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Multicentre Evaluation of an Enzyme-Immunoassay for Cortisol Determination

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Summary: The present paper describes a multicentre evaluation of a one step enzyme-immunoassay for the determination of cortisol in serum or plasma. Data from the investigation were analysed in terms of imprecision, detection limit, and correlation with other test methods.

Within-run and between-run imprecisions (coefficient of variation) of Enzymun-Test® Cortisol were less than 8% and 12%, respectively. The detection limit was 30 nmol/l (11 µg/l). With the exception of prednisolone, only low interference was found with other endogenous steroids. A good correlation between Enzymun-Test® Cortisol and HPLC, LIA, FPIA and RIA was registered, although the latter two methods showed a scattering of regression lines from the different evaluators.

The results show that Enzymun-Test® Cortisol can be recommended as an alternative for the measurement of cortisol. As the method is calibrated against isotope dilution-mass spectrometry, results obtained with Enzymun-Test® Cortisol are in agreement with the reference method.

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Introduction

Cortisol is the major glucocorticoid produced and secreted by the adrenal gland. It is involved in the regulation of protein, fat and carbohydrate metabolism, electrolyte balance, body water distribution, blood pressure regulation and immunosuppressant antiinflammatory action. For the assessment of adrenocortical function the estimation of serum cortisol is essential (1). Thus, various procedures have been described for the determination of cortisol, such as different radioimmunoassays (RIA) (2), high performance liquid chromatography (HPLC) (3), luminescence immunoassay (LIA) (4), and fluorescence polarisationimmunoassay (FPIA) (5). Recently, a new method for the determination of cortisol was developed, namely a competitive one-step enzyme-immunoassay, Enzymun-Test® Cortisol. In this test, serum/plasma cortisol and peroxidase-labelled cortisol compete for cortisol antibodies coated to the tube wall. After addition of chromogen the absorbance of the developing colour complex is measured and the cortisol concentration calculated from a standard curve.

The aim of this study was to investigate the imprecision and detection limit of this new Enzymun-Test® as well as its suitability for daily laboratory routine use. Furthermore, the investigation was aimed at comparing cortisol levels determined by this test with those determined by the above-mentioned methods. The following paper describes the results of a multicentre evaluation of Enzymun-Test® in nine laboratories.

Materials and Methods

Materials

Each of the nine laboratories used serum samples from the routine workload. For investigating within-run imprecision and between-run imprecision serum pools were used. For the purpose of quality control, two control sera (Precinorm®-IM, lot 09, cortisol 331 nmol/l (120 µg/l); Precipath®-IM, lot 10, cortisol 751 nmol/l (272 µg/l); Boehringer Mannheim GmbH, FRG) were used throughout.

The determinations were performed either with the batch analyser ES 22 or the fully automated system ES 600 (Boehringer Mannheim GmbH, FRG) (6).

Analytical procedures

Enzymun-Test® Cortisol is a competitive one-step enzyme-immunoassay. The detailed assay protocol is shown in figure 1. In the first step, cortisol from serum or plasma samples and horseradish peroxidase-labelled cortisol compete for a limited quantity of polyclonal cortisol antibodies coated to the tube wall. During this step, sample cortisol is displaced from its serum binding protein using a steroid (first progesterone, in later experiments danazole) contained in the incubation buffer. Bound/free separation is achieved by a simple washing step. Then, the substrate, di-ammonium 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulphonate) (ABTS®) is added and the absorbance is measured at 420 nm (ES 600) or Hg 405 nm (ES 22). Serum cortisol concentration is calculated from a standard curve. The total assay time is about one hour.

Calibration

The Enzymun-Test® Cortisol was calibrated using isotope dilution-mass spectrometry (ID-MS) recommended by the "Deutsche Gesellschaft für Klinische Chemie" and the "Commission of the European Communities" (7) as reference method. Masterlot calibrators were determined by ID-MS using the method of *Siekmann & Breuer* (8). The concentrations of testkit calibrators are determined from the masterlot calibration curve.

Specificity

Antibodies to cortisol were raised in sheep using cortisol coupled to keyhole limpet haemocyanin as the immunogen. Cross-reactivities of cortisol-related substances are shown in table 1. For a better interpretation of cross-reactivity data, the reported maximal serum concentrations are listed in the right-hand column.

Recovery

As shown in table 2, the recovery of reference materials with Enzymun-Test® Cortisol was found to vary from 98.9% to 109.4%.

Imprecision

Two control sera were analysed by each laboratory with Enzymun-Test® Cortisol at two different concentration levels (331 and 751 nmol/l corresponding to 120 µg/l, and 272 µg/l). Furthermore, the cortisol content was measured in two or three individual human serum pools (concentration range: 55 to 864 nmol/l ≙ 20 to 313 µg/l for within-run imprecision and 74 to 751 nmol/l ≙ 27 to 272 µg/l for between-run imprecision). Within-run imprecision was determined from 20 measurements at each concentration level. Between-run imprecision was derived from measurements over ten days.

Lower detection limit, linearity

In order to ascertain the lower detection limit the absorbance was measured in each laboratory 20 times with the zero calibrator of the kit (17 nmol/l ≙ 6 µg/l) as sample. The value of the lower detection limit was estimated by reading the mean plus three S. D. of the absorbance from the corresponding calibration curve (9). Linearity of Enzymun-Test® Cortisol was determined in each laboratory by diluting specimens containing high concentrations of cortisol (in human serum and/or the highest standard) with increasing amounts (n = 10) of specimens containing low cortisol concentrations (9 g/l NaCl or the lowest standard). Linearity was checked by linear regression analysis and visual inspection of the relative differences between the calculated and measured concentrations.

Method comparison

The Boehringer Mannheim Enzymun-Test® Cortisol was compared in serum samples with the reference method ID-MS (eight different series in four laboratories of Boehringer Mannheim GmbH). Furthermore, Enzymun-Test® was compared in serum samples with RIA (Diagnostic Products Corporation, DPC; Immunochem Corp., Imm.; DRG Instruments, DRG), HPLC (Laboratory method), LIA (Baxter), and FPIA (Abbott Diagnostics).

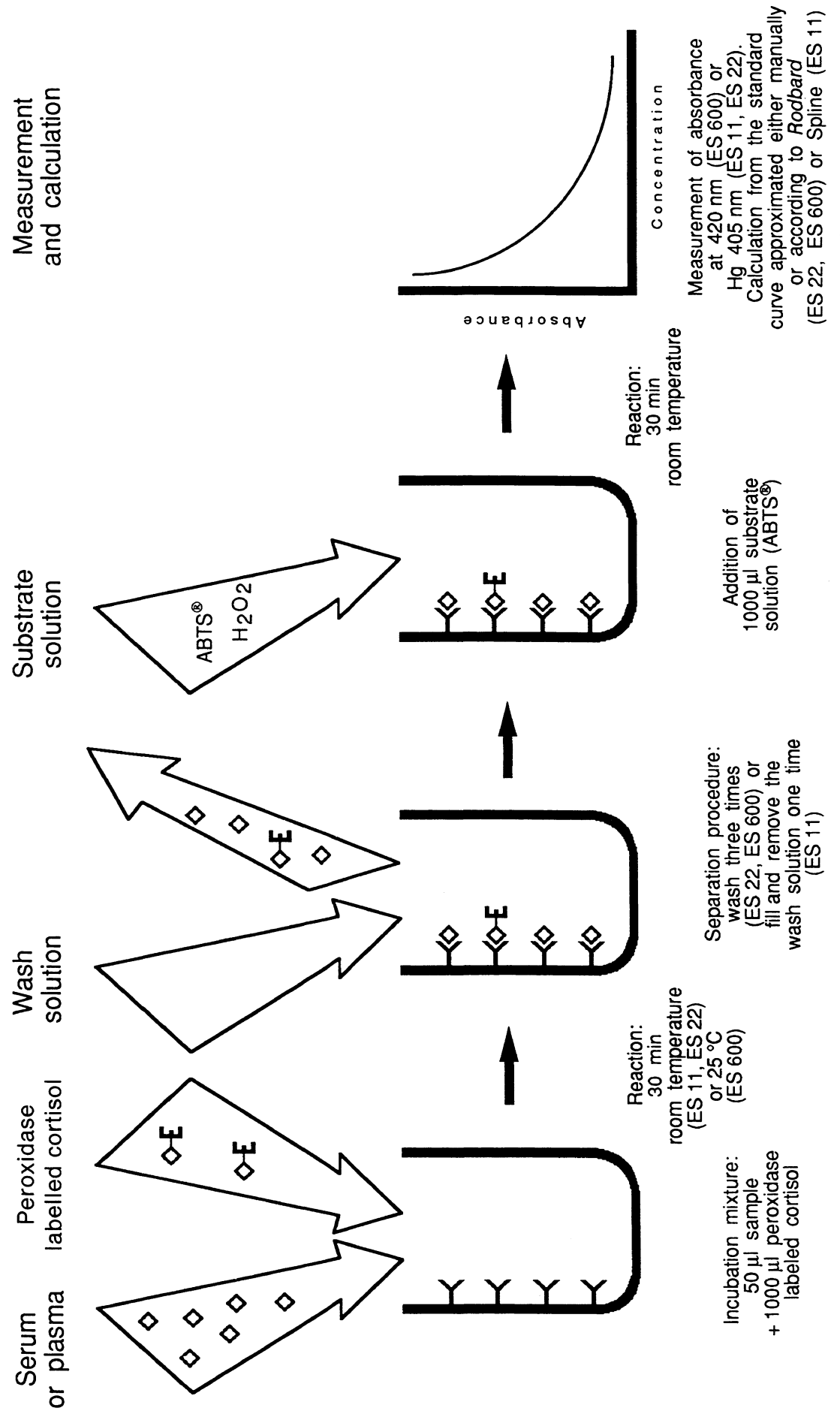

Fig. 1. Assay protocol of Enzymun-Test® Cortisol.

Statistics

Regression parameters were calculated using a biometric regression procedure according to *Passing & Bablok* (10). Significances of the differences in the dexamethasone test were calculated according to *Student*'s t-test.

Tab. 1. Assay specificity

Substance	Cross-reaction [%]	Maximal expected values in serum [µg/l]
Cortisol	100.0	600
Prednisolone	70.2	500
Prednisone	0.2	–[a]
11-Desoxycortisol	4.7	20 (350[b])
21-Desoxycortisol	13.8	–[c]
Cortisone	0.3	20
Corticosterone	1.5	50
11-Desoxycorticosterone	0.1	0.2
6β-Hydroxycortisol	52.4	–[d]
5β-Pregnene-tetrol	0.3	<100
20α-Dihydrocortisol	0.2	–[d]
20β-Dihydrocortisol	0.1	–[d]
Tetrahydrocortisone	<0.1	–[d]
α-Cortol	<0.1	–[d]
α-Cortolone	<0.1	–[d]
Dexamethasone	<0.1	–[a]
Betamethasone	<0.1	–[a]
Triamcinolone	<0.1	–[a]
Progesterone	<0.1	150
Danazol	<0.1	–[a]

[a] Data not available; [b] during metyrapone-test; [c] only minor product during steroid synthesis; [d] significant amounts only in urine samples

Results

Imprecision

The coefficient of variation (CV) obtained by the nine laboratories with the Enzymun-Test® Cortisol for within-run imprecision (n = 20) ranged between 1.3 and 5.6% for control sera (tab. 3 A), and between 1.7 and 11.7% for human serum pools (fig. 2 a). The CV-values for the between-run imprecision (n = 10) ranged between 2.3 and 9.6% for control sera (tab. 3 B), and between 3.3 and 20.8% for human serum pools (fig. 2 b). There were no significant differences in CV between ES 22 and ES 600.

Lower detection limit, linearity

The detection limit of cortisol is defined as the threefold standard deviation of absorbance of a zero standard calculated from the calibration curve. Based on nine laboratories, a mean lower detection limit (± SEM) of Enzymun-Test® Cortisol of 30 ± 8 nmol/l ≙ 11 ± 3 µg/l (range: 22 to 50 nmol/l ≙ 8 to 18 µg/l) was calculated.

The linearity of Enzymun-Test® Cortisol was assessed by using human serum or standard containing varying amounts of cortisol. Within the tested concentration range (110 to 1460 nmol/l ≙ 40 to 530 µg/l) in all

Tab. 2. Recovery of reference materials with Enzymun-Test® Cortisol

Sample	ID-MS		Enzymun-Test®		Cortisol recovery [%]
	[nmol/l]	[µg/l]	[nmol/l]	[µg/l]	
BCR No 192	273	98.8	273	98.8	100.0
BCR No 193	765	277.0	770	278.9	100.7
DGKCh No 13	557	201.9	552	200.0	99.1
DGKCh No 15	346	125.4	353	127.9	102.0
DGKCh No 20	292	105.8	303	109.9	103.9
DGKCh No 21	349	126.5	382	138.4	109.4
DGKCh No 2241	480	174.0	475	172.0	98.8
DGKCh No 2242	405	146.8	420	152.3	103.8

BCR: Community Bureau of Reference, Commission of the European Communities, Brussels, Belgium
DGKCh: Deutsche Gesellschaft für Klinische Chemie

Tab. 3. Within-run imprecision (A) and between-run imprecision (B) from control sera

Laboratory		Precinorm®-IM			Precipath®-IM				Precinorm®-IM			Precipath®-IM		
		[nmol/l]	Mean [µg/l]	CV [%]	[nmol/l]	Mean [µg/l]	CV [%]		[nmol/l]	Mean [µg/l]	CV [%]	[nmol/l]	Mean [µg/l]	CV [%]
1	A	331	120	2.0	795	288	1.5	B	348	126	2.3	820	297	3.9
2	Within-	375	136	5.6	792	287	5.4	Between-	378	137	7.3	800	290	3.3
3	run	348	126	2.1	745	270	1.9	run	342	124	4.0	773	280	6.1
4	(n = 20)	342	124	1.5	781	283	1.3	(n = 10)	334	121	7.7	787	285	8.8
5		362	131	3.5	789	286	2.1		337	122	5.0	753	273	3.8
7		395	143	2.9	748	271	2.9		367	133	9.5	751	272	5.3
8		342	124	4.5	762	276	3.5		342	124	4.7	792	287	5.5
9		304	110	2.3	751	272	3.2		331	120	1.9	792	287	3.6
10		331	120	4.7	759	275	4.4		334	121	3.9	773	280	3.4

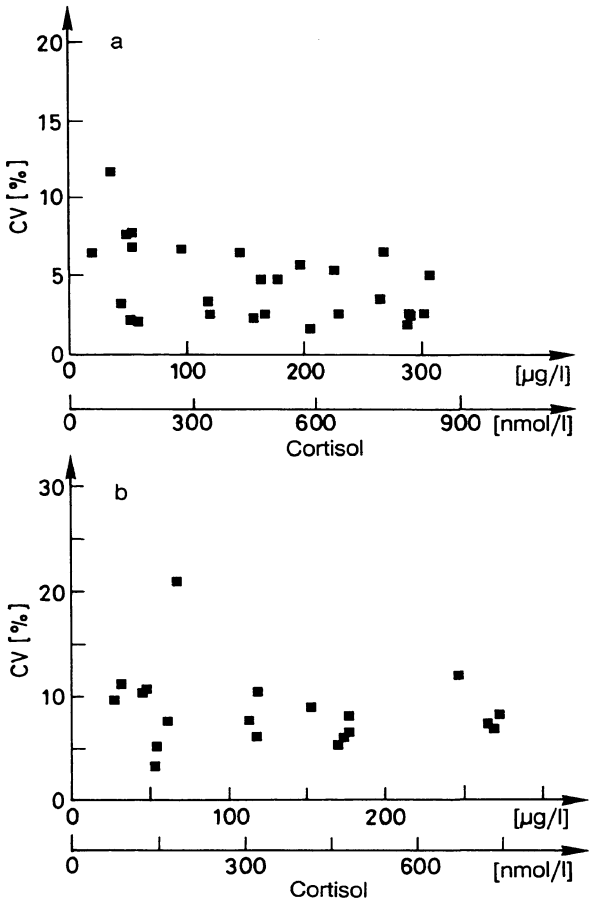

Fig. 2. Within-run imprecision (a) and between-run imprecision (b) of Enzymun-Test® Cortisol in serum pools with cortisol concentrations ranging from 55 to 847 nmol/l ≙ 20 to 307 µg/l and from 75–751 nmol/l ≙ 27 to 272 µg/l, respectively.

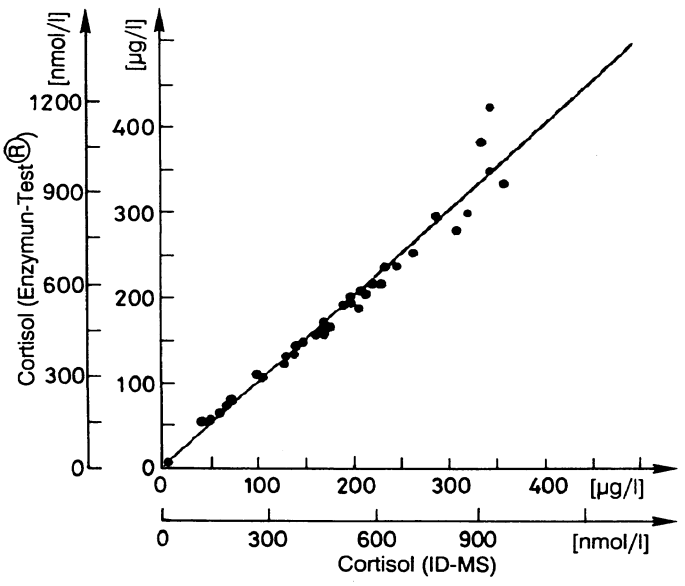

Fig. 3. Correlation of Enzymun-Test® Cortisol (y) with isotope dilution-mass spectrometry (ID-MS; x) in 38 serum samples with cortisol concentrations ranging from 25 to 1100 nmol/l ≙ 9 to 400 µg/l. The solid line represents the regression line ($y = 1.02x - 0.45$; $r = 0.98$) according to *Passing & Bablok* (10).

laboratories the method was proven to be linear in 12 separate experiments, the slope of the regression line being 0.88 to 1.07.

Method comparison

A method comparison was performed by determining serum samples with Enzymun-Test® Cortisol and the reference method ID-MS. As shown in figure 3, a good agreement between the Enzymun-Test® Cortisol and the reference method is demonstrated.

Furthermore, human serum samples were analysed with the proposed method and four other cortisol assays. Table 4 shows a comparison of the results from the different laboratories. Four examples of graphical correlation are demonstrated in figures 4a–d. A good correlation was observed for Enzymun-Test® Cortisol and LIA, HPLC, RIA and FPIA, although the latter two methods showed some variation from laboratory to laboratory.

In one laboratory, the cortisol concentration was measured simultaneously with Enzymun-Test® Cortisol and RIA in the sera of 10 patients before and after administration of dexamethasone (fig. 5). The cortisol concentration measured with both methods showed no significant differences, either before or after dexamethasone application.

Tab. 4. Correlation between Enzymun-Test® Cortisol and four different methods using a biometric regression procedure

Laboratory	y	x	n	Slope	Intercept	Range tested		Mean concentration [µg/l]		r
						[nmol/l]	[µg/l]	y	x	
1	Enzymun-Test®	DPC-RIA	202	0.92	+ 0.8	14–1190	5–430	141	146	0.905
2	Enzymun-Test®	LIA	99	1.05	+ 1.0	25–1270	9–460	161	148	0.839
3	Enzymun-Test®	DPC-RIA	60	0.85	+ 0.8	14–1210	5–440	107	121	0.959
4	Enzymun-Test®	DPC-RIA	59	0.73	+30.7	69–1080	25–390	173	201	0.892
5	Enzymun-Test®	Imm-RIA	68	0.95	− 9.2	14– 770	5–280	96	111	0.965
7	Enzymun-Test®	LIA	36	1.07	−15.6	55–1210	20–440	136	146	0.959
7	Enzymun-Test®	FPIA	70	0.84	+ 6.6	28–1460	10–530	127	135	0.977
8	Enzymun-Test®	HPLC	54	0.91	− 5.5	28– 990	10–360	157	179	0.878
9	Enzymun-Test®	FPIA	95	0.71	+13.0	14–2620	5–950	153	164	0.912
10	Enzymun-Test®	DRG-RIA	93	0.95	+ 6.4	14–1320	5–480	123	121	0.960

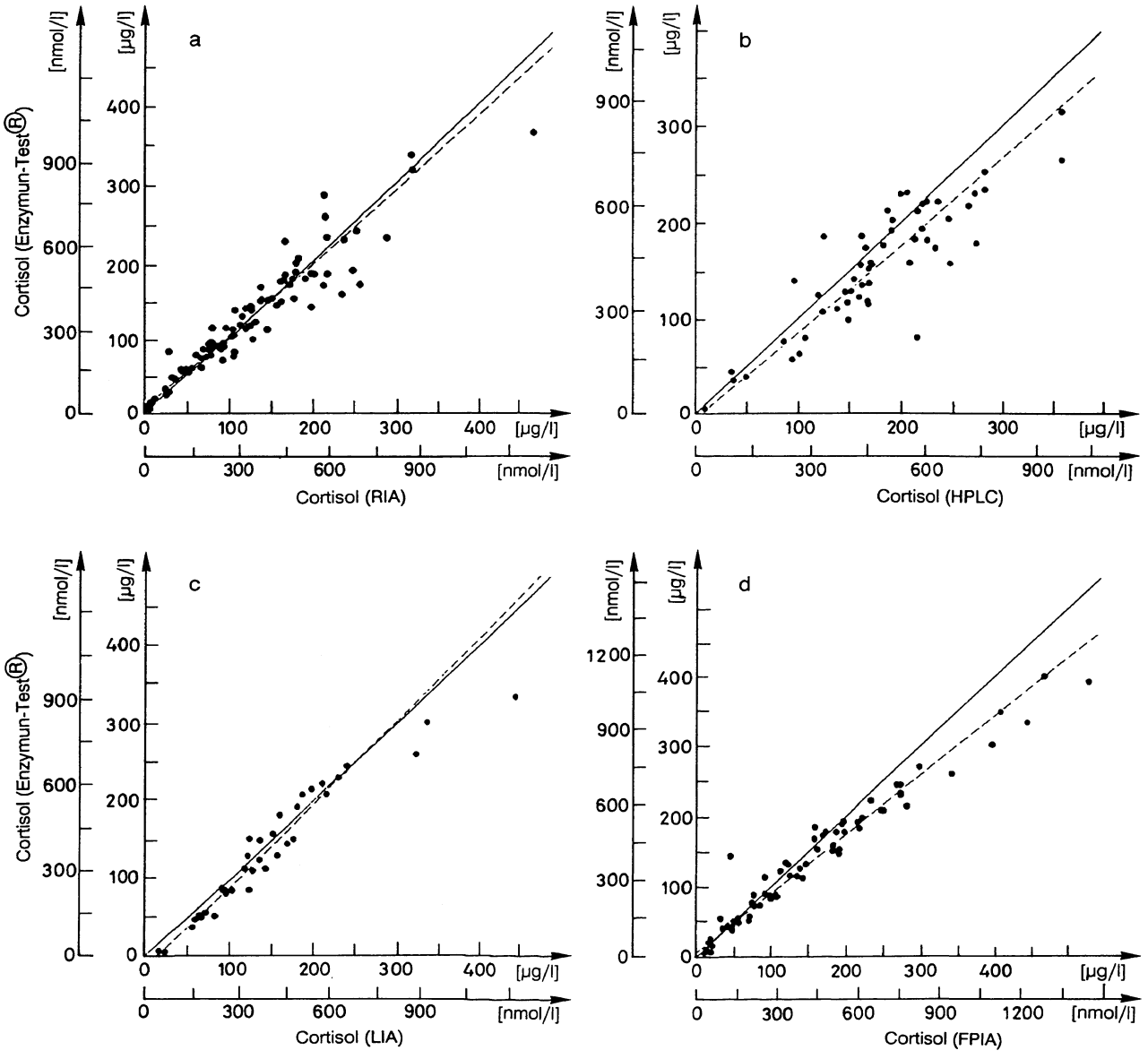

Fig. 4. Correlation of Enzymun-Test® Cortisol (y) with four different methods (x): RIA (a), HPLC (b), LIA (c), and FPIA (d). The solid lines represent identity lines (y = x), the dashed lines the regression lines according to *Passing & Bablok* (10).

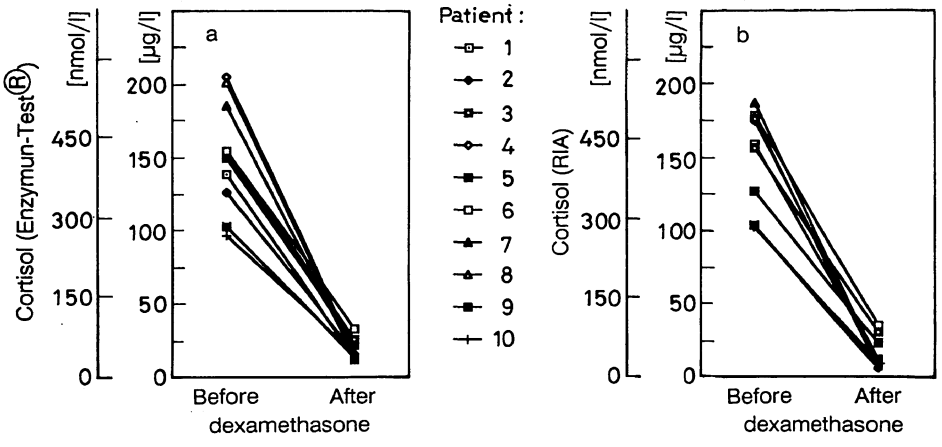

Fig. 5. Comparison of cortisol concentrations measured in serum samples of 10 patients with Enzymun-Test® Cortisol (a) and RIA (b) before and after administration of dexamethasone.

Discussion

The present paper describes a multicentre evaluation of a one-step enzyme-immunoassay for the determination of cortisol in serum or plasma samples. The easy handling and short measuring time prove the suitability of Enzymun-Test® Cortisol for daily laboratory routine work. The imprecision of this assay is rather low; with the exception of one evaluator, the coefficient of variation for within-run imprecision was less than 8% and for between-run imprecision less than 12%. These values are comparable with those reported for RIA (2, 11), HPLC (3), LIA (4), and FPIA (5). The measuring range of Enzymun-Test® Cortisol is sufficient for low cortisol levels in hypocortisolism or after dexamethasone suppression and for high cortisol levels in hypercortisolism or after corticotropin stimulation.

In eight different reference materials Enzymun-Test® Cortisol gave satisfactory recoveries (98.9 to 109.2%) in comparison with isotope dilution-mass spectometric analysis. The results show only low interferences by cortisol metabolites and other endogenous steroids, indicating that the employed antibody has an excellent specificity. Furthermore, dexamethasone did not interfere. Thus, Enzymun-Test® Cortisol can be utilized for differential diagnosis of hypercortisolism (dexamethasone-test). However, there is a cross-reaction with prednisolone. Serum cortisol concentrations measured with Enzymun-Test® Cortisol from patients treated with prednisolone, therefore, should be interpreted with caution.

The cortisol levels determined with Enzymun-Test® Cortisol exhibit a high degree of correlation with other assay methods. For LIA and HPLC the statistical evaluation according to *Passing & Bablok* (10) yielded slopes for the regression lines which are close to unity. For the RIA systems, however, the slopes were found to vary between 0.95 and 0.73. Similar differences were found concerning FPIA. This, in part, may be due to a change in the standard material used during this multicentre evaluation.

In conclusion, Enzymun-Test® Cortisol permits in general a precise and specific determination of cortisol in serum or plasma. Furthermore, it offers the advantages of a non-radioactive assay and the possibility of mechanization, using the ES 600 equipment.

References

1. Jenkins, J. S. (1975) The assessment of adrenal function. Brit. J. Hosp. Med. *14*, 373–380.
2. Wilke, T. J. & Hirning, D. A. (1984) The evaluation of five commercial serum cortisol kits regarding precision and accuracy. Clin. Biochem. *17*, 311–316.
3. Lambert, W. E., De Slypere, J.-P. M., Jonckheere, J. A., Vermeulen, A. & De Leenheer, A. P. (1984) Improved liquid chromatographic determination of serum cortisol with double internal standardization compared to radioimmunoassay and fluorometry, and elevated by isotope dilution/mass spectrometry. Anal. Biochem. *134*, 216–223.
4. Westerhuis, L. W. (1988) Measurement of cortisol with EMIT and Amerlite immunoassays. Clin. Chem. *34*, 2374.
5. Ayers, G. J., Clark, J. & Burnett, D. (1989) An evaluation of the Abbott TD_x serum cortisol assay. Ann. Clin. Biochem. *26*, 294–295.
6. Schmidt, H. & Heuer, M. (1986) ES 600. Lab. Clin. Pract. *4*, 83–87.
7. Lawson, A. M., Calam, D. H. & Colinet, E. S. (1985) BCR Information, "The Certification of Cortisol in two Lyophilized Serum Materials, BCR No. 192 and 193", Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium.
8. Siekmann, L. & Breuer, H. (1982) Determination of cortisol in human plasma by isotope dilution – mass spectrometry. J. Clin. Chem. Clin. Biochem. *20*, 883–892.
9. Kaiser, H. (1965) Zum Problem der Nachweisgrenze. Z. Analyt. Chem. *209*, 1–18.
10. Passing, H. & Bablok, W. (1983) A new biological procedure for testing the equality of measurements from two different analytical methods. J. Clin. Chem. Clin. Biochem. *21*, 709–720.
11. Lantto, O., Björkhem, I., Blomstrand, R. & Kallner, A. (1980) Interlaboratory evaluation of four kits for determination of plasma cortisol, with special reference to accuracy: Influence of matrix in calibration standards. Clin. Chem. *26*, 1899–1902.

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