those obtained at the beginning of the series; in addition, a standard curve was calculated from the results each time.

Method comparison

In order to rule out effects due to reagent differences, about 80 human sera were determined in parallel with the same reagents on the ES 600 and the ES 22 (1) using duplicate determinations. The same sera were also determined using the routine method of the respective laboratory.

Linearity

Each laboratory prepared a 10-step dilution series from a human serum with a high analyte content and a human serum with a low analyte content. This procedure was carried out for all five analytes. The dilutions were measured in series using two duplicate determinations each time.

Carry-over

The carry-over experiments were carried out in such a way that three duplicate determinations of a human serum with the highest possible analyte concentration were followed by three duplicate determinations of a serum having a low concentration. This sequence was measured ten times successively within a series.

Tab. 2. Instrument adjustment data for Enzymun-Test® parameters on the ES 600 at an incubation temperature of 25 °C.

Analyte	Type of Test	Sample volume (µl)	Reagent volume (ml)	Intensity of washing	Time of incubation
Thyroxine	competitive	20	1	9	30/25 min
Thyroxine binding globulin	competitive	50	1	6	30/25 min
Digoxin	competitive	100	1	4	25/20 min
Thyrotropin	2-step-sandwich	200	1	6	60/60/45 min
Carcinoembryonic antigen	1-step-sandwich	100	1	6	120/45 min

Results and Discussion

Within-series precision

The results are given in table 3 and for the measured human serum pools in figures 1–5.

Mean coefficients of variation of 3% were found over the entire measuring range for the competitive methods for thyroxine (T_4) (fig. 1), thyroxine binding globulin (TBG) (fig. 2) and digoxin (fig. 3). Also in the lower and higher measuring ranges, no values were found above 8.5% for thyroxine, 6.4% for thyroxine binding globulin and 8.0% for digoxin.

For the determinations based on the sandwich principle, i.e. carcinoembryonic antigen (CEA) (fig. 4) and thyrotropin (TSH) (fig. 5), the coefficient of variation showed a greater dependence on the concentration of the sample measured; this was due to the large measuring range and the position of the calibration curve. As this effect only becomes manifest in the lower range, the mean coefficients of variation are generally about 2% for carcinoembryonic antigen and 5% for thyrotropin. Coefficients of variation of 20% were only found in samples containing the analyte in the lower concentration range in the vicinity of the method detection limit in individual series.

Between-series precision

The results are summarized in table 4 and presented graphically in figures 6–10 for the human serum pools measured.

Here, too, the competitive methods show only a slight dependence upon concentration. For thyroxine (fig. 6) the mean CV is 7%, with the values from all of the laboratories involved being below 10%.

Thyroxine binding globulin measurements in human serum pools (fig. 7) yielded a mean CV of 7% although three values were distinctly higher (up to 15%). For digoxin (fig. 8), the mean CV was 6% whereas in human serum pools values of up to 17% were obtained at the lower detection limit.

For the thyrotropin and carcinoembryonic antigen protein determination used within the framework of the multicentre study, a greater concentration-dependence of the between-series imprecision was measured. For carcinoembryonic antigen (fig. 9), the mean value is 7% (with the very good precision in the higher measuring range contributing to this), whereas in the lowest measuring range the CV is up to 16%. One serum pool was considerably above the measuring range of the method (50 µg/l). The concentration-

Tab. 3. Within-series precision.
The range of coefficients of variation gives the lowest and highest value obtained each time.

Enzymun-Test®	Precinorm®-IM	Precipath®-IM	Pack control	Human serum low	Human serum intermediate	Human serum high
T_4						
N	12	12	12	6	16	12
Concentration (µg/dl)	8.3	15.9	8.6	< 5	5 –11	>11
Median of CV (%)	4.6	4.1	4.3	4.9	3.5	4.1
Range of CV (%)	3.1–6.6	2.9–6.7	2.8–6.7	2.7–8.8	2.5– 6.4	2.7–8.4
TBG						
N	12	12	11	7 <15	13	8
Concentration (mg/l)	14.5	28.8	16.9	3.4	15 –25	>25
Median of CV (%)	3.3	2.4	3.2	2.3–4.0	2.9	1.8
Range of CV (%)	2.1–5.2	1.3–4.3	1.9–6.3		1.7– 5.2	1.1–6.4
Digoxin						
N	12	12	12	8	9	11
Concentration (µg/l)	0.90	3.36	2.0	< 1	1 –2	> 2
Median of CV (%)	4.4	2.1	3.0	4.4	3.3	2.4
Range of CV (%)	3.0–8.0	1.4–2.8	1.7–3.9	3.0–5.6	1.9–4.5	1.6–4.1
CEA						
N	12	12	11	14	6	9
Concentration (µg/l)	6.0	51.8	11.5	< 3	3 –25	>25
Median of CV (%)	2.3	1.6	1.9	6.5	1.7	1.7
Range of CV (%)	1.5–4.6	0.7–2.8	1.3–6.3	2.3–21.8	0.9– 2.9	0.9–2.3
TSH						
N	11	10	11	16	6	9
Concentration (mU/l)	3.3	9.9	5.3	< 4	4 –20	>20
Median of CV (%)	4.1	2.7	5.0	10.3	6.7	2.5
Range of CV (%)	3.0–6.2	1.8–6.4	1.6–8.8	2.7–21.2	1.4– 9.6	1.4–5.0

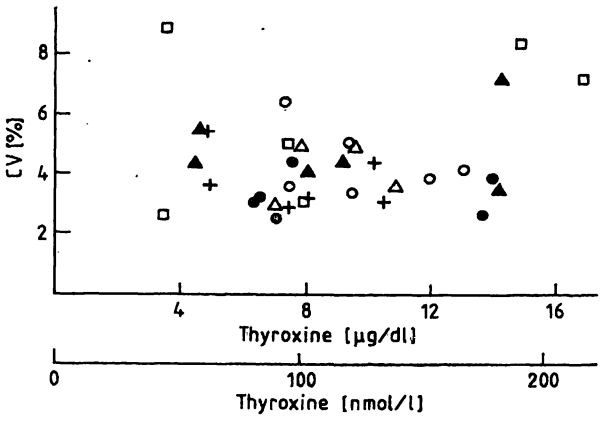


Fig. 1. Within-series precision for Enzymun-Test® T_4
The precision profile shows all measurements obtained from human serum pools in the participating laboratories.
△: Laboratory 1 ○: Laboratory 4
□: Laboratory 2 +: Laboratory 5
●: Laboratory 3 ▲: Laboratory 6

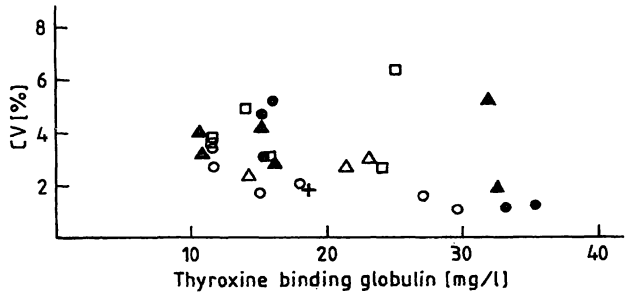


Fig. 2. Within-series precision for Enzymun-Test® TBG
For explanation and symbols see figure 1.

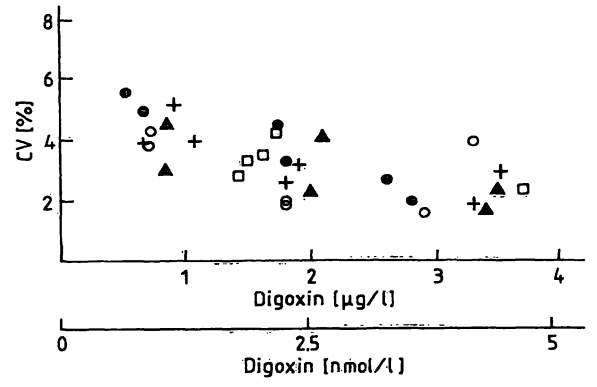


Fig. 3. Within-series precision for Enzymun-Test® Digoxin
For explanation and symbols see figure 1.

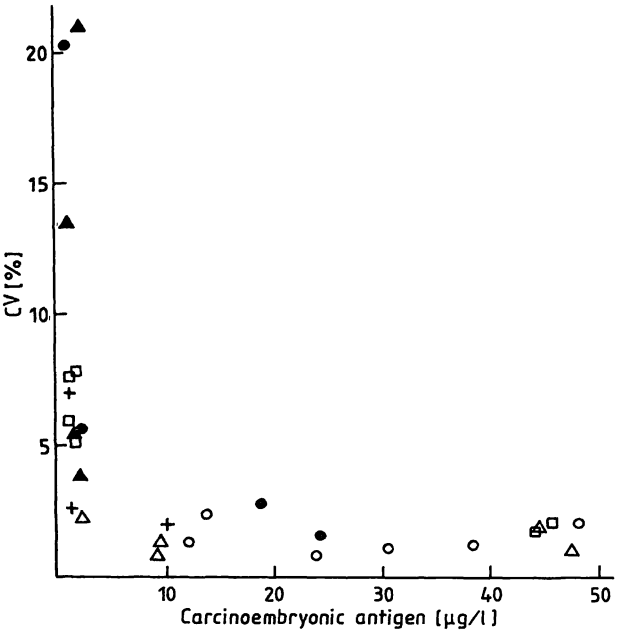


Fig. 4. Within-series precision for Enzymun-Test® CEA
For explanation and symbols see figure 1.

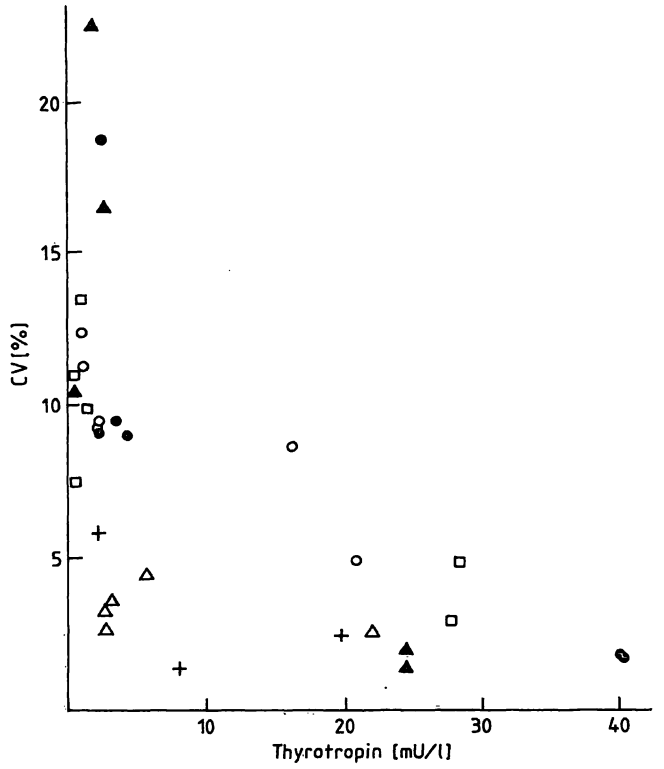


Fig. 5. Within-series precision for Enzymun-Test® TSH
For explanation and symbols see figure 1.

dependency in the thyrotropin test (fig. 10) is somewhat more clear. In the lower measuring range, the CV's are above 10% with the individual values generally differing considerably. Here too, however, for values above 4 mU/l the coefficients of variation are on average around 5%.

Collaborative study

Within the scope of the determination of between-series precision, control sera (Precinorm® IM, Precipath® IM and the respective pack controls) were sent to all of the participating laboratories. The results for Precinorm® IM are shown in figures 11–15; similar

Tab. 4. Between-series precision.
The range of concentrations and coefficients of variation gives the highest and lowest values determined each time in a laboratory.

Enzymun-Test®	Precinorm®-IM	Precipath®-IM	Pack Control	Human serum low	Human serum intermediate	Human serum high
T_4						
N	6	6	6	3	6	3
Target value (µg/dl)	8.3	15.9	8.6	< 5	5 –11	>11
Median of recovery (µg/dl)	8.5	16.3	8.4			
Range of recoveries (µg/dl)	7.0–9.8	12.3–20.2	6.7–10.2	3.2–4.4	7.5– 9.7	12.8–14.9
Median of CV (%)	7.0	8.6	6.9	6.6	7.2	5.8
Range of CVs (%)	5.1–9.2	5.4–10.3	5.6– 9.5	6.6–8.2	6.5–10.0	5.1– 6.3
TBG						
N	6	6	6	3	4	3
Target value (mg/l)	14.5	28.8	16.9	<15	15 –25	>25
Median of recovery (mg/l)	14.9	28.5	17.4			
Range of recoveries (mg/l)	12.1–18.1	25.7–33.6	12.6–21.0	10.0–14.2	15.1–19.2	24.4–32.3
Median of CV (%)	7.9	5.6	6.7	11.4	11.7	4.9
Range of CVs (%)	4.9–10.9	5.2– 7.7	5.0–10.0	9.3–15.6	8.5–15.2	4.2– 5.7
Digoxin						
N	6	6	6	6	2	4
Target values (µg/l)	0.90	3.36	2.00	< 1.00	1 – 2	> 2
Median of recovery (µg/l)	0.98	3.57	2.01			
Range of recoveries (µg/l)	0.83– 1.16	2.64– 4.07	1.59– 2.67	0.59– 0.90	1.11– 1.21	3.03–4.75
Median of CV (%)	7.5	5.1	6.4	12.4	10.8	4.6
Range of CVs (%)	3.2–10.4	3.1–10.0	2.0–10.2	6.5–17.1	8.3–13.2	3.3–5.6
CEA						
N	6	6	6	5	3	3
Target values (µg/l)	6.0	51.8	11.5	< 3	3 –25	>25
Median of recovery (µg/l)	6.4	54.5	11.1			
Range of recoveries (µg/l)	4.3– 7.6	48.0–69.5	8.7–13.5	1.3– 2.9	10.5–18.7	33.3–164.2*
Median of CV (%)	7.7	5.8	7.3	14.6	5.5	5.8
Range of CVs (%)	5.2–13.0	2.3–10.5	4.1–15.2	8.2–15.6	3.2–10.1	4.6– 14.9*
TSH						
N	6	6	6	7	2	3
Target values (mU/l)	3.3	9.9	5.3	< 4	4 –20	>20
Median of recovery (mU/l)	3.2	9.3	5.2			
Range of recoveries (mU/l)	2.3– 5.6	7.3–11.2	3.8– 7.2	0.5*– 2.6	17.8–18.9	24.7–32.9
Median of CV (%)	14.2	8.7	8.8	23.0	3.9	5.0
Range of CVs (%)	5.8–23.5	3.9–10.4	5.0–12.2	12.6 –34.9*	2.7– 5.0	4.1– 9.0

* Value out of measuring range

results were obtained for the other control sera. The presentations contain all of the values found in 10 series in the laboratories.

For thyroxine (fig. 11; target value 8.3 µg/dl), the median value was 8.5 with a scatter of 7.0–9.0 µg/dl for the individual values. For thyroxine binding globulin (fig. 12; target value 14.5 mg/l), the median value was 14.9 with a scatter of 12.1–18.1 for the individual values. For digoxin (fig. 13; target value 0.9 µg/l), the corresponding figures are 0.98 and 0.83–1.16. The following were determined for the sandwich assays of carcinoembryonic antigen and thyrotropin: for carcinoembryonic antigen (fig. 14; target value 6.0 µg/l), a median of 6.4 and a scatter of 4.3–7.6; for thyrotropin (fig. 15; target value 3.3 mU/l), a median of 3.2 and a scatter of 2.3–5.6 for individual values from all of the laboratories.

In all laboratories, the individual values show a normal distribution and only differ slightly from the common mean.

Measurement trend

Because of the calibration necessary in each run, the procedure recommended by the ECCLS was modified somewhat so that the standards used for calibration were again employed as samples at the end of lengthy measurement series.

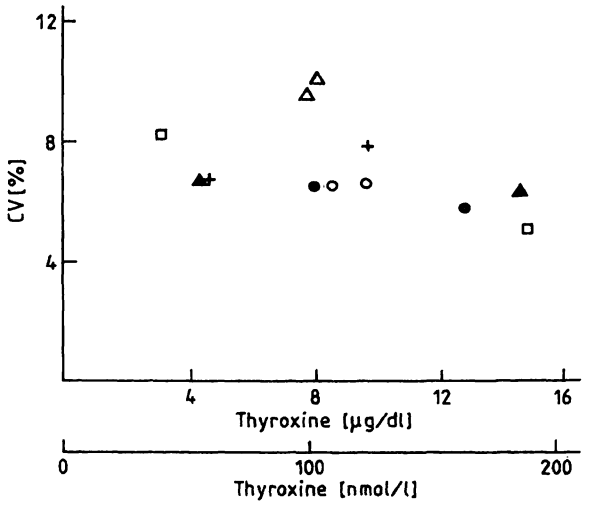


Fig. 6. Between-series precision for Enzymun-Test® T_4
For explanation and symbols see figure 1.

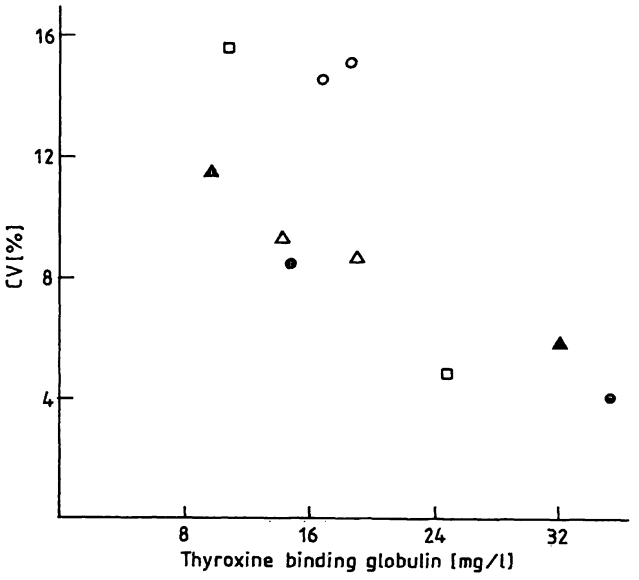


Fig. 7. Between-series precision for Enzymun-Test® TBG
For explanation and symbols see figure 1.

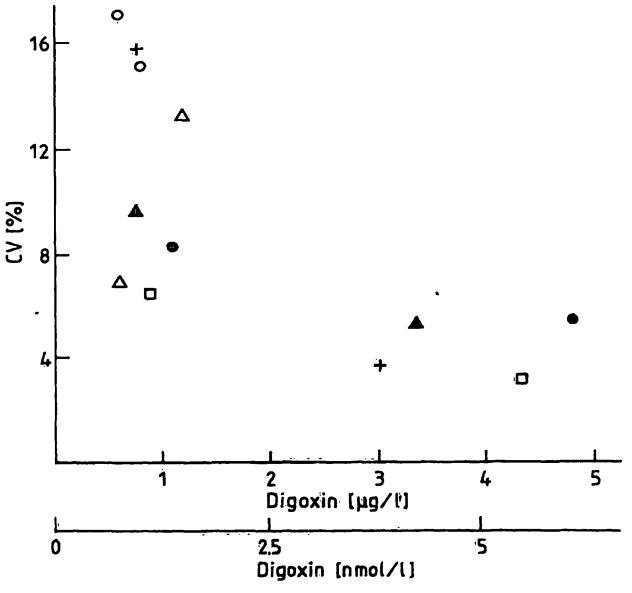


Fig. 8. Between-series precision for Enzymun-Test® Digoxin
For explanation and symbols see figure 1.

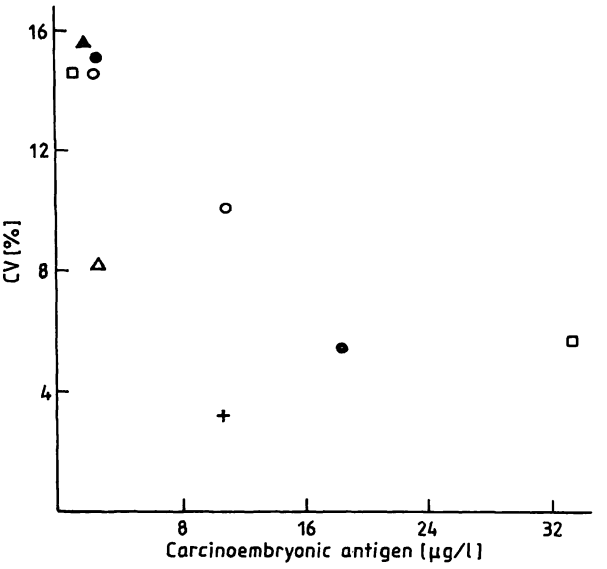


Fig. 9. Between-series precision for Enzymun-Test® CEA
For explanation and symbols see figure 1.

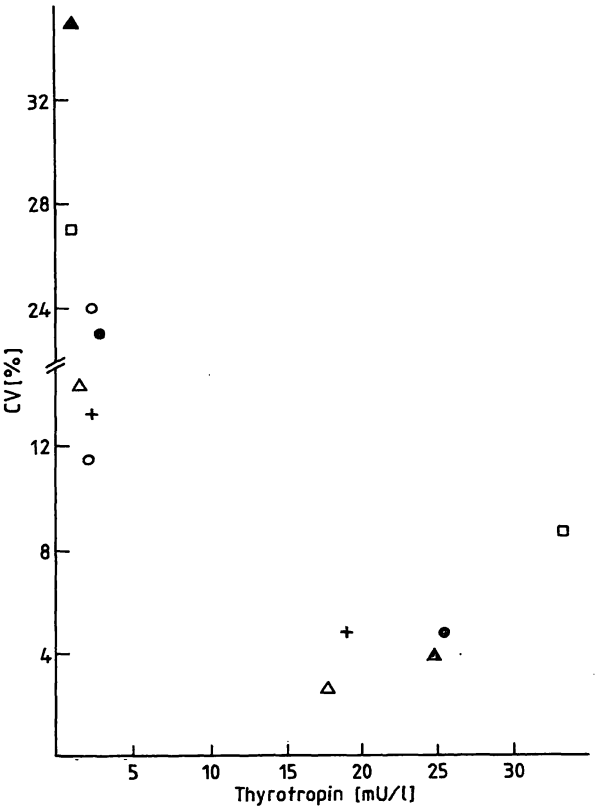


Fig. 10. Between-series precision for Enzymun-Test® TSH
For explanation and symbols see figure 1.

As an example figure 16 shows the calibration curves for the absorbances of the standards at the beginning and end of the series for Enzymun-Test® T_4.

No trend in the measurements can be seen for any of the methods. The absorbances of the standards at the beginning and end of the series lie within the measurement tolerances and show no systematic differences.

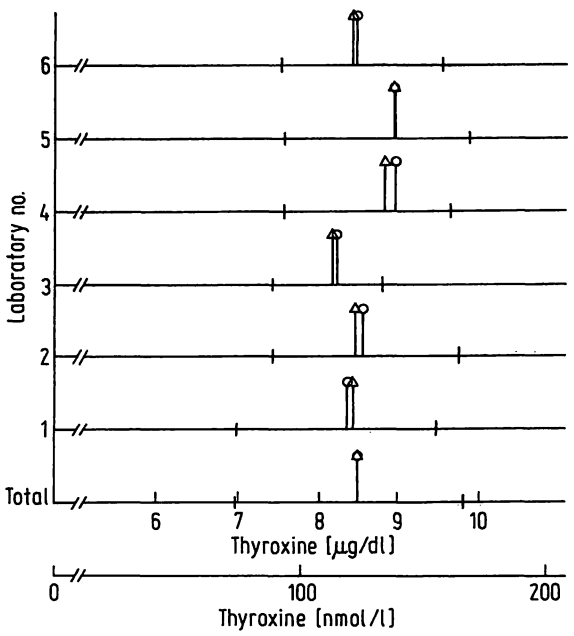


Fig. 11. Between-series precision for Enzymun-Test® T_4;
Sample: Precinorm® IM
The results of n = 10 series are plotted in the line for each laboratory. The respective mean, median, lowest and highest values are marked on each line.
○: Mean
△: Median

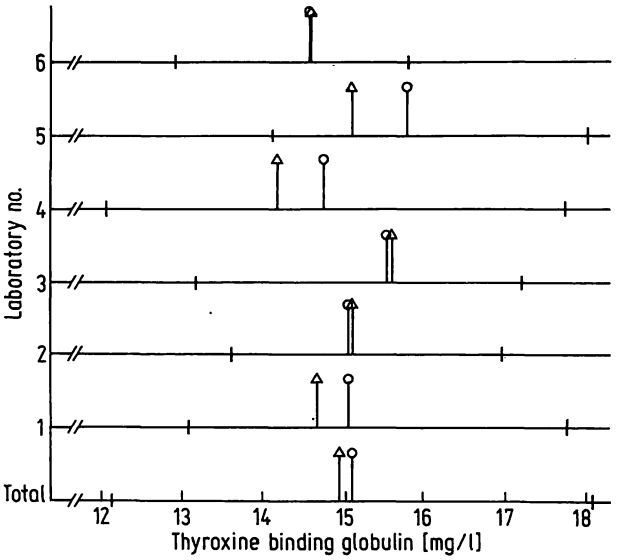


Fig. 12. Between-series precision for Enzymun-Test® TBG;
Sample: Precinorm® IM
For details see figure 11.

Fig. 15. Between-series precision for Enzymun-Test® TSH;
Sample: Precinorm® IM
For details see figure 11.

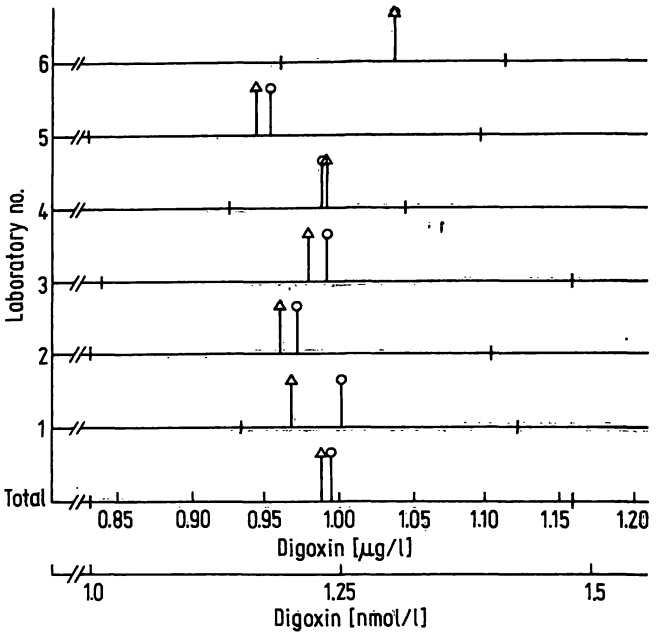


Fig. 13. Between-series precision for Enzymun-Test® Digoxin;
Sample: Precinorm® IM
For details see figure 11.

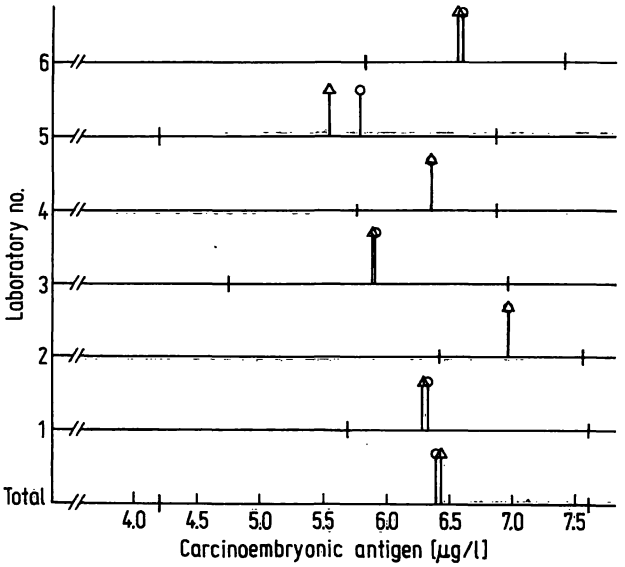
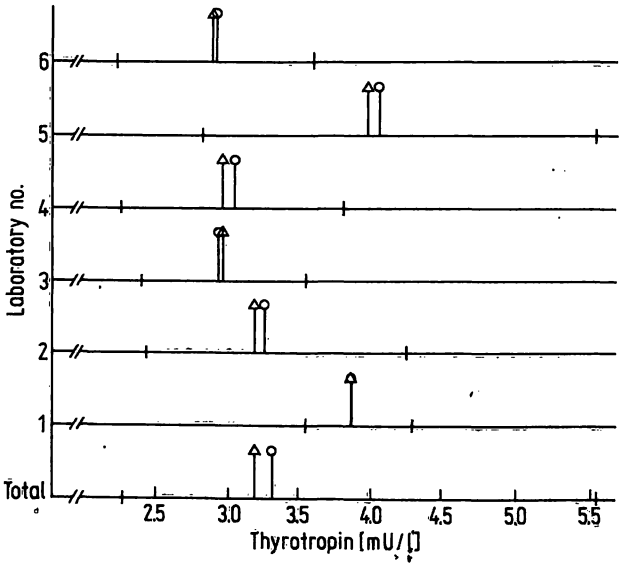


Fig. 14. Between-series precision for Enzymun-Test® CEA;
Sample: Precinorm® IM
For details see figure 11.



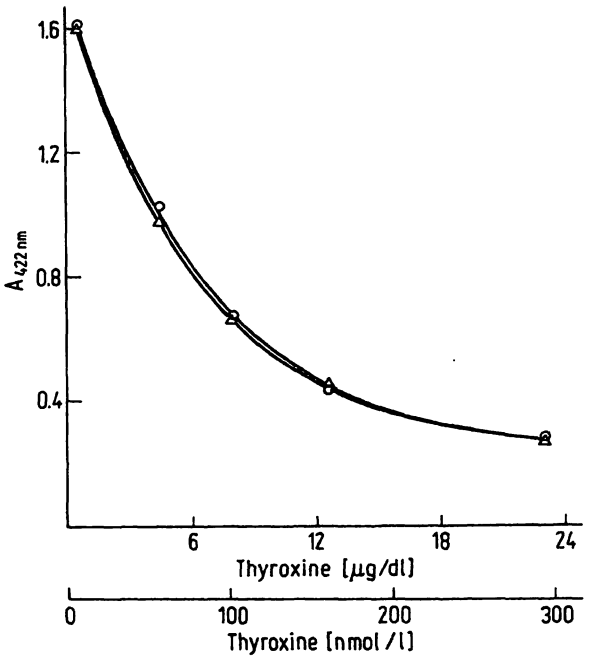


Fig. 16. Measurement trend for Enzymun-Test® T_4;
The standard curves measured at the beginning and end of a series are plotted. The length of the series was > 120 determinations.
o: Start
Δ: End of series

Method comparison

The ECCLS guidelines for the evaluation of analysers in clinical chemistry recommend that the conditions for the comparison should be, as far as possible, the same, i.e. using the same reagents (including standards). For this reason comparisons with the results obtained using the procedure on the ES 22 were used to assess the instruments. As a generality it should be noted that the precision of determinations using the ES 22 is not as good as that obtained using the ES 600. The results for the individual tests are given in tables 5–9 for all laboratories and also graphically (figs 17–21).

Statistical evaluation was made according to the non-parametric procedure given by *Bablok & Passing* (12, 13).

The following individual results were obtained:

Enzymun-Test® T_4 (fig. 17)
The measurements on the two instruments show very good agreement. No systematic deviation can be seen.

Enzymun-Test® TBG (fig. 18)
The results basically correspond to those obtained for thyroxine. No systematic difference can be seen.

Tab. 5. Method comparisons for Enzymun-Test® T_4.
Thyroxine concentration µg/dl.

Laboratory	Number of paired values	$\bar{x}$ (µg/dl)	$\bar{y}$ (µg/dl)	Range (µg/dl)	*Bablok & Passing* y = a + bx	Coefficient of correlation
1	50	8.4	8.8	2.8–17.6	y = −2.58 + 1.374x	0.934
2	79	8.1	8.3	2.8–13.5	y = 0.245 + 1.000x	0.851
3	80	8.0	8.4	4.0–19.5	y = 0.976 + 0.932x	0.935
4	80	8.0	8.5	0.3–15.9	y = −0.122 + 1.083x	0.883
5	76	7.9	7.4	1.1–16.0	y = 0.059 + 0.933x	0.963
6	80	8.3	8.2	4.1–20.1	y = 0.735 + 0.903x	0.884
Median					y = 0.152 + 0.967x	0.909

Tab. 6. Method comparisons for Enzymun-Test® TBG.
Thyroxine binding globulin concentration mg/l.

Laboratory	Number of paired values	$\bar{x}$ (mg/l)	$\bar{y}$ (mg/l)	Range (mg/l)	*Bablok & Passing* y = a + bx	Coefficient of correlation
1	50	17.3	17.1	11.0–30.0	y = −3.765 + 1.19x	0.954
2	75	16.5	15.7	6.5–27.0	y = 2.639 + 0.788x	0.644
3	80	14.5	16.8	9.0–28.0	y = −3.878 + 1.412x	0.796
4	80	15.1	15.3	6.0–25.0	y = 1.646 + 0.904x	0.919
5	101	17.5	17.5	9.5–29.0	y = 0.790 + 0.947x	0.873
6	80	14.8	15.6	8.0–29.0	y = 2.740 + 0.856x	0.809
Median					y = 1.218 + 0.926x	0.841

Tab. 7. Method comparisons for Enzymun-Test® Digoxin.
Digoxin concentration µg/l.

Laboratory	Number of paired values	$\bar{x}$ (µg/l)	$\bar{y}$ (µg/l)	Range (µg/l)	*Bablok & Passing* y = a + bx	Coefficient of correlation
1	78	0.78	0.91	0.00–4.48	y = 0.284 + 0.833x	0.981
2	79	1.44	1.41	0.43–6.35	y = −0.023 + 0.980x	0.953
3	78	1.66	1.65	0.26–4.10	y = 0.057 + 0.958x	0.968
4	79	1.40	1.34	0.19–6.48	y = 0.060 + 1.010x	0.981
5	74	1.32	1.36	0.13–5.15	y = −0.036 + 1.076x	0.975
6	60	1.10	1.15	0.00–5.0	y = 0.238 + 0.791x	0.905
Median					y = 0.058 + 0.939x	0.972

Tab. 8. Method comparisons for Enzymun-Test® CEA.
Carcinoembryonic antigen concentration µg/l.

Laboratory	Number of paired values	$\bar{x}$ (µg/l)	$\bar{y}$ (µg/l)	Range (µg/l)	*Bablok & Passing* y = a + bx	Coefficient of correlation
1	71	3.5	3.4	0.5–10.0	y = 0.805 + 0.750x	0.912
2	52	2.0	2.5	0.0– 4.7	y = 0.891 + 0.789x	0.763
3	79	4.0	3.8	0.0–44.0	y = 0.150 + 0.887x	0.986
4	74	20.2	20.5	0.0–60.0	y = 0.868 + 0.967x	0.989
5	52	14.9	13.2	0.5–60.0	y = 0.150 + 0.863x	0.985
6	78	2.6	3.6	0.0–10.0	y = 1.535 + 0.864x	0.861
Median					y = 0.632 + 0.864x	0.949

Tab. 9. Method comparisons for Enzymun-Test® TSH.
Thyrotropin concentration mU/l.

Laboratory	Number of paired values	$\bar{x}$ (mU/l)	$\bar{y}$ (mU/l)	Range (mU/l)	*Bablok & Passing* y = a + bx	Coefficient of correlation
1	46	11.9	9.4	1.0–59.0	y = −0.549 + 0.808x	0.962
2	80	1.6	1.7	0.0– 5.0	y = 0.181 + 0.901x	0.634
3	73	6.0	6.1	0.0–51.0	y = 0.158 + 0.981x	0.997
4	79	6.9	8.3	0.0–70.0	y = 0.239 + 1.101x	0.986
5	64	10.2	10.1	0.0–33.0	y = −0.224 + 0.971x	0.994
6	80	2.1	2.2	0.0– 9.0	y = 0.496 + 0.832x	0.901
Median					y = 0.170 + 0.940x	0.975

Enzymum-Test® Digoxin (fig. 19)
In this test also, the results on the ES 22 agree very well with those obtained on the ES 600.

Enzymum-Test® CEA (fig. 20)
When assessing the results in table 8, the small concentration range used in some cases (laboratories 1, 2 and 6) must be taken into account. On the whole, however, no systematic deviation can be observed here either.

Enzymum-Test® TSH (fig. 21)
Here, too, the result is influenced by the use of sample materials in a very narrow measuring range (laboratories 2 and 6). The agreement is, however, also very good.

Linearity/measuring range

This investigation was carried out by blending a human serum containing a high analyte concentration with a human serum containing as low an analyte concentration as possible.

The results are given in figures 22–26. No deviation within the measuring ranges defined by the standards were found for any of the methods. As a result of the measuring conditions (3 mm path length of the cuvette) and the excellent linearity of the photometer, it would have been possible to make measurements over a greater measuring range; however, interpolation beyond the range defined by the standards cannot be carried out with non-linear calibration curves.

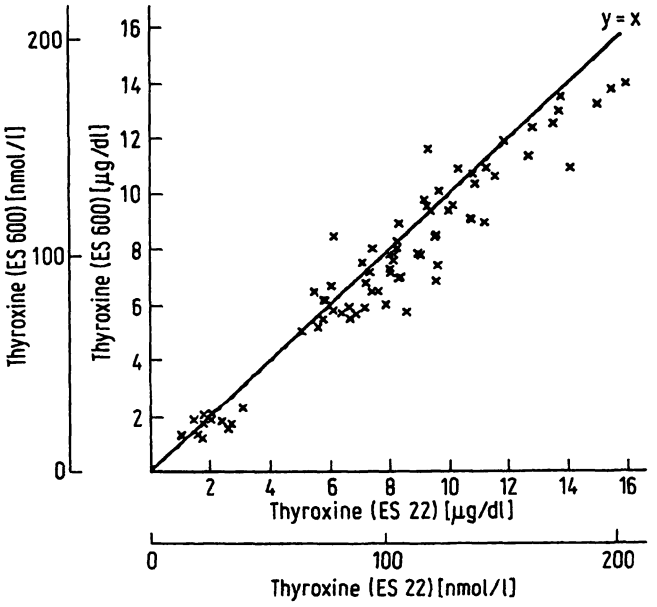


Fig. 17. Instrument comparison for Enzymun-Test® T_4
Comparative measurements were carried out using the same method on ES 22 Enzymun-Test® System.
Number of paired values: 76
y = 0.059 + 0.933 x
Coefficient of correlation: 0.963

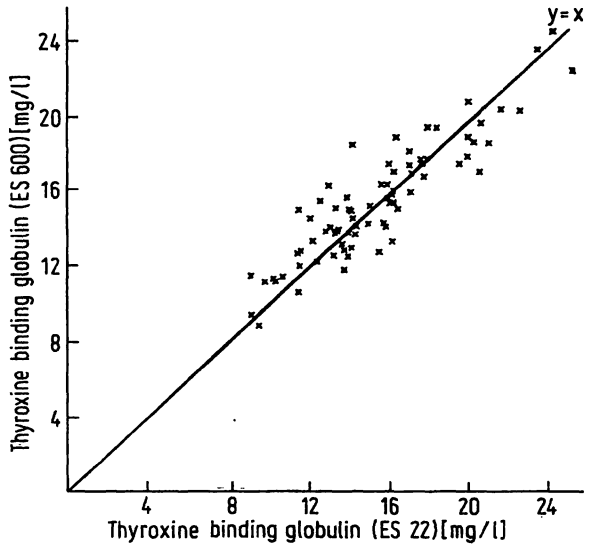


Fig. 18. Instrument comparison for Enzymun-Test® TBG
Comparative measurements were carried out using the same method on ES 22 Enzymun-Test® System.
Number of paired values: 80
y = 1.646 + 0.904 x
Coefficient of correlation: 0.919

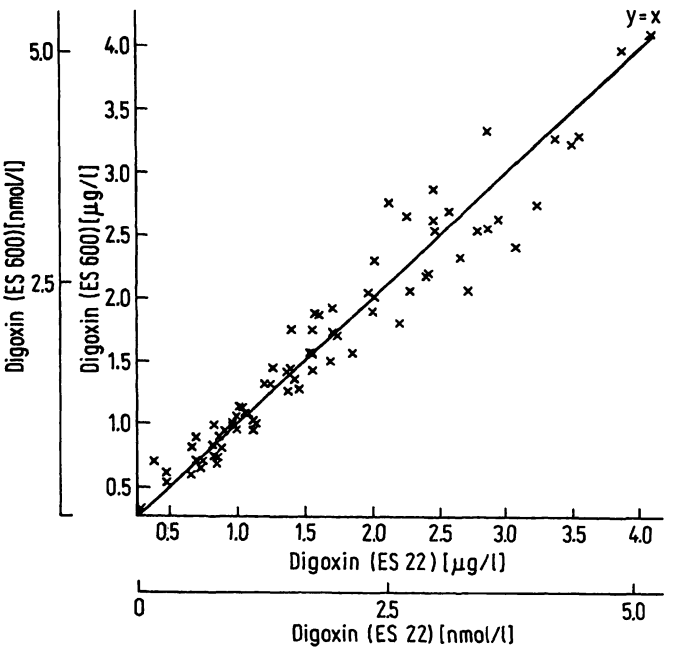


Fig. 19. Instrument comparison for Enzymun-Test® Digoxin.
Comparative measurements were carried out using the same method on ES 22 Enzymun-Test® System.
Number of paired values: 78
y = 0.057 + 0.958 x
Coefficient of correlation: 0.968

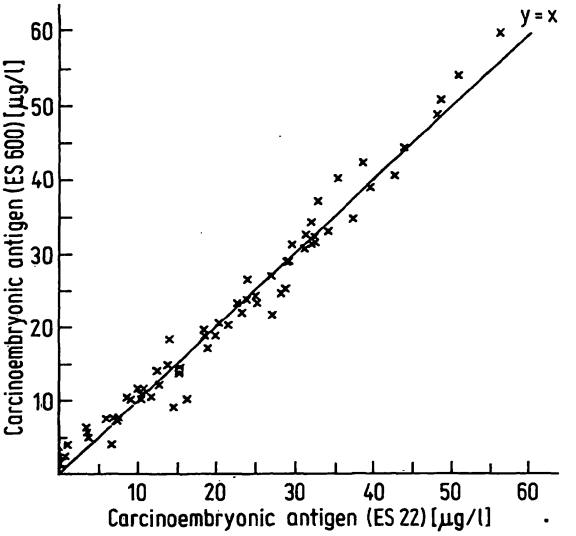


Fig. 20. Instrument comparison for Enzymun-Test® CEA
Comparative measurements were carried out using the same method on ES 22 Enzymun-Test® System.
Number of paired values: 74
y = 0.868 + 0.967 x
Coefficient of correlation: 0.987

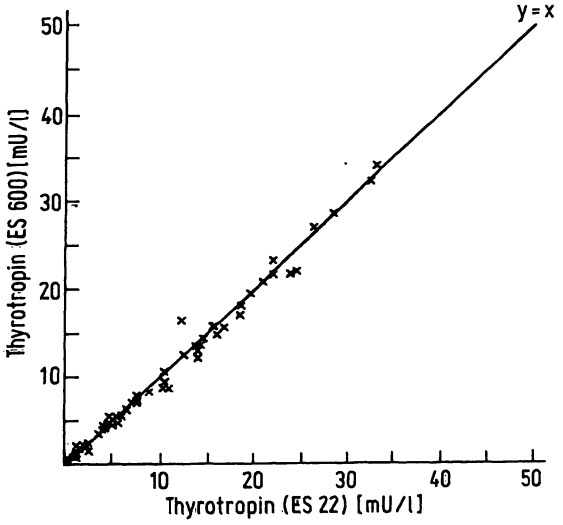


Fig. 21. Instrument comparison for Enzymun-Test® TSH
Comparative measurements were carried out using the same method on ES 22 Enzymun-Test® System.
Number of paired values: 64
y = 0.224 + 0.971 x
Coefficient of correlation: 0.993

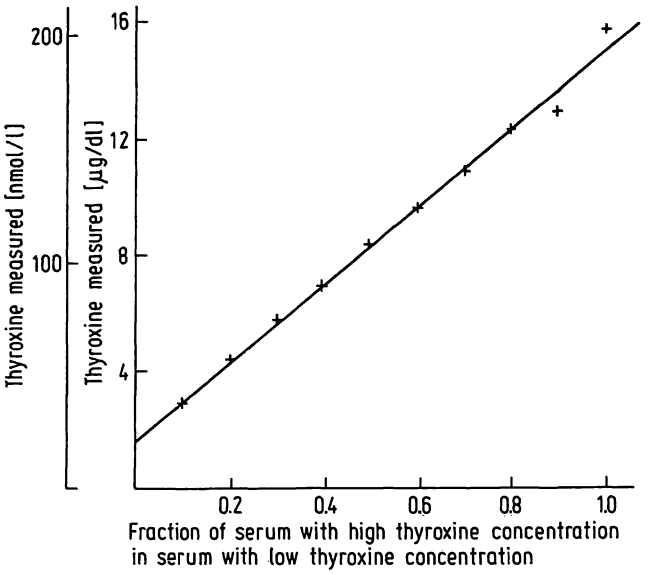


Fig. 22. Linearity of Enzymun-Test® T_4

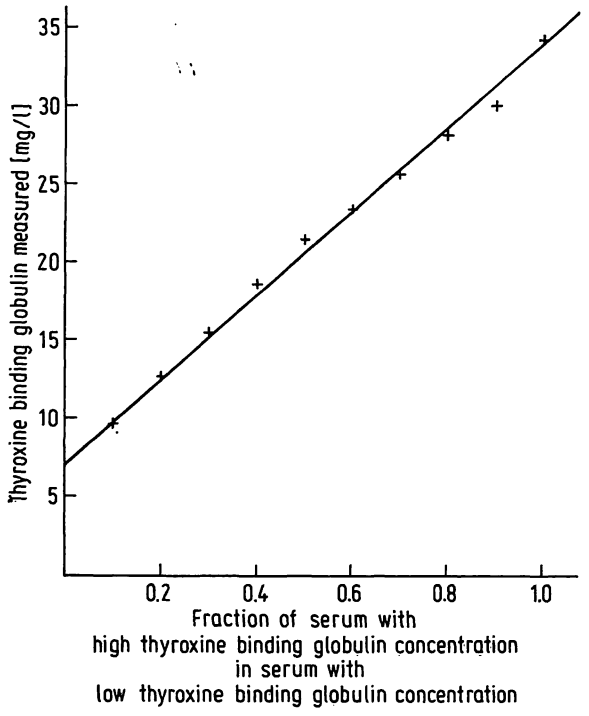


Fig. 23. Linearity of Enzymun-Test® TBG

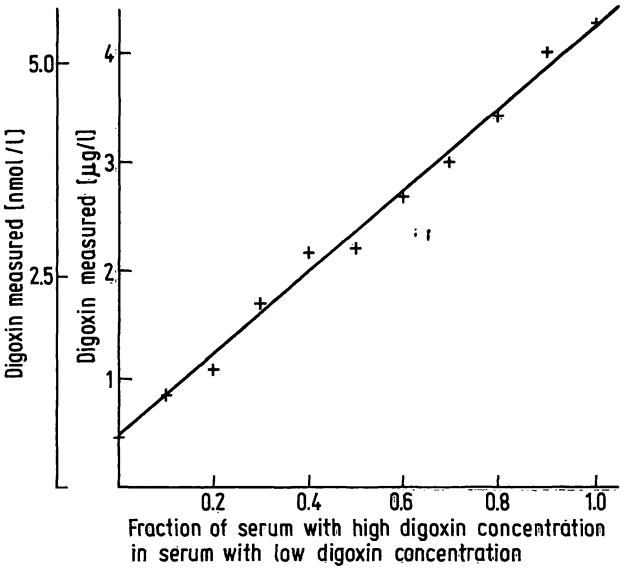


Fig. 24. Linearity of Enzymun-Test® Digoxin

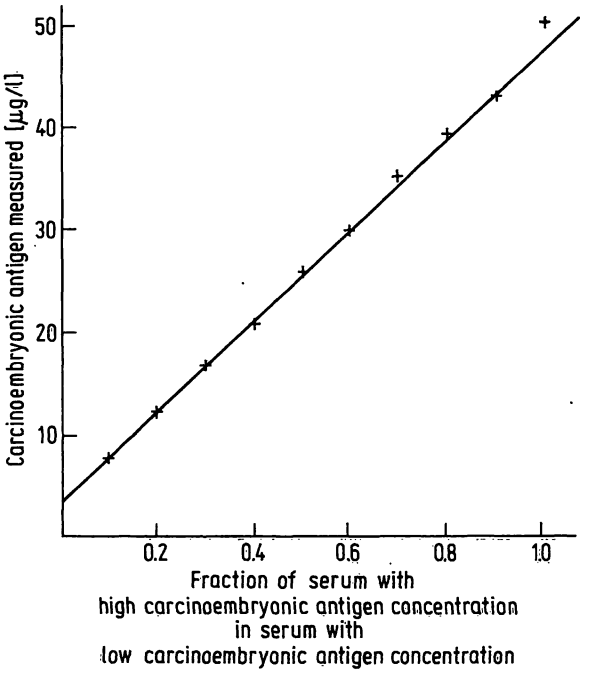


Fig. 25. Linearity of Enzymun-Test® CEA

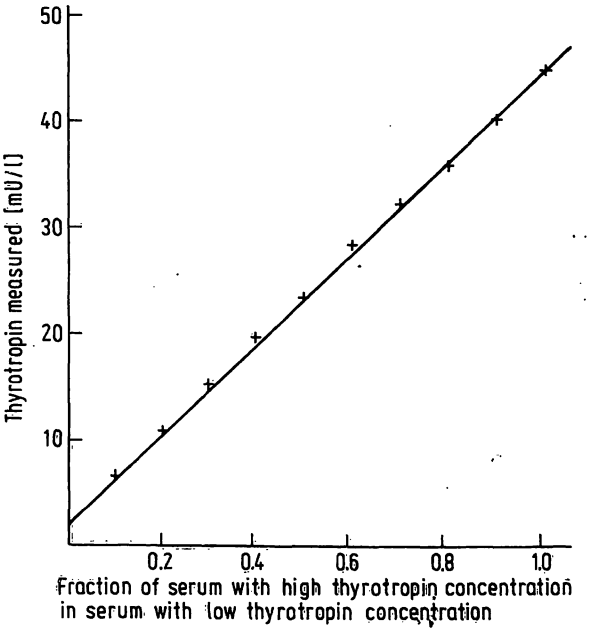


Fig. 26. Linearity of Enzymun-Test® TSH

Carry-over

The results are summarized in table 10. In making an assessment it should be remembered that because of the measuring range or the physiological range of the sample concentrations there are in some instances only very small differences between the "high" and "low" samples. This directly affects the evaluation which is based on concentration differences.

For this reason, in table 10 the coefficients of variation are given for comparison purposes in the low concentration range. It is evident here, that even in the most unfavourable case (for Enzymun-Test® T_4) a calculated carry-over of 0.6% is still well below the precision of the method in this measuring range; hence, no significant alteration of the values determined is to be expected. It can be concluded from the results that overall the carry-over is around 0.05% in the ES 600.

Tab. 10. Carry-over on the ES 600.
The procedure is described in the text.

Enzymun-Test®	$\bar{x}$ high	$\bar{x}$ low	x_1 low	Carry-over (%) high → low	CV (%) $\bar{x}$ low
T_4	22.2	6.2	6.3	0.6	4
TBG	33.3	3.0	3.1	0.3	4
Digoxin	13.7	0.97	1.00	0.2	4
CEA	444.3	0.8	0.9	0.02	8
TSH	28.6	0.8	0.9	0.4	10

Investigator's Summary

The results show that with the help of the ES 600, analytical quality in terms of precision and reliability can be achieved in the field of immunochemistry which is comparable to the state of the art in clinical chemistry. It is hence possible to carry out immunoassays with Enzymun-Test® diagnostics as single determinations on the ES 600 instrument without loss of accuracy and precision.

The multicentre study was carried out with instruments from the first production series. As a result of this early period in the development of the instrument, it became obvious during the evaluation that modifications were necessary, although no basic changes to the instrument concept were required for the production series. The alterations can be summarized into two areas:

1. technical changes to the instrument, and
2. alterations to the user software.

The technical changes to the instrument mainly concern modifications to increase the reliability of instrument function (particularly concerning tube transport and the wash station). These changes were carried out immediately. The corrections to the user software took somewhat more time, but were not decisive for the reliability of instrument function.

In addition to the less serious defects, the modifications are mainly related to the installation of a bidirectional interface, which for differently organized laboratories greatly simplifies and accelerates the necessary input for the RUN definition. In addition to the normal input via keyboard and display screen, it is also possible to enter requests for analyses from patient samples directly via the laboratory electronic data processing or with the aid of a barcode reader, thereby substantially shortening the preparation time. This additionally increases the rationalization effect by a further saving in laboratory capacity.

The ES 600 instrument provides a further possibility for lowering analysis costs, namely by carrying out determinations as single analyses and employing recalibration with only one standard in association with stored calibration curves. From the present evaluation one can conclude that this possibility will be exploited.

Follow-Up Measurement Programme

After instrument modifications, a follow-up measuring programme was carried out in four laboratories in order to check the improvement in instrument reliability. For this purpose, measurements aimed at determining the within-series and between-series precisions were generally carried out using control sera (Precinorm® IM and Precipath® IM) together with human serum pools.

The results are summarized in tables 11 and 12. Particularly for the between-series precision (tab. 12), lower coefficients of variation were found. These values are as a rule well below 10%; for the medium concentration range the coefficients of variation were generally found to be below 5%. In addition the reliability of the instrument was clearly improved.

Tab. 11. Within-series precision.
Recovery after instrument modification.

Enzymun-Test®	Precinorm®-IM	Precipath®-IM	Human serum 1	Human serum 2
T_4				
N	20	20	20	20
Concentration (µg/dl)	8.6	16.0	5.2	11.8
CV (%)	3.9	3.9	5.1	3.8
TBG				
N	20	20	20	20
Concentration (mg/l)	16.0	36.7	7.8	19.0
CV (%)	2.4	1.5	3.4	2.4
Digoxin				
N	20	20	20	20
Concentration (µg/l)	1.08	3.62	0.99	2.72
CV (%)	3.5	2.0	4.0	2.0
CEA				
N	20	20	20	20
Concentration (µg/l)	6.2	54.9	4.0	32.9
CV (%)	1.5	1.0	1.5	1.5
TSH				
N	20	20	20	20
Concentration (mU/l)	3.1	6.4	1.9	13.1
CV (%)	1.8	1.3	3.3	1.6

Tab. 12. Between-series precision.
Repetition of measurements after instrument modification.

Enzymun-Test®	Precinorm®-IM	Precipath®-IM	Human serum
T_4			
N	10	10	10
Concentration (µg/dl)	7.4	18.7	6.1
CV (%)	3.4	5.7	3.3
TBG			
N	9	9	9
Concentration (mg/l)	16.9	34.7	8.8
CV (%)	6.6	4.1	11.4
Digoxin			
N	10	10	10
Concentration (µg/l)	1.04	3.61	1.28
CV (%)	5.9	4.0	5.5
CEA			
N	10	10	10
Concentration (µg/l)	6.4	55.5	4.2
CV (%)	4.6	3.3	2.7
TSH			
N	9	9	9
Concentration (mU/l)	3.7	6.3	8.2
CV (%)	12.1	6.0	2.8

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