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Analyses with the KODAK-Ektachem. Accuracy Control Using Reference Method Values and the Influence of Protein Concentration

Part II. Substrates

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Summary: The reliability of the determination of the most common substrates with the Ektachem 700 was evaluated. Accuracy control was performed in various ways including the comparison of results with reference method values. The influence of protein concentration was investigated systematically using sera containing varying amounts of protein obtained by ultracentrifugation.

Bilirubin. Deviation from the reference method values was within limits of the guidelines (Dt. Ärztebl. *85* (1988) B 517–B 532). The influence of protein concentration was negligible.

Cholesterol. The results agreed well with the reference method values. The method was not influenced by different protein concentrations.

Creatinine. The results were in good agreement with reference method values, when the method was calibrated with the primary assigned values of the calibrators. At high protein concentrations, the results were higher than those of the comparative method.

Glucose. The mean deviation from the reference method values was 1.0%. There was a clear positive bias at high protein concentrations.

Protein. The results from the Ektachem deviated from the reference method values by −6.8%.

Total glycerol (triacylglyerols). In the analysis of control sera, the Ektachem values differed greatly (+32.1%) from the reference method values. This difference was not found in a comparative study with native sera. At high protein concentrations the Ektachem results were higher than those of the comparative method.

Urea. The results were lower than the method-dependent assigned values (−16.6%). This deviation was not observed in a comparative study with native sera. Interference by protein was not observed.

Uric acid. In accuracy control with reference method values, a small bias was observed (+3.3%), which increased at high protein concentrations.

Introduction

The new guidelines for quality assessment in clinical laboratories of the Federal Republic of Germany (1) became valid in 1989. For accuracy control, the guidelines are based on reference method values as far as these are applicable. As methods using carrier-bound reagents become more popular, they should be evaluated to determine whether the results agree with those of reference methods for the analysis of control sera, or whether e. g. matrix effects impede their application.

An important interfering constituent of the matrix may be protein, especially in dry-phase technology. An experimental design using ultracentrifugation was evaluated for a systematic study of the influence of protein concentration on the measurement of substrates.

Materials and Methods

Methods

Bilirubin

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading and reaction layer and a control layer are held on a film support. After application of the sample, bilirubin is dissociated from protein by dyphylline and surfactant. All bilirubin fractions subsequently react with a diazonium salt to yield azobilirubin chromophores. The buffered control layer stabilizes the azo dyes, which are measured at 520 nm.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). In the comparative study (tab. 3) total bilirubin was determined, after dissociation from protein with caffeine-sodium benzoate, by reaction with diazotised sulphanilic acid, with correction for the sample blank.

3. Hitachi 704 (Boehringer Mannheim, Mannheim, F. R. G.). Measurement was performed at two wavelengths after reaction with diazotised sulphanilic acid in the presence of an accelerator.

Cholesterol

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading and reaction layer and a gelatine sublayer are coated on a film support. Serum cholesterol is dissociated from lipoproteins by a surfactant; cholesterol esters are hydrolysed enzymatically. Cholesterol is oxidized, forming hydrogen peroxide, which reacts with a leuco dye. The coloured product is measured at 540 nm.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). Cholesterol esters are hydrolysed by cholesterol esterase; free cholesterol is oxidized enzymatically to produce hydrogen peroxide, which in turn forms a quinoneimine dye from 4-aminoantipyrine and 4-hydroxybenzoic acid.

Creatinine

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer and two reagent layers are coated on a film support. Creatinine is hydrolysed enzymatically to form creatine, which is split into sarcosine and urea. Sarcosine is oxidized to glycine, formaldehyde, and hydrogen peroxide, which in turn forms a dye from a triarylimidazol derivative. Measurement is performed at 670 nm.

2. Hitachi 737 (Boehringer Mannheim, Mannheim, F. R. G.). Creatinine and creatine are hydrolysed enzymatically to sarcosine and urea. Sarcosine is oxidized to form glycine, formaldehyde and hydrogen peroxide, which forms a quinoneimine dye from 4-aminoantipyrine and a benzoic acid derivative.

3. SMA-C (Technicon, Bad Vilbel, F. R. G.). Creatinine was determined according to *Jaffe*, using alkaline picrate solution.

4. High performance liquid chromatography. The method was performed as described (2, 9).

Glucose

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer and a reagent layer are held on a polyester support. In the reagent layer glucose is oxidised enzymatically, forming gluconate and hydrogen peroxide, which reacts with 4-aminoantipyrine and 1,7-dihydroxynaphthalene to produce a red dye. The latter is measured at 540 nm by reflected light.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). Glucose was determined enzymatically, using the coupled enzyme system, hexokinase/glucose-6-phosphate dehydrogenase.

Protein, total

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer and a reagent layer are held on a film support. When the fluid penetrates the reagent layer, biuret reagent diffuses to react with the protein in the spreading layer. The coloured complex is measured by reflected light at 540 nm.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). Protein was determined by the biuret reaction and corrected for the sample blank.

Total glycerol/Triacylglycerols

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer, scavenger layer and reagent layer are coated on a film support. When the serum is deposited on the analytical element, a surfactant aids in dissociating the triacylglycerols from lipoproteins. Triacylglycerols are hydrolysed by lipase. Glycerol diffuses to the reagent layer to be phosphorylated by adenosine triphosphate. Glycerophosphate is oxidised to form dihydroxyacetone phosphate and hydrogen peroxide, which reacts with a triarylimidazole derivative to produce a dye (wavelength of measurement: 540 nm). Its intensity correlates with the total glycerol content of the sample.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). Triacylglycerols were determined enzymatically.

Urea

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer, a semipermeable layer, and two reagent layers are held on a film support. In the upper reagent layer urea is split to ammonia and carbon dioxide. Ammonia diffuses through the semipermeable membrane to react with an indicator dye. Its intensity is measured at 670 nm.

2. SMA-C (Technicon, Bad Vilbel, F. R. G.). In the comparative study (tab. 3) urea was determined by reaction with diacetyl monoxime after dialysis.

3. Hitachi 704 (Boehringer Mannheim, Mannheim, F. R. G.). Measurement was performed after reaction with the coupled enzyme system, urease/glutamate dehydrogenase.

Uric acid

1. Ektachem slide (KODAK, Stuttgart, F. R. G.). A spreading layer, a scavenger layer, and a reagent layer are held on a film support. Uric acid migrates through the scavenger layer to the reagent layer, where it is oxidised to allantoin, carbon dioxide and hydrogen peroxide, which produces a chromophoric product from a leuco dye (wavelength of measurement: 670 nm).

2. Cobas Bio (Hoffmann-La Roche, Grenzach-Wyhlen, F. R. G.). Uric acid was determined enzymatically, using the coupled enzyme system, uricase/aldehyde dehydrogenase (Human, Taunusstein, F. R. G.).

Calibration

Calibration of the Ektachem 700 was performed according to the recommendations of the manufacturer, if not otherwise stated.

Sample preparation

Samples with different amounts of protein were prepared as described (see Part I)[1]).

Quality assessment

The accuracy of the bilirubin, cholesterol, creatinine, glucose, protein, total glycerol ("triacylglycerols") and uric acid analyses was controlled with the aid of reference method values, which were determined by the authors as published (see references in the tables). Urea values for control sera, obtained with the Ektachem, were compared with the mean of the pertinent assigned values from field methods (urease/glutamate dehydrogenase), because reference method values were not available as a reference method is not yet established.

Statistics

In the comparative study (tab. 3) the standardized principal component analysis was used. The lines in the figures were constructed by linear regression analysis.

Results

Substrates

Bilirubin

Precision was adequate at high and even at low concentrations (tab. 1). In accuracy control the mean bias from the reference method values was +0.7% (tab. 2); no result exceeded the allowable limit of the guidelines (±21%) (1). The results from the Ektachem agreed well with those from the comparative method, when 129 native sera were analysed by both procedures (tab. 3). From experiments using ultracentrifuged sera, one may conclude that the bilirubin results are not influenced by the protein concentration (fig. 1).

Cholesterol

The mean relative standard deviation (1.9%) is far below the limit of the guidelines (1) (tab. 1). The mean deviation from the reference method values was +0.3% (tab. 4) (allowable limits of the guidelines (1): ±18%). When native sera were analysed with the Ektachem in a comparative study, the mean values differed by −8.2% (tab. 3). There was no detectable influence of protein concentration on the Ektachem results (fig. 2).

[1] This J. *28* (1990) 825–833.

Tab. 1. Imprecision between days of Ektachem 700

Analyte	Number of determinations	Mean value $\bar{x}$	Relative standard deviation CV %	Allowable relative standard deviation[5]) %
Bilirubin	10	36 [1])	2.9[1])	≤7.0
µmol/l	10	251 [2])	1.8[2])	
Cholesterol	10	3.01[1])	2.4[1])	≤6.0
mmol/l	10	2.92[2])	1.4[2])	
Creatinine	10	86 [1])	1.4[1])	≤6.0
µmol/l	10	941 [2])	1.9[2])	
Glucose	10	4.6 [1])	1.1[1])	≤5.0
mmol/l	10	23.3 [2])	0.9[2])	
Protein	10	34 [1])	1.5[1])	≤3.0
g/l	10	68 [2])	1.9[2])	
Total glycerol	10	1.48[3])	1.8[3])	≤7.0
mmol/l	10	1.59[4])	2.2[4])	
Urea	10	5.9 [3])	2.2[3])	≤8.0
mmol/l	10	12.3 [4])	1.6[4])	
Uric acid	10	409 [3])	1.7[3])	≤6.0
µmol/l	10	545 [4])	1.1[4])	

[1]) as determined by use of Kodatrol level I.
[2]) as determined by use of Kodatrol level II.
[3]) as determined by Kontrollogen L (Behring, Frankfurt, F. R. G.).
[4]) as determined by Kontrollogen LP (Behring, Frankfurt, F. R. G.).
[5]) according to the new guidelines of quality assessment (1).

Tab. 2. Bilirubin

Control serum[2])	Reference method value[3]) µmol/l	d %[1])
L	39.5	−5.8
M	44.3	+6.1
N	64.8	+6.0
O	70.2	+1.7
P	77.0	−1.4
Q	273.9	+0.9
R	314.8	−0.9
S	331.9	−0.7

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (6, 7)).

Creatinine

Creatinine determination with the Ektachem 700 was rather precise (tab. 1). When the analyser was calibrated with "primary assigned values", the accuracy control showed satisfactory results (tab. 5). Calibration according to the recommendations of the manufacturer by use of "the supplementary assigned values" yielded results that differed by +11.7% (range

Tab. 3. Comparison of results obtained by Ektachem 700 and field methods

Quantity		Number of determinations	Standardized principal component analysis		$s_{y \cdot x}$[1])	$\bar{y}$[2])	$\bar{x}$[3])	t[4])	r[5])
			Slope	Intercept					
S-Bilirubin	µmol/l	129	0.934	0.94	1.6	29.3	30.3	2.7[8])	0.999
S-Cholesterol	mmol/l	104	0.928	−0.05	0.11	5.04	5.49	22.8[8])	0.996
S-Creatinine[6])	µmol/l	103	0.982	2.47	6.9	108.1	108.6	0.6	0.997
S-Creatinine[7])	µmol/l	117	0.982	−5.35	15.6	228.8	238.6	4.7[8])	0.996
S-Glucose	mmol/l	133	0.995	0.06	0.16	6.50	6.47	1.4	0.998
S-Protein	g/l	132	1.119	−6.06	1.5	66.8	65.1	8.4[8])	0.974
S-Triacylglycerols/ Total glycerol	mmol/l	104	1.078	−0.09	0.17	1.89	1.84	2.0	0.974
S-Urea	mmol/l	85	1.021	−0.04	0.35	9.54	9.39	2.8[8])	0.999
S-Uric acid	µmol/l	70	1.036	0.76	11.9	327.1	314.9	5.9[8])	0.994

[1]) standard error of residuals.
[2]) arithmetic mean of results obtained by Ektachem 700.
[3]) arithmetic mean of results obtained by field method.
[4]) t-value (paired t-test).
[5]) correlation coefficient.
[6]) x-values determined by high performance liquid chromatography.
[7]) x-values determined enzymatically (Hitachi 737).
[8]) statistically significant ($\alpha < 0.01$)

Tab. 4. Cholesterol

Control serum[2])	Reference method value[3]) mmol/l	d %[1])
DA	2.622	−2.0
DB	2.799	−1.0
DC	2.826	−1.6
DD	2.893	−0.1
DE	3.013	+3.2
DF	3.045	+6.1
DG	3.084	−0.5
DH	3.126	+0.1
DI	3.177	−4.3
DJ	3.288	+1.0
DK	3.367	−1.4
DL	3.566	+4.3
DM	3.603	−0.6
DN	3.793	+1.0
DO	3.815	+0.1

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (8)).

Tab. 5. Creatinine
Calibration according to reference method values

Control serum[2])	Reference method value[3]) µmol/l	d %[1])
AA	61.4	+7.5
AB	112.3	+8.6
AC	132.3	+4.3
AD	170.4	−2.0
AE	173.3	+0.4
AF	176.3	−5.8
AG	177.6	−3.7
AH	180.0	−2.2
AI	180.7	−1.0
AJ	181.9	+0.6
AK	271.7	+3.8
AL	295.8	+5.1
AM	303.2	+4.9
AN	351.7	+7.2
AO	352.0	−2.8
AP	356.3	+3.3

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (9)).

−4.8% to +22.8%) from the reference method value (tab. 6). According to the guidelines (1) deviations up to ±18% are allowed. In a comparative study (2), the Ektachem results were in good agreement with results obtained by high performance liquid chromatography and by an enzymatic procedure (tab. 3).

From experiments with ultracentrifuged sera, it was clear that the results were influenced by the protein concentration (fig. 3). At a protein concentration of 120 g/l, the results were 29% higher than those obtained with an enzymatic procedure, and they finally even exceeded the values obtained by the *Jaffe*-reaction. Paraproteinaemic sera yielded similar results. These findings were confirmed by analyses of native sera of different protein concentration (fig. 4).

Tab. 6. Creatinine
Calibration as recommended by the manufacturer

Control serum[2])	Reference method value[3]) µmol/l	d %[1])
D	111.8	+17.2
B	112.3	+22.8
E	132.3	+13.3
C	150.2	− 4.8
H	180.0	+ 8.3
K	180.7	+ 8.4
G	295.8	+17.0

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (9)).

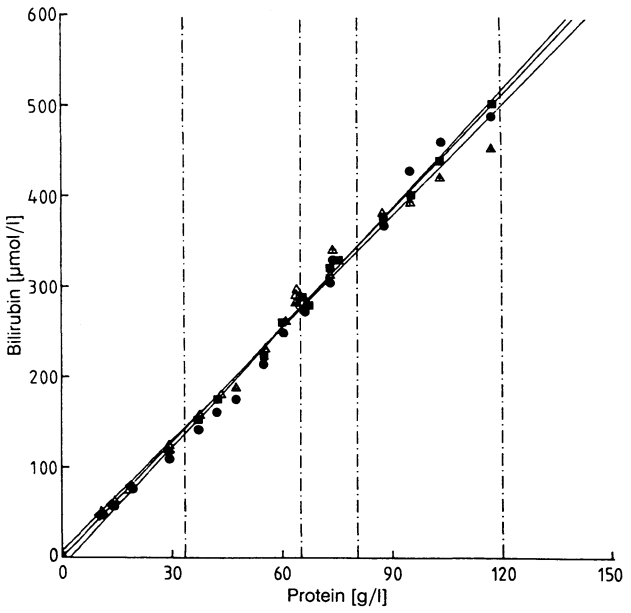

Fig. 1. Determination of bilirubin in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using diazo-methods (SMA-C ■, Hitachi 704 △) and the Ektachem 700 (●). The reference interval (65–80 g/l) of total protein and of the extreme concentrations found in every thousandth patient according to our files, are shown by the dotted line. Evaluation by linear regression analysis.

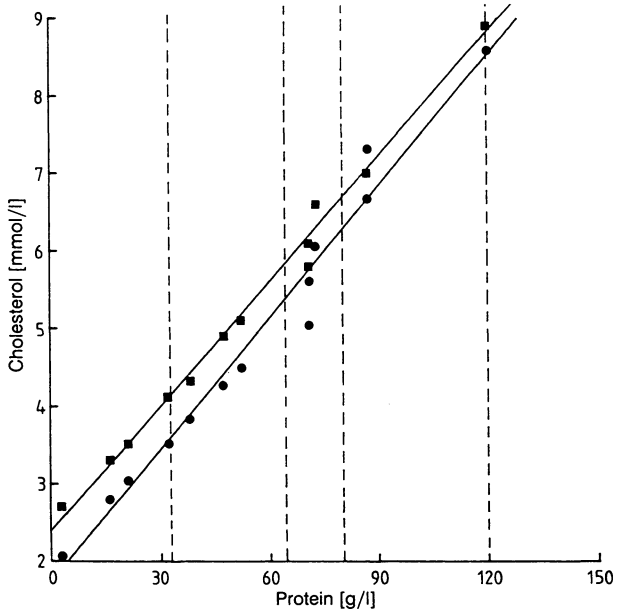

Fig. 2. Determination of cholesterol in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using an enzymatic reaction (SMA-C ■) and the Ektachem 700 (●). Dotted line: see fig. 1. Evaluation by linear regression analysis.

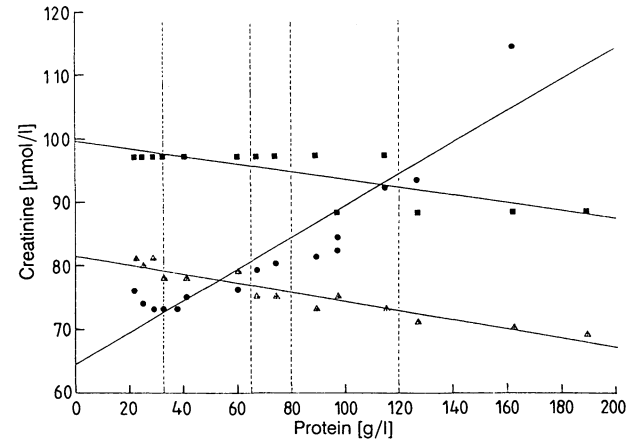

Fig. 3. Determination of creatinine in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods") by the *Jaffe*-reaction (SMA-C ■), an enzymatic reaction (Hitachi 704 △) and the Ektachem 700 (●). Dotted line: see fig. 1. Evaluation by linear regression analysis.

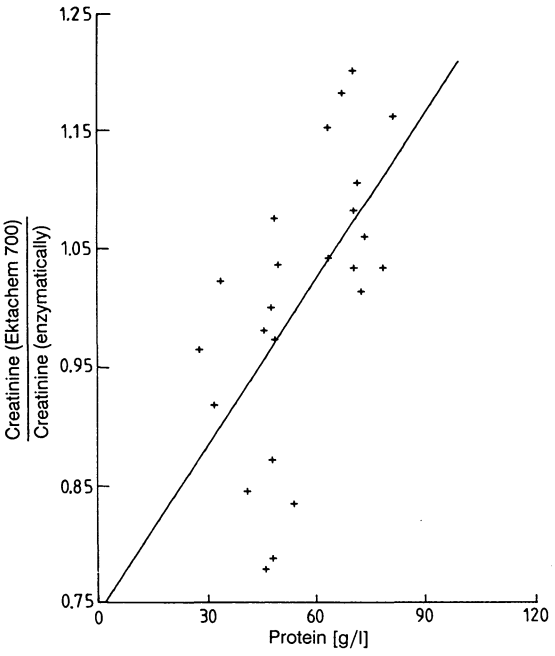

Fig. 4. Effect of protein concentration on the determination of creatinine in samples of native sera, using an enzymatic reaction (Hitachi 704) and the Ektachem 700 (n = 24). Evaluation by linear regression analysis.

Glucose

The mean coefficient of variation was 1.0% (tab. 1). The mean bias was +1.0% in accuracy control with reference method values of control sera (tab. 7) (maximally allowable bias (1): ±15%). In a study with native sera (n = 133), the Ektachem results agreed well with those of the comparative method (tab. 3). At high protein concentrations, however, the Ektachem results showed a positive bias. In the analysis of ultracentrifuged sera, the Ektachem results were 7% higher than those obtained with a comparative method at 120 g/l protein (fig. 5). Similar results were obtained with paraproteinaemic sera and with native sera of high protein concentration (fig. 6).

Tab. 7. Glucose

Control serum[2])	Reference method value[3]) mmol/l	d %[1])
E	4.79	+2.3
C	4.90	+0.1
D	5.00	+0.4
B	5.80	+1.8
H	6.27	+3.6
K	6.54	+2.4
G	13.60	−3.7

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (10)).

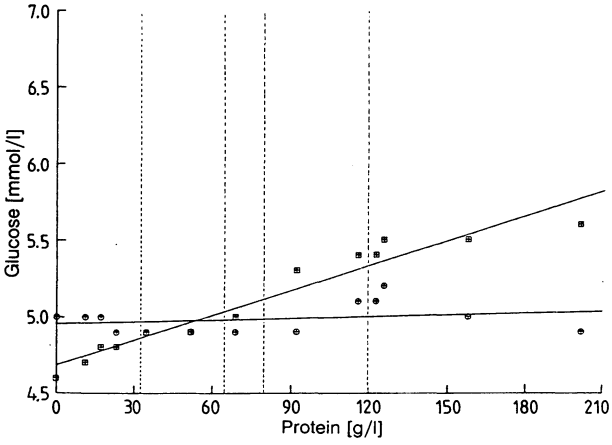

Fig. 5. Determination of glucose in serum preparations (pH 7.41) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using an enzymatic reaction (SMA-C ⊕) and the Ektachem 700 (⊞). Dotted line: see fig. 1. Evaluation by linear regression analysis.

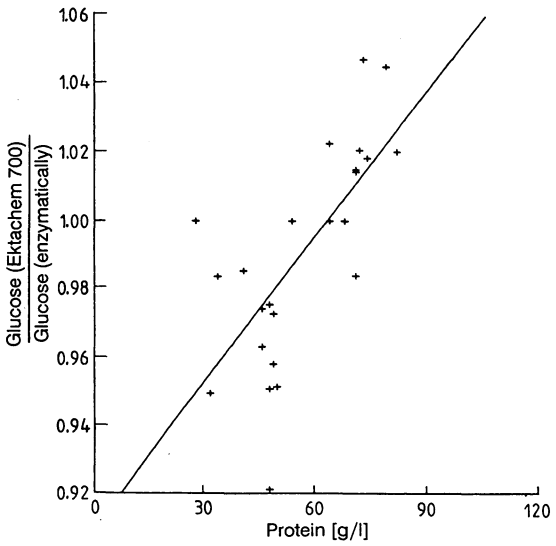

Fig. 6. Effect of protein concentration on the determination of glucose in samples of native sera, using an enzymatic reaction (SMA-C) and the Ektachem 700 (n = 24). Evaluation by linear regression analysis.

Protein

Imprecision was sufficiently low (tab. 1). Compared with reference method values, there was a mean deviation of −6.8% (tab. 8). Two out of nine results were outside the allowable limits (±9%) (1). In a comparative study with native sera, the mean values differed by +2.6% (tab. 3). A proportional and a constant error were observed.

Total glycerol/Triacylglycerols

The determination of total glycerol was precise (tab. 1). In accuracy control, a large bias was obtained (tab. 9). The mean deviation from the reference method values was +32.1%, far exceeding the limits of the guidelines (1): ±21%. In a comparative study,

Tab. 8. Protein

Control serum[2])	Reference method value[3]) g/l	d %[1])
K	48.7	− 5.5
H	49.0	− 4.1
G	51.9	− 9.4
C	53.7	− 6.9
D	59.2	− 8.8
F	60.4	− 9.0
E	62.3	−10.0
A	62.9	− 3.0
B	71.2	− 4.5

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (11, 12)).

Tab. 9. Total glycerol

Control serum[2])	Reference method value[3]) mmol/l	d %[1])
CA	0.919	+ 8.8
CB	1.103	+33.3
CC	1.110	+31.5
CD	1.121	+32.9
CE	1.126	+39.4
CF	1.167	+32.8
CG	1.176	+42.0
CH	1.208	+34.9
CI	1.230	+33.3
CJ	1.297	+34.9
CK	1.325	+35.8
CL	1.353	+22.0
CM	1.493	+36.6
CN	1.646	+31.8

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (13)).

however, which was performed with native sera, mean values differed only by +2.7%, even though the comparative results were obtained by SMA-C determining triacylglycerols (tab. 3). The influence of protein on the results was investigated by the analysis of ultracentrifuged sera; a bias of 10.7% at 120 g/l protein was observed (fig. 7).

Urea

Imprecision was small and the coefficient of variation far below the limit (8%) (1) (tab. 1). As reference method values are not available, the accuracy of the

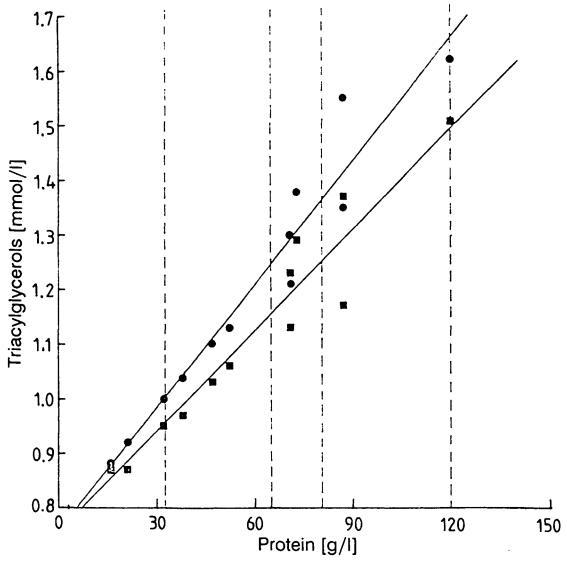

Fig. 7. Determination of total glycerol/triacylglycerols in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using an enzymatic reaction (SMA-C ■) and the Ektachem 700 (●). Dotted line: see fig. 1. Evaluation by linear regression analysis.

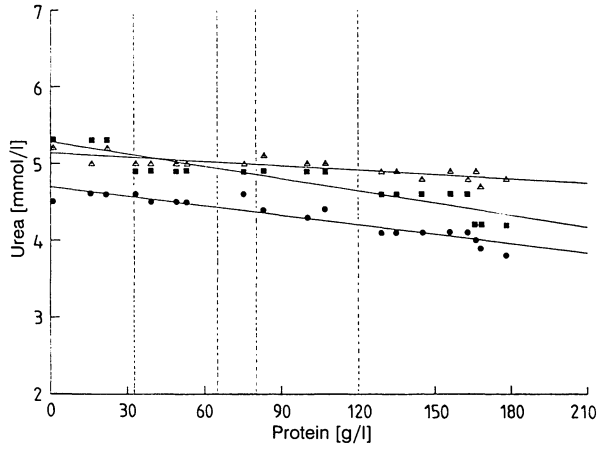

Fig. 8. Determination of urea in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using an enzymatic reaction (Hitachi 704 △), diacetyl monoxime (SMA-C ■) and the Ektachem 700 (●). Dotted line: see fig. 1. Evaluation by linear regression analysis.

Tab. 10. Urea

Control serum	Method-dependent assigned value mmol/l	d %[1])
EK[4])	2.63	−27.8
EF[5])	3.4 [2])	− 8.8
EC[6])	5.11	−21.7
EE[7])	5.5 [2])	−16.4
EG[8])	6.7	−20.9
EA[9])	7.99	− 2.4
EI[10])	8.24	−24.8
EL[11])	9.64[3])	−20.1
EJ[12])	17.5	−23.2
EH[13])	18.0	−12.2
ED[14])	18.6	−15.1
EB[15])	26.8	− 5.2

[1]) Deviation of the value obtained by Ektachem from the method-dependent assigned value in %.
[2]) Arithmetic mean of the contributing method-dependent assigned values.
[3]) Definitive value.
[4]) Pathonorm L (Merck, Darmstadt, F. R. G.).
[5]) PED (Ciba Corning, Fernwald, F. R. G.).
[6]) Moni-trol I (Baxter Deutschland, Unterschleißheim, F. R. G.).
[7]) QCS Normal Control Serum Assayed (Ciba Corning, Fernwald, F. R. G.).
[8]) Validate-N (Organon Teknika, Eppelheim, F. R. G.).
[9]) Seronorm (Merck, Darmstadt, F. R. G.).
[10]) Kontrollogen L (Behring, Frankfurt, F. R. G.).
[11]) Standard Reference Material 909 (National Institute of Standards and Technology, Washington, D. C.).
[12]) Kontrollogen LP (Behring, Frankfurt, F. R. G.).
[13]) Validate-A (Organon Teknika, Eppelheim, F. R. G.).
[14]) Moni-trol II (Baxter Deutschland, Unterschleißheim, F. R. G.).
[15]) Pathonorm H (Merck, Darmstadt, F. R. G.).

Ektachem measurements was evaluated by comparison with method-dependent assigned values (method: urease/glutamate dehydrogenase). A negative bias was found (−16.6%), which ranged from −2.4% to −27.8% (tab. 10). According to the guidelines (1), a bias up to ±24% is acceptable. In a comparative study with native sera, good agreement was observed (tab. 3), the mean values differing by +1.6%. An influence of protein was not evident (fig. 8).

Uric acid

The mean relative standard deviation was 1.4% (tab. 1). A small, but constantly positive bias was obtained in accuracy control with reference method values (+3.3%) (tab. 11), but never exceeding the limits of the guidelines: ±18% (1). In a comparative study, the mean values differed by 3.9% due to a proportional error (tab. 3). At high protein concentration, uric acid is overestimated by 17.2% (fig. 9), as shown by the analysis of ultracentrifuged sera. This was confirmed by the determination of native sera containing different protein concentrations (fig. 10).

Tab. 11. Uric acid

Control serum[2])	Reference method value[3]) µmol/l	d %[1])
FA	280.4	+4.0
FB	292.1	+3.7
FC	292.7	+3.5
FD	295.2	+1.1
FE	299.2	+1.5
FF	321.8	+2.5
FG	329.7	+5.0
FH	394.0	+3.5
FI	499.3	+2.0
FJ	499.7	+3.2
FK	530.0	+3.7
FL	547.7	+2.2
FM	629.1	+4.5
FN	649.7	+3.3
FO	662.2	+5.8

[1]) Deviation of the value obtained by Ektachem from the reference method value in %.
[2]) Control sera not yet commercially available.
[3]) Reference method value as determined by the reference method (l. c. (14)).

Discussion

Bilirubin

Obviously accuracy control can be performed according to the new concept of quality assessment based on reference method values, as there were no signs of interference due to matrix effects from control sera.

Cholesterol

The Ektachem results agreed well with the reference method values of the control sera and were independent of the protein concentration.

Creatinine

As creatinine determinations are performed by a specific enzymatic reaction, it is not reasonable to calibrate the analyser by using "supplementary assigned values" obtained by *Jaffe* analyses, and which are contradictory to the intentions of the new concept (1). After calibration by "primary assigned values", good agreement with the reference method value was observed, i.e. Ektachem results approach the true values rather well. These findings were confirmed by studies with native sera using the *Fuller*'s earth method, high performance liquid chromatography and an enzymatic procedure for comparison (2). In the meantime, the Ektachem slides, which were used in this study (GEN 07), were replaced by slides GEN 05. GEN 05 slides do not show unlinearity at concentrations below 50 µmol/l (2) and are as accurate as the GEN 07 series. The influence of protein has to be considered, at least when its concentration exceeds 90 g/l.

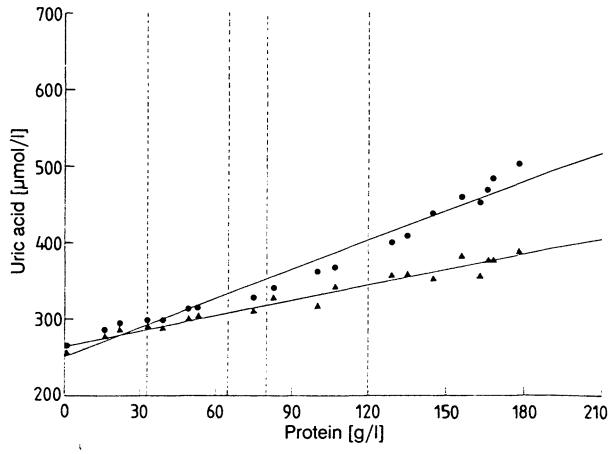

Fig. 9. Determination of uric acid in serum preparations (pH 7.40) of different protein concentration (prepared by ultracentrifugation, see "Methods"), using an enzymatic reaction (Cobas Bio ▲) and the Ektachem 700 (●). Dotted line: see fig. 1. Evaluation by linear regression analysis.

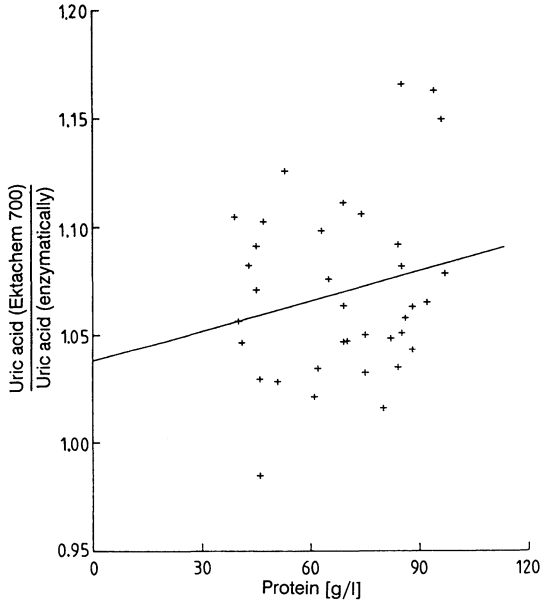

Fig. 10. Effect of protein concentration on the determination of uric acid in samples of native sera, using an enzymatic reaction (Cobas Bio) and the Ektachem 700 (n = 36). Evaluation by linear regression analysis.

Glucose

The influence of protein must also be taken into account in the determination of glucose, in cases of hyperproteinaemia due to polyclonal as well as to monoclonal globulins.

Protein

A negative bias was expected in accuracy control using reference method values. From a previous study (3), it was concluded that methods using a correction

with a sample blank underestimate the target values, whereas those without correction overestimate them. Hence the negative bias is absent in the study with native sera, where both field methods use a correction for sample blank.

Total glycerol/Triacylglycerols

Total glycerol measurements with Ektachem could not be evaluated conclusively by using reference method values of control sera: All control sera of both manufacturers (Behring-Werke, Boehringer Mannheim) seem to contain additives. These grossly interfere with the Ektachem method, which is well suited to the determination of native sera. At high protein concentrations, a small positive bias was found.

Urea

The negative bias in accuracy control using method-dependent assigned values is thought to be attributable to interfering compounds of the matrix, and it can be avoided by reconstitution of control sera with sodium carbonate solution (4). In any case, analyses of native sera were unaffected.

Uric acid

The small bias, which was observed with control sera and native sera, could be due to the assigned value of the Ektachem calibrator.

On the whole, one may conclude that accuracy control of the Ektachem system can be performed by using the reference method values of the control sera, with the exception of total glycerol and, to a lesser extent, of protein. The influence of protein can be studied by using samples prepared by ultracentrifugation, which is more realistic than using hyaluronate (5). The results were confirmed by the analysis of native sera of different protein concentration and composition. The "ultracentrifugation" approach seems to be more conclusive than the use of native sera, as the analyte concentration changes homogeneously. In native sera both analyte and protein concentration vary unsystematically. The deviating results in hyperproteinaemia are probably due to an increase of viscosity, which alters the time course of the chemical reactions.

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