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(54) **PROCEDE POUR SUPPRIMER LES ERREURS DUES A L'HEMOGLOBINE LORS DE L'ANALYSE D'ECHANTILLONS MEDICAUX**

(54) **PROCESS TO ELIMINATE HAEMOGLOBIN ERRORS WHEN ANALYSING MEDICAL SAMPLES**

(57) L'invention concerne un procédé pour déterminer la présence d'une substance dans un échantillon contenant de l'hémoglobine libre, par mesure bichromatique optique à une longueur d'onde de mesure principale et une longueur d'onde de mesure secondaire. Selon ce procédé, on utilise une longueur d'onde de mesure secondaire supérieure à 475 nm comportant des bandes d'absorption d'hémoglobine.

(57) The present invention relates to a process for determining an analyte in a sample containing free haemoglobin by optical bichromatic measurement for a main and a secondary measuring wave length. A secondary measuring wave length of above 475 nm is used containing absorption bands of haemoglobin.

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(54) Title: PROCESS TO ELIMINATE HAEMOGLOBIN ERRORS WHEN ANALYSING MEDICAL SAMPLES

(54) Bezeichnung: VERFAHREN ZUR BESEITIGUNG VON HÄMOGLOBINSTÖRUNGEN BEI DER ANALYSE MEDIZINISCHER PROBEN

(57) Abstract

The present invention relates to a process for determining an analyte in a sample containing free haemoglobin by optical bichromatic measurement for a main and a secondary measuring wave length. A secondary measuring wave length of above 475 nm is used containing absorption bands of haemoglobin.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft ein Verfahren zur Bestimmung eines Analyten in einer freies Hämoglobin enthaltenden Probe durch optische bichromatische Messung bei einer Haupt- und einer Nebenmeßwellenlänge, wobei man eine Nebenmeßwellenlänge von oberhalb 475 nm verwendet, bei der sich Absorptionsbanden von Hämoglobin befinden.

Method for the elimination of haemoglobin interferences in the analysis of medical samples

Description

The invention concerns a method for the determination of an analyte in a sample containing free haemoglobin in which the determination is carried out by an optical bichromatic measurement at a main and a secondary wavelength. In particular this method is suitable for the determination of the parameters ammonia, creatine kinase and isoenzymes thereof and lactate dehydrogenase and isoenzymes thereof in a medical sample e.g. a serum or plasma sample.

It is generally known that haemolysis interferes with the determination of many analytes in some cases to a considerable extent. In order to nevertheless obtain measured values which are unfalsified various methods have been published in the past for eliminating haemolysis interference.

One of these methods is that when measuring in automated analyzers a second wavelength (secondary wavelength) is used in addition to the first wavelength (primary wavelength) by means of which the interfering influence of interfering substances such as haemoglobin, bilirubin and lipaemia can be eliminated or at least minimized.

One requirement for this is that the substance to be measured absorbs as little as possible at the secondary wavelength but that the interfering substance absorbs at the same level as possible as at the main wavelength

("Praxistechnik: Photometer für die Ärztliche Praxis, Deutscher Ärzteverlag" 1977, pages 41-42).

In the DIA letter (Boehringer Mannheim) No. 70 (1985) it is mentioned that the secondary wavelength should be as close as possible to the main wavelength since as a rule the interfering substance then has similar absorbances at the main and secondary wavelengths.

In Clin. Chem. 25/6, 951-959 (1979) it is pointed out that the secondary wavelength should be selected so that it is near to the absorption minimum of the chromogen and near to the absorption maximum of the interfering substance. In this connection a secondary wavelength of 380 nm is recommended for the determination of glucose (main wavelength 340 nm) since here the absorbance of the interfering substances is similar to that at 340 nm.

In contrast in the Eur. J. Clin. Chem. Clin. Biochem. 31/9, 595-601 (1993) it is regarded as critical to measure UV tests at a secondary wavelength of 380 nm since in this case the conversion of Hb-O_2 into Meth-Hb leads to spectral changes at 380 nm and thus to errors in the measurements. Therefore a secondary wavelength is recommended for tests which are based on the measurement of a decrease or increase in NAD(P)H which lies behind the so-called Soret region such as e.g. 475 nm.

All previously described methods relate to the elimination of interference in erroneous measurements caused by haemolysis. The availability of blood substitutes based on haemoglobin makes the issue of removing interferences by native or synthetic haemoglobin or compounds analogous to Hb much more acute

than hitherto. Such interferences then on the one hand also occur in non-haemolytic sample material and on the other hand also to a much greater extent than in native haemolysis since the haemoglobin content of blood serum or plasma can be up to 2000 mg/dl in blood substitute therapy.

In addition it was found that when measuring certain analytes such as ammonia, creatine kinase and isoenzymes thereof as well as lactate dehydrogenase and isoenzymes thereof it is not easily possible to achieve an adequate elimination of haemoglobin interference by using a secondary wavelength of 475 nm or higher e.g. at 480, 505, 600, 660 or 700 nm. Since these parameters are of essential importance in the context of cardiovascular and emergency diagnostics as well as for the diagnosis of patients treated with blood substitutes, the object of the present invention was to provide a simple method for eliminating interferences which are caused by native haemoglobin or by blood substitutes based on synthetic haemoglobin or compounds similar to haemoglobin, in particular when measuring the above-mentioned analytes.

The object of the invention is achieved by a method for the determination of an analyte in a sample containing free haemoglobin by optical bichromatic measurement at a main and a secondary wavelength wherein a secondary wavelength above 475 nm is used in which the absorption bands of haemoglobin are located.

Preferred secondary wavelengths for the method according to the invention are in the range of 546 ± 10 nm, in particular 546 ± 5 nm as well as in the range of 570 ± 10 nm and in particular 570 ± 5 nm. The wavelengths 546

and 570 nm are most preferred.

The selection of the wavelengths according to the invention as secondary wavelengths was surprising since the greatest interferences by haemoglobin are obtained at the secondary wavelength of 405 nm known from the state of the art (for example instrument settings for the Boehringer Mannheim/Hitachi 717-analyser according to the instructions of the kit insert for the reagent to determine creatine kinase, order No. 1 273 248, Boehringer Mannheim Diagnostica Catalogue 1997) at which haemoglobin also absorbs. Furthermore in the aforementioned publication Eur. J. Clin. Chem. Clin. Biochem. it is pointed out that a secondary wavelength should be used to eliminate interference by haemoglobin which lies beyond the Soret region (the main absorption band of haemoglobin) so that it would at most have been obvious to use those wavelengths as a secondary wavelength where there are no absorption bands at all of haemoglobin.

The method for eliminating interference according to the invention is suitable for methods in which the analyte is determined by optical measurement in particular by optical measurement at a main wavelength in the UV range. The method is particularly preferably carried out for tests which are based on a measurement of the increase or decrease of the concentration of NADH or NADPH in the sample. In this case one preferably uses a main wavelength in the range of 340 ± 10 nm.

The method according to the invention is suitable for the determination of any samples in which free haemoglobin is present. Examples of such samples are

haemolytic serum or plasma samples or samples which contain a blood substitute. Examples of blood substitutes which fall under the term "free haemoglobin" within the sense of the present invention are derivatized, polymerized, modified or cross-linked derivatives of haemoglobins in particular of human haemoglobin or bovine haemoglobin such as DCL haemoglobin (diaspirin cross-linked haemoglobin) and recombinantly produced haemoglobin.

In a preferred embodiment of the method according to the invention the content of an analyte selected from the group comprising ammonia, creatine kinase and isoenzymes thereof and lactate dehydrogenase and isoenzymes thereof is determined.

The determination of ammonia by the method according to the invention is preferably carried out according to the enzymatic UV method (Da Fonseca-Wollheim F., Z. Klin. Chem. Klin. Biochem. 11 (1973) 421).

Creatine kinase (CK) is preferably determined according to the "optimized standard method" of the German Society for Clinical Chemistry (J. Clin. Chem. Clin. Biochem. 15 (1977), 249). The creatine kinase isoenzyme CK-MB is preferably determined by the immunological UV method (Würzburg U. et al., Klin. Wschr. 54 (1976), 357).

The determination of lactate dehydrogenase (LDH) or of the lactate dehydrogenase isoenzyme (HBDH) (1-hydroxy-butyrate dehydrogenase) is preferably carried out according to the "optimized standard method" of the German Society for Clinical Chemistry (Z. Klin. Chem. Klin. Biochem. 8 (1970), 658 and 10 (1972), 182).

A serum or plasma sample is preferably used as the sample in the method according to the invention in particular a human serum or plasma sample.

A particular advantage of the method according to the invention is that it can be carried out in an automated analyzer such as a Boehringer Mannheim/Hitachi 704 or 717 analyzer. In such analyzers it is easily possible to set the particularly preferred secondary wavelengths of 546 or 570 nm.

The invention is further elucidated by the following examples.

General methods

A solution containing haemoglobin was added to one portion of a serum pool such that a haemoglobin content of 2000 mg/dl was reached. Another equal portion of the serum pool was admixed with an equivalent amount of a NaCl solution (154 mmol/l). Both portions were subsequently mixed together in different ratios in such a way that a Hb concentration series of 11 samples was formed, whereby one sample contained no Hb and the highest sample contained 2000 mg/dl Hb.

Example 1

Determination of ammonia

The determination was carried out on a Boehringer Mannheim/Hitachi 717 analyzer. The following reagents were used:

Reagent 1: 150 mmol/l triethanolamine buffer, pH 8.6; 15 mmol/l α-ketoglutarate; 1.5 mmol/l ADP

Reagent 2: 150 mmol/l triethanolamine buffer; pH 8.5; 15 mmol/l α-ketoglutarate; 1.5 mmol/l ADP; 0.31 mmol/l NADPH; ≥ 24 U/ml glutamate dehydrogenase (GLDH)

The test procedure was as follows: 200 µl reagent 1 and after 5 min 50 µl reagent 2 were added to 20 µl sample. The analyte was determined after a period of a further 40 sec. A main wavelength of 340 nm and secondary wavelengths of 405 nm, 480 nm, 505 nm, 600 nm, 660 nm and 700 nm (comparison) as well as of 546 nm and 570 nm (invention) were used for the measurement.

The result of this determination is shown in table 1. It can be seen that when using the measurement wavelengths of 546 and 570 nm according to the invention a considerably improved recovery was achieved than with the other wavelengths.

Example 2

Determination of creatinine kinase

The determination was carried out on a Boehringer Mannheim/Hitachi 717 analyzer. The following reagents were used:

Reagent 1: 110 mmol/l imidazole buffer; pH 6.7; 20.5 mmol/l glucose; 2.05 mmol/l EDTA; 2.5 mmol/l ADP; 6.1 mmol/l AMP; 12 µmol/l diadenosine pentaphosphate; 2.5 mmol/l NADP; 25 mmol/l N-acetylcysteine; ≥ 3.1 U/ml hexokinase (HK); ≥ 1.8 U/ml glucose-6-phosphate dehydrogenase (G6P-DH)

Reagent 2: 25 mmol/l imidazole buffer; pH 7.5; 20.5 mmol/l glucose; 2.05 mmol/l EDTA; 61 mmol/l Mg^{2+}; 184 mmol/l creatine phosphate

The test procedure was as follows: 250 µl reagent 1 and after 5 min 50 µl reagent 2 were added to 7 µl sample. The analyte was determined after a period of a further 2 min. A main wavelength of 340 nm and secondary wavelengths of 405 nm, 480 nm, 505 nm, 600 nm, 660 nm and 700 nm (comparison) as well as of 546 nm and 570 nm (invention) were used for the measurement.

The result of this determination is shown in table 2. It can be seen that when using the measurement wavelengths of 546 and 570 nm according to the invention a considerably improved recovery was achieved than with

the other wavelengths.

Example 3

Determination of the creatine kinase isoenzyme CK-MB

The determination was carried out on a Boehringer Mannheim/Hitachi 717 analyzer. The following reagents were used:

Reagent 1: 110 mmol/l imidazole buffer; pH 6.7; 21 mmol/l glucose; 11 mmol/l Mg^{2+}; 2.1 mmol/l EDTA; 2.4 mmol/l ADP; 6.0 mmol/l AMP; 12 µmol/l diadenosine pentaphosphate; 2.4 mmol/l NADP; 24 mmol/l N-acetyl cysteine; ≥ 3.0 U/ml HK; ≥ 1.8 U/ml G6P-DH; antibody, inhibitory capacity towards CK-M up to 2000 U/l.

Reagent 2: 110 mmol/l imidazole buffer; pH 6.7; 21 mmol/l glucose; 2.1 mmol/l EDTA; 11 mmol/l Mg^{2+}; 186 mmol/l creatine phosphate

The test procedure was as follows: 250 µl reagent 1 and after 5 min 50 µl reagent 2 were added to 12 µl sample. The analyte was determined after a period of a further 3 min. A main wavelength of 340 nm and secondary wavelengths of 405 nm, 480 nm, 505 nm, 600 nm, 660 nm and 700 nm (comparison) as well as of 546 nm and 570 nm (invention) were used for the measurement.

The result of this determination is shown in table 3. It

can be seen that when using the measurement wavelengths of 546 and 570 nm according to the invention a considerably improved recovery was achieved than with the other wavelengths.

Example 4

Determination of lactate dehydrogenase

The determination was carried out on a Boehringer Mannheim/Hitachi 717 analyzer. The following reagents were used:

Reagent 1: 68 mmol/l phosphate buffer; pH 7.5;
≥ 0.73 mmol/l pyruvate

Reagent 2: ≥ 1.1 mmol/l NADH

The test procedure was as follows: 250 µl reagent 1 and after 5 min 50 µl reagent 2 were added to 5 µl sample. The analyte was determined after a period of a further 60 sec. A main wavelength of 340 nm and secondary wavelengths of 405 nm, 480 nm, 505 nm, 600 nm, 660 nm and 700 nm (comparison) as well as of 546 nm and 570 nm (invention) were used for the measurement.

The result of this determination is shown in table 4. It can be seen that when using the measurement wavelengths of 546 and 570 nm according to the invention a considerably improved recovery was achieved than with the other wavelengths.

Example 5

Determination of the LDH isoenzyme HBDH

The determination was carried out on a Boehringer Mannheim/Hitachi 717 analyzer. The following reagents were used:

Reagent 1: 68 mmol/l phosphate buffer; pH 7.5;
3.7 mmol/l α-oxobutyrate

Reagent 2: ≥ 1.1 mmol/l NADH

The test procedure was as follows: 250 µl reagent 1 and after 5 min 50 µl reagent 2 were added to 5 µl sample. The analyte was determined after a period of a further 60 sec. A main wavelength of 340 nm and secondary wavelengths of 405 nm, 480 nm, 505 nm, 600 nm, 660 nm and 700 nm (comparison) as well as of 546 nm and 570 nm (invention) were used for the measurement.

The result of this determination is shown in table 5. It can be seen that when using the measurement wavelengths of 546 and 570 nm according to the invention a considerably improved recovery was achieved than with the other wavelengths.

Table 1

Sample	Hb content [mg/dl]	Content at 405 nm [µg/dl]	Content at 480 nm [µg/dl]	Content at 505 nm [µg/dl]	Content at 546 nm [µg/dl]	Content at 570 nm [µg/dl]	Content at 600 nm [µg/dl]	Content at 660 nm [µg/dl]	Content at 700 nm [µg/dl]
1	0	157	163	160	159	158	154	158	154
2	200	145	162	162	157	156	158	157	155
3	400	140	165	163	162	158	162	158	160
4	600	133	166	162	161	153	163	162	161
5	800	145	172	171	164	158	168	166	158
6	1000	144	173	172	162	155	168	171	167
7	1200	160	171	175	167	156	175	166	164
8	1400	159	177	177	166	155	177	171	170
9	1600	176	180	178	162	152	178	178	180
10	1800	175	183	177	163	157	188	180	183
11	2000	178	189	192	172	159	199	190	187

Sample	Hb content [mg/dl]	Recovery 405 nm [%]	Recovery 480 nm [%]	Recovery 505 nm [%]	Recovery 546 nm [%]	Recovery 570 nm [%]	Recovery 600 nm [%]	Recovery 660 nm [%]	Recovery 700 nm [%]
1	0	100	100	100	100	100	100	100	100
2	200	92	99	101	99	99	103	99	101
3	400	89	101	102	102	100	105	100	104
4	600	85	102	101	101	97	106	103	105
5	800	92	106	107	103	100	109	105	103
6	1000	92	106	108	102	98	109	108	108
7	1200	102	105	109	105	99	114	105	106
8	1400	101	109	111	104	98	115	108	110
9	1600	112	110	111	102	96	116	113	117
10	1800	111	112	111	103	100	122	114	119
11	2000	113	116	120	108	101	129	120	121

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Table 2

Sample	Hb content [mg/dl]	Content at 405 nm [U/l]	Content at 480 nm [U/l]	Content at 505 nm [U/l]	Content at 546 nm [U/l]	Content at 570 nm [U/l]	Content at 600 nm [U/l]	Content at 660 nm [U/l]	Content at 700 nm [U/l]
1	0	175	175	177	175	177	176	179	174
2	200	181	176	177	175	180	176	180	178
3	400	189	177	179	178	180	176	179	178
4	600	182	165	165	168	171	165	170	166
5	800	191	164	166	168	170	162	166	166
6	1000	185	164	164	170	172	163	166	163
7	1200	196	163	164	170	174	163	166	165
8	1400	205	163	163	170	174	160	165	164
9	1600	212	163	163	173	174	161	164	162
10	1800	214	160	163	172	177	161	165	163
11	2000	224	160	161	173	178	160	164	164

Sample	Hb content [mg/dl]	Recovery 405 nm [%]	Recovery 480 nm [%]	Recovery 505 nm [%]	Recovery 546 nm [%]	Recovery 570 nm [%]	Recovery 600 nm [%]	Recovery 660 nm [%]	Recovery 700 nm [%]
1	0	100	100	100	100	100	100	100	100
2	200	103	101	100	100	102	100	101	102
3	400	108	101	101	102	102	100	100	102
4	600	104	94	93	96	97	94	95	95
5	800	109	94	94	96	96	92	93	95
6	1000	111	94	93	97	97	93	93	94
7	1200	112	93	93	97	98	93	93	95
8	1400	117	93	92	97	98	91	92	94
9	1600	121	93	92	99	98	91	92	93
10	1800	122	91	92	98	100	91	92	94
11	2000	128	91	91	99	101	91	92	94

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Table 3

Sample	Hb content [mg/dl]	Content at 405 nm [U/l]	Content at 480 nm [U/l]	Content at 505 nm [U/l]	Content at 546 nm [U/l]	Content at 570 nm [U/l]	Content at 600 nm [U/l]	Content at 660 nm [U/l]	Content at 700 nm [U/l]
1	0	40.6	40.2	40.7	41.5	40.1	40.7	40.8	38.1
2	200	49.7	41.2	39.4	40.7	42.6	39.1	39.7	37.9
3	400	58.9	37.3	38.2	41.8	41.6	38.8	36.0	37.2
4	600	68.1	38.5	36.8	43.1	40.8	36.1	37.7	36.5
5	800	75.3	36.6	35.7	42.6	42.4	32.7	36.3	32.5
6	1000	82.4	34.9	35.4	44.1	43.4	30.8	32.6	36.5
7	1200	82.5	39.6	33.9	44.4	44.5	27.5	36.7	32.8
8	1400	95.0	35.5	34.5	44.8	43.0	26.1	33.4	33.9
9	1600	99.2	37.3	35.0	44.1	44.3	25.9	31.7	29.4
10	1800	98.9	33.9	29.8	46.7	42.9	24.8	27.1	29.4
11	2000	97.1	33.2	29.1	48.1	44.1	22.4	27.9	29.9

Sample	Hb content [mg/dl]	Recovery 405 nm [%]	Recovery 480 nm [%]	Recovery 505 nm [%]	Recovery 546 nm [%]	Recovery 570 nm [%]	Recovery 600 nm [%]	Recovery 660 nm [%]	Recovery 700 nm [%]
1	0	100	100	100	100	100	100	100	100
2	200	122	102	97	98	106	96	97	99
3	400	145	93	94	101	104	95	88	98
4	600	168	96	90	104	102	89	92	96
5	800	185	91	88	103	106	80	89	85
6	1000	203	87	87	106	108	76	80	96
7	1200	203	98	83	107	111	68	90	86
8	1400	234	88	85	108	107	64	82	89
9	1600	244	93	86	106	110	64	78	77
10	1800	244	84	73	113	107	61	66	77
11	2000	239	83	71	116	110	55	68	78

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Table 4

Sample	Hb content [mg/dl]	Content at 405 nm [U/l]	Content at 480 nm [U/l]	Content at 505 nm [U/l]	Content at 546 nm [U/l]	Content at 570 nm [U/l]	Content at 600 nm [U/l]	Content at 660 nm [U/l]	Content at 700 nm [U/l]
1	0	196	197	199	197	199	199	199	205
2	200	162	202	206	203	198	208	200	206
3	400	127	210	215	199	195	219	207	215
4	600	95	218	223	202	196	229	217	224
5	800	72	220	228	200	192	225	222	222
6	1000	45	224	226	199	193	230	226	222
7	1200	27	223	232	201	190	236	228	233
8	1400	11	237	240	203	192	243	234	236
q	1600	-13	230	238	199	190	242	233	236
10	1800	-1	234	244	198	192	245	237	238
11	2000	-15	233	246	197	191	247	238	241

Sample	Hb content [mg/dl]	Recovery 405 nm [%]	Recovery 480 nm [%]	Recovery 505 nm [%]	Recovery 546 nm [%]	Recovery 570 nm [%]	Recovery 600 nm [%]	Recovery 660 nm [%]	Recovery 700 nm [%]
1	0	100	100	100	100	100	100	100	100
2	200	83	103	104	103	99	104	101	100
3	400	65	107	108	101	98	110	104	105
4	600	48	111	112	103	98	115	109	109
5	800	37	112	115	102	96	113	112	108
6	1000	23	114	114	101	97	116	114	108
7	1200	14	113	117	102	95	119	115	114
8	1400	6	120	121	103	96	122	118	115
9	1600	-7	117	120	101	95	122	117	115
10	1800	-1	119	123	101	96	123	119	116
11	2000	-8	118	124	100	96	124	120	118

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Table 5

Sample	Hb content [mg/dl]	Content at 405 nm [U/l]	Content at 480 nm [U/l]	Content at 505 nm [U/l]	Content at 546 nm [U/l]	Content at 570 nm [U/l]	Content at 600 nm [U/l]	Content at 660 nm [U/l]	Content at 700 nm [U/l]
1	0	88.0	93.4	93.8	92.6	90.8	92.1	93.0	91.0
2	200	83.5	91.1	99.4	97.4	90.3	94.3	91.9	93.1
3	400	68.5	95.0	97.5	90.6	92.5	95.6	87.1	99.3
4	600	58.0	97.0	98.7	94.4	90.2	97.6	103.6	99.4
5	800	45.0	100.2	99.1	91.3	85.6	94.2	100.7	103.0
6	1000	38.6	101.8	101.5	91.4	92.3	105.3	100.9	99.4
7	1200	38.1	101.4	101.1	90.3	87.8	104.7	98.0	100.8
8	1400	18.0	101.4	104.8	92.0	91.6	104.9	109.3	104.6
q	1600	22.6	104.0	104.4	94.9	90.3	103.0	99.4	104.7
10	1800	20.2	102.9	102.2	91.9	94.3	102.8	106.8	94.0
11	2000	18.7	103.4	102.7	91.8	89.2	100.3	106.2	102.0

Sample	Hb content [mg/dl]	Recovery 405 nm [%]	Recovery 480 nm [%]	Recovery 505 nm [%]	Recovery 546 nm [%]	Recovery 570 nm [%]	Recovery 600 nm [%]	Recovery 660 nm [%]	Recovery 700 nm [%]
1	0	100	100	100	100	100	100	100	100
2	200	95	98	106	105	99	102	99	102
3	400	78	102	104	98	102	104	94	109
4	600	66	104	105	102	99	106	111	109
5	800	51	107	106	99	94	102	108	113
6	1000	44	109	108	99	102	114	109	109
7	1200	43	109	108	98	97	114	105	111
8	1400	20	109	112	99	101	114	118	115
9	1600	26	111	111	102	99	112	107	115
10	1800	23	110	109	99	104	112	115	103
11	2000	21	111	110	99	98	109	114	112

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Claims

1. Method for the determination of an analyte in a sample containing free haemoglobin by optical bichromatic measurement at a main and secondary wavelength,
wherein
a secondary wavelength above 475 nm is used at which absorption bands of haemoglobin are located.

2. Method as claimed in claim 1,
wherein
a secondary wavelength in the range of 546 ± 10 nm is used.

3. Method as claimed in claim 1,
wherein
a secondary wavelength in the range of 570 ± 10 nm is used.

4. Method as claimed in one of the claims 1-3,
wherein
a test is carried out which is based on a measurement of the increase or decrease of the concentration of NADH or NADPH in the sample.

5. Method as claimed in claim 4,
wherein
a main wavelength in the range of 340 ± 10 nm is used.

6. Method as claimed in one of the claims 1-5,
wherein
the content of an analyte is determined selected from the group comprising ammonia, creatine kinase and isoenzymes thereof and lactate dehydrogenase and isoenzymes thereof.

7. Method as claimed in claim 6,
wherein
ammonia is determined.

8. Method as claimed in claim 6,
wherein
creatine kinase or/and the creatine kinase isoenzyme CK-MB is determined.

9. Method as claimed in claim 6,
wherein
lactate dehydrogenase or/and the lactate dehydrogenase isoenzyme HBDH is determined.

10. Method as claimed in one of the claims 1-9,
wherein
a sample is determined which contains a blood substitute.

11. Method as claimed in one of the claims 1-10,
wherein
the determination is carried out on a serum or plasma sample.

12. Method as claimed in one of the claims 1-11, **wherein**
the determination is carried out in an automated analyzer.