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Stability of heparin blood samples during transport based on defined pre-analytical quality goals

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Abstract

Background: In many countries and especially in Scandinavia, blood samples drawn in primary healthcare are sent to a hospital laboratory for analysis. The samples are exposed to various conditions regarding storage time, storage temperature and transport form. As these factors can have a severe impact on the quality of results, we wanted to study which combination of transport conditions could fulfil our predefined goals for maximum allowable error.
Methods: Samples from 406 patients from nine general practitioners (GPs) in two Danish counties were sent to two hospitals for analyses, during two periods (winter and summer). Transport conditions (mail, courier pick-up, or brought to hospital by public coach), storage time, storage temperature and centrifugation requirements were different in the two counties. Results were tested for deviation from a ''0-sample'', the blood sample taken, centrifuged and separated at the doctor's office within 45–60 min. This sample was considered as the best estimate of a comparison value.
Results: The pre-set quality goals were fulfilled for all the investigated components for samples transported to hospital by courier either as whole blood or as ''on gel'' after centrifugation, as long as the samples were stored at 20–25°C and centrifuged/analysed within 5–6 h. A total of 4% of the samples sent by mail had mismatched identity, probably due to plasma being transferred to a new tube.
Conclusions: Samples can be sent as unprocessed anticoagulated whole blood if the above mentioned conditions are met. There is no need for centrifugation in the primary sector. Neither mailing of samples with plasma ''on gel'' nor public transport by coach bus fulfil our analytical goals.
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Introduction

In Denmark with a population of 5.5 million, approximately 3 million blood samples are drawn each year in General Practice (GP) and sent to hospital laboratories for analyses.

The transport logistics are different for various counties in Denmark. Samples (whole blood or plasma) are transported by mail, by ''hospital car'', taxi, coach or private transport. In at least one county all these possibilities are used. Pre-analytical factors also vary: the samples are drawn in tubes with or without separation gel, sent with or without centrifugation, or with centrifugation followed by plasma separation (1, 2). Some hospital laboratories keep the samples at a constant temperature of 21±1°C (1). Also, different brands of tubes are used.

The pre-analytical conditions have a decisive impact on the final result and may lead to diagnostic mistakes. Sinclair et al. (3) describe seasonal pseudohyperkalaemia during transport of samples. A recently published paper (4) deals with spurious hyperkalaemia caused by storage of the sample and transport conditions.

Centrifugation of blood samples before transport is time consuming and expensive. Furthermore, transfer of plasma to another tube increases both the risk of sample mismatch and infection. Therefore, it is preferable to transport whole blood. As both the economical and quality consequences of pre-analytical solutions are considerable, our aim was to investigate which combination of storage time, storage temperature and transport method would allow the use of primary tubes with whole blood or ''on gel'' after centrifugation. All existing procedures in two counties were investigated.

The focus was on combinations of transport conditions as such and not on a single factor.

Materials and methods

Subjects

Adult patients that were undergoing routine venous puncture at their GP were asked to participate. Extra tubes (6 or 7) were drawn and submitted to different pre-analytical conditions. The sample handling and the pre-analytical procedures were carried out by the GP's staff, nurses, secretaries or laboratory technologists.

In Centre A, four GPs recruited a total of 101 patients during March 2006 and 105 patients during August 2006. The samples were drawn between 08.00 and 09.30 am.

In Centre B, five primary healthcare centres each drew samples from 20 patients (a total of 100 patients) in April 2006 and from 100 patients in September 2006.

Transport

The centres existing transportation means were examined. For study purposes, some additional situations were investigated.

Centre A: Transport by coach The samples were placed in styrofoam transport boxes. They were delivered in special boxes located at bus stops and were picked up by a public bus driver who delivered them to hospital porters. The samples transported this way were positioned randomly and exposed to outdoor temperatures for approximately 30 min. The duration of transport was approximately 60 min.

Centre A: Transport by courier The samples were collected at the primary care centre. These samples were placed in racks and were positioned upright. The duration of transport was approximately 10–60 min. The samples were collected from the GPs twice a day.

Centre A: Transport by mail Samples were sent after centrifugation, with plasma ''on gel''. They normally reached the hospital the next morning. Samples were transported in a random position. The exposure to outdoor temperature was variable.

Centre B There is an established routine courier service, which picks up the blood samples within 8 h after sampling. In the meantime, all samples are stored at a controlled temperature of 21±1°C in a special thermostated box, and the stable temperature is maintained during transport. During transport, the samples are positioned upright. This procedure is a follow-up of the results of the previous investigation described in 2005 (1). Duration of transport during this investigation was 10–15 min.

As an additional transport form we investigated the stability of samples stored for 4 and 8 h at room temperature. These samples were transported together and in the same way as the thermostated samples.

Another possibility for GPs located far from Centre B's laboratory is to centrifuge the blood samples, transfer the plasma to another tube and mail them to the laboratory. Here, an additional situation was undertaken: mailing of centrifuged samples ''on gel'' without plasma separation.

Temperature

Centre A The average temperature for the period in which the samples were collected during the winter was –4.3°C at night and 1.5°C during the day. In August, average temperatures were above 30°C during the investigation. The GPs that participated had no air-conditioning.

Centre B Samples were stored either at room temperature or at 21±1°C in the special thermostated boxes for 4 or 8 h. The average temperature for the period in which the samples were sent by mail during the winter was +1°C at night and 5.0°C during the day.

In the summer period, the average night temperature was 13°C and 20°C during the day.

The sampling time, the arrival of the sample at the hospital and the analysing time were recorded in the laboratory information system.

Abbreviations

The investigated combinations of pre-analytical circumstances are designated A1 to A7 and B1 to B6 for Centre A and Centre B, respectively. Explanation of these codes is given in Tables 1 and 2.

Analytical methods

The GPs in Centre A used VenoSafe Lithium Heparin gel tubes (Lot 0510051) from Terumo Medical Incorporation, Leuven, Belgium. In Centre B, Lithium Heparin tubes with gel from Becton Dickinson, BD Diagnostics, Plymouth, England were used.

All involved participants centrifuged the samples for 7–10 min at 1300–2000×*g* according to tube manufacturer's instructions.

The investigated components are listed in Table 3. The inclusion criterion was that all components could be analysed in a single tube. Thus, components such as glucose, demanding a special blood sampling tube were not considered.

Plasma concentrations of all investigated components were analysed in both centres on Modular P from Roche Diagnostics, with commercial kits from Roche Diagnostics GmbH, Mannheim, Germany.

A ''0-sample'' was the blood sample taken, centrifuged and separated at the doctor's office within 45–60 min and considered as the best estimate of a ''true'' comparison value. Thereafter, the plasma is considered stable during the designed protocol period (5–7). The ''0-samples'' were handled by laboratory (temperature regulated=thermostated) boxes, able to keep a temperature of 21±1°C, and were purchased from ViboCold, Viborg, Denmark.

Analytical quality goals

Limits for acceptable deviation from the 0-sample results were pre-defined by the authors of this paper (Table 3). CLIA rules [as cited in Tietz (8)] and the relevance of the components for the GPs were taken into consideration.

Because the analytical coefficient of variation (CV)% for the single component in the laboratories influences both the comparison sample ''0'' and the transported samples, and because the biological and sampling variations are eliminated as samples are taken in the same puncture, the total acceptable difference was defined as follows:

$$SD_{\text{Total acceptable deviation}}{}^2 = SD_{\text{Acceptable pre-analytical deviation}}{}^2 + 2 \times SD_{\text{analytical}}{}^2.$$

The quality demand was that at least 95% of the measurements had to be within 2 $SD_{\text{Total deviation}}$ from the 0-sample.

Outliers The 0-sample results were checked by Burnett's model (9) and no outliers were found. Outliers for the single test sample were not excluded and the sample was not re-analysed.

Statistics

The number of results exceeding quality limits for the single transport form was tested for significance against transport

Table 1 Percentage of results within the limits for maximal acceptable deviation: winter.

Transport to		Centre A							Centre B						Centre A+B	
Centrifugation at		GP		Hospital		GP	Hospital	GP	Hospital				GP			
Transport by		Courier	Coach	Courier	Coach	Courier		Mail	Courier				Mail			
Blood sample		On gel		Whole blood		On gel	Whole blood	On gel	Whole blood				On gel	Pipetted		
Lowest temperature		10°C	−1.7°C	10°C	−1.7°C	10°C	10°C	−8.6°C	21±1°C	21±1°C	21±1°C	21±1°C	−3.0°C			
Time to centrifugation (mean), h		3/4	3/4	6	6	3/4	8	3/4	4		8		4			
Time to analysing (mean), h		6				8		30	6		10		30			
Number		50	51	50	51	101		101	100	100	100	100	100	100	Patient results	
Component	Allowable deviation, %	A1	A2	A3	A4	A5	A6	A7	B1	B2	B3	B4	B5	B6	Median	Range
Alanine aminotransferase, U/L	24	100	86	98	100	99	99	94	98	98	100	99	91	99	23	13–84
Albumin, g/L	17	100	100	100	100	100	100	100	100	100	100	100	100	100	44	37–50
Alkaline phosphates, U/L	20	100	100	100	100	100	100	100	100	100	100	100	100	100	65	29–181
Bilirubin, μmol/L	36	100	100	98	100	99	99	100	100	100	100	100	100	99	9	4–31
Calcium, mmol/L	7	100	96	100	94	99	99	89	99	100	99	100	98	98	2.33	2.00–2.61
Total cholesterol, mmol/L	8	100	100	100	98	100	100	100	99	99	97	98	100	100	5.1	3.3–8.6
Cobalamin (B_{12}), pmol/L	19								100	100	100	100	100	98	363	55–1400
C-Reactive protein, mg/L	36	100	100	100	100	100	100	100	100	100	100	100	100	100	<5	<5–93
Creatinine, μmol/L	11	100	98	100	100	100	100	100	100	100	100	100	100	99	86	67–160
Creatine kinase, U/L	16	100	96	100	100	100	98	100	100	99	100	100	98	99	82	22–555
Free thyroxine, pmol/L	12								99	100	100	99	97	96	16	11–26
γ-Glutamyltransferase, U/L	28	100	75	100	100	100	100	68	100	100	100	100	74	100	32	11–552
HDL-cholesterol, mmol/L*	13	100	100	100	100	100	100	100	100	100	100	100	100	99	1.64	0.94–3.3
Lactate dehydrogenase, U/L	18	100	80	100	96	98	100	13	98	96	98	86	0	99	144	73–425
LDL-cholesterol, mmol/L	9	96	96	96	98	99	99	90							2.8	1.2–5.6
Phosphate, mmol/L	14	100	98	68	59	100	62	92	99	90	70	37	93	96	1.03	0.48–1.47
Potassium, mmol/L	10	98	96	98	90	99	93	87	97	98	88	94	73	100	3.9	2.9–5.5
Sodium, mmol/L	6	100	100	100	100	100	100	100	100	100	100	100	100	100	141	133–145
Triglyceride, mmol/L	12	99	100	100	100	100	100	99	98	97	98	97	94	99	1.23	0.41–11.78
Thyreotropin, mU/L	10								100	100	100	100	98	99	1.45	0.03–7.6
Urate, mmol/L	21	100	100	100	100	100	100	100	100	100	100	100	100	100	0.31	0.16–0.71

HDL, high-density lipoprotein; LDL, low-density lipoprotein. *HDL-cholesterol in winter, Centre B (n=83).

Table 2 Percentage of results within the limits for maximal acceptable deviation: summer.

Transport to		Centre A							Centre B						Centre A+B	
Centrifugation at		GP		Hospital		GP	Hospital	GP	Hospital				GP			
Transport by		Courier	Coach	Courier	Coach	Courier		Mail	Courier				Mail			
Blood sample		On gel		Whole blood		On gel	Whole blood	On gel	Whole blood				On gel	Pipetted		
Highest temperature		>30°C	>30°C	>30°C	>30°C	>30°C	>30°C	>30°C	21±1°C	26°C	21±1°C	26°C	26°C			
Time to centrifugation (mean), h		3/4	3/4	6	6	3/4	8	3/4	4		8		4			
Time to analysing (mean), h		6				8		30	6		10		30			
Number		51	54	51	54	105		105	100	100	100	100	100*	100	Patient results	
Component	Allowable deviation, %	A1	A2	A3	A4	A5	A6	A7	B1	B2	B3	B4	B5	B6	Median	Range
Alanine aminotransferase, U/L	24	96	80	94	96	96	97	75	98	98	98	98	97	98	23	5–111
Albumin, g/L	17	100	100	100	100	100	100	100	100	100	100	100	100	100	44	35–51
Alkaline phosphates, U/L	20	100	100	100	100	100	100	100	100	100	100	100	100	100	71	14–173
Bilirubin, μmol/L	36	90	100	96	98	99	99	98	100	100	100	100	100	94	9	4–103
Calcium, mmol/L	7	100	100	100	98	99	98	98	100	100	100	100	100	100	2.36	2.15–2.72
Total cholesterol, mmol/L	8	100	98	100	91	100	99	98	100	99	99	99	100	98	5.2	2.6–9.0
Cobalamin (B_{12}), pmol/L	19								100	100	100	100	100	96	299	131–656
C-Reactive protein, mg/L	36	100	100	100	100	100	100	100	100	100	100	100	100	100	<5	<5–205
Creatinine, μmol/L	11	100	98	98	98	99	99	97	100	100	100	100	100	97	90	68–206
Creatine kinase, U/L	16	100	100	98	96	97	98	98	100	100	100	100	100	95	96	19–496
Free thyroxine, pmol/L	12								99	98	97	88	100	100	14	11–23
γ-Glutamyltransferase, U/L	28	98	74	96	100	97	99	52	99	99	98	99	79	99	33	8–611
HDL-cholesterol, mmol/L*	13	100	100	100	100	100	99	100	100	100	100	100	100	100	1.54	0.8–2.93
Lactate dehydrogenase, U/L	18	92	68	98	94	99	98	26	95	96	98	93	0	99	159	105–306
LDL-cholesterol, mmol/L	9	98	94	98	94	94	95	79	100	100	99	99	100	96	2.8	1.3–6.3
Phosphate, mmol/L	14	96	94	69	40	99	56	97	96	67	60	27	97	80	1.03	0.62–1.75
Potassium, mmol/L	10	100	100	77	50	100	71	95	99	96	87	91	97	95	4.1	3.0–5.4
Sodium, mmol/L	6	100	100	100	100	100	100	100	100	100	100	100	100	100	140	134–146
Triglyceride, mmol/L	12	100	100	100	100	100	100	98	100	97	100	100	95	99	1.56	0.38–11.41
Thyreotropin, mU/L	10								96	95	98	97	95	92	1.3	0.03–4.7
Urate, mmol/L	21	100	100	100	100	100	100	100	100	100	100	100	100	100	0.32	0.15–0.68

*In the summer period only 78 samples were collected at B5 in Centre B. The number indicates the percentage of results fulfilling the goals.

Table 3 Biochemical components, critical level and maximal deviation from 0-sample which can be accepted.

Component	Critical level	Clinical acceptable pre-analytical deviation		CV% analytical	Total CV for deviation, %	Allowed deviation (%) between 0-sample and test sample 95.5% results within ±2 SD
		conc. (=2 SD)	CV%			
Alanine aminotransferase, U/L	70	±15	±10.7	4	±12.1	±24.2
Albumin, g/L	35	±3	±4.3	5	±8.3	±16.5
Alkaline phosphates, U/L	105	±20	±9.5	2.5	±10.2	±20.3
Bilirubin, μmol/L	20	±7	±17.5	3.5	±18.2	±36.4
Calcium, mmol/L	2.1	±0.1	±2.4	2	±3.7	±7.4
Total cholesterol, mmol/L	5	±0.3	±3.0	2	±4.1	±8.2
Cobalamin (B_{12}), pmol/L	150	±20	±6.7	5	±9.7	±19.4
C-Reactive protein (CRP) mg/L	20	±7	±17.5	2	±17.7	±35.5
Creatinine, μmol/L	100	±7	±3.5	3	±5.5	±11.0
Creatine kinase, U/L	150	±20	±6.7	3	±7.9	±15.8
Free thyroxine, pmol/L	22	±2	±4.5	3	±6.2	±12.4
γ-Glutamyltransferase, U/L	75	±20	±13.3	3	±14.0	±28.0
HDL-cholesterol, mmol/L	1	±0.1	±5.0	3	±6.6	±13.1
Lactate dehydrogenase (LD), U/L	200	±35	±8.8	2	±9.2	±18.4
LDL-cholesterol, mmol/L	3	±0.2	±3.3	2	±4.4	±8.7
Phosphate, mmol/L	0.8	±0.1	±6.3	2	±6.9	±13.7
Potassium, mmol/L	3	±0.3	±5.0	1	±5.2	±10.4
Sodium, mmol/L	140	±7	±2.5	1	±2.9	±5.7
Thyreotropin, mU/L	4	±0.3	±3.8	2.5	±5.2	±10.3
Triglyceride, mmol/L	2	±0.2	±5.0	2	±5.7	±11.5
Urate, mmol/L	0.25	±0.05	±10.0	2	±10.4	±20.8

Critical level is the level at which the maximal allowed deviation is defined. Clinical acceptable pre-analytical deviation (95% CI) is given as a concentration (=2 SD) in reported units and as CV%. CV% analytical is the imprecision in the laboratories. Total CV for deviation is the combined CV-analytical for both 0-sample and the test sample and the CV% of accepted deviation. Maximal allowed deviation is the 95% CI limits for difference between 0-sample and test sample which fulfil the goals at the critical level.

form B1, where the criteria were met using Fisher's exact χ^2-test.

Ethics

The study was presented to and accepted by the Regional Danish Science Ethics Committee as a technical and quality investigation. Oral informed consent was obtained from all participants.

Results

Tables 1 and 2 show how the components were affected by transport and the percentage of results fulfilling quality goals. The most sensitive components were potassium, phosphate, alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) and lactate dehydrogenase (LD), especially if specimens were exposed to high temperatures during the summer period (A2, A7, B5).

Potassium stability is strongly influenced by prolonged storage times and temperature (A3, A4, A6, A7, B3–B5).

Phosphate represents a serious problem for almost all combinations of time and temperature (A2, A7 and B2–B6). Analysis shows that these transport forms give results that differ significantly from those obtained under the optimal transport and storage combination B1 (whole blood, thermostated storage for maximum 6 h, courier transport) (see Table 4).

ALT, GGT and LD cannot be transported by coach or sent by mail ''on gel'' (A2, A7, B4 and B5).

There are also minor deviations for some other components, especially for samples transported by couch or sent by mail ''on gel''.

Table 4 summarises the evaluation of the different modes of transport of tubes. Results are arranged from the highest to the lowest degree of fulfilment of quality goals in the winter and in the summer periods, separately and also in total, with transport form B1 yielding the best sample quality. It can be observed that all components in transport form B1 fulfil quality goals. All other transport forms produce several results falling outside acceptance limits and they are all of worse quality than transport form B1 (A5 vs. B1 tested with Fisher's exact χ^2-test is significant: $p=0.014$, all others $p<0.00005$).

Table 5 is the ''opposite'' of Tables 1 and 2 and shows what the acceptance limits should be if 95% of the results were to fall within these limits in summer, as well as in winter.

Discussion

Several papers (10–13) describe influences of different pre-analytical factors on stability of blood samples. Each factor and its effect have been examined separately and exhaustively. In contrast to analytical quality goals, pre-analytical quality goals are seldom

Table 4 Transport form ranked according to the number of components not fulfilling the pre-defined goals.

Mode	Time to centrifugation, h	Sample	Temperature, °C		Transport by	Time to analyse, h	Component[a]	Number results not fulfilling goals		
			Storage	Transport				Winter	Summer	Total
B1	4	Whole blood	21±1	21±1	Courier	6	All fulfil	0	0	0
A5	3/4	On gel	w: 20–22 s: 25–>30	Outdoor	Courier	8	LDL	1	6	7
A1	3/4	On gel	w: 20–22 s: 25–>30	Outdoor	Courier	6	LD Bilirubin	0 0	8 10	8 10
B6	ca. 4	Plasma	Room temp. 20–26	Outdoor	Mail	30	Bilirubin TSH P	1 1 4	6 8 20	7 9 24
B2	4	Whole blood	Room temp. 20–26	21±1	Courier	6	P	10	33	43
B3	8	Whole blood	21±1	21±1	Courier	10	K P	12 30	13 40	25 70
A6	8	Whole blood	w: 20–22 s: 25–>30	Outdoor	Courier	8	K P	7 28	29 44	36 72
A3	6	Whole blood	w: 20–22 s: 25–>30	Outdoor	Courier	6	ALT K P	2 2 32	6 23 31	8 25 63
B4	8	Whole blood	Room temp. 20–26	21±1	Courier	10	K LD P	6 14 63	9 7 73	15 21 136
A7	3/4	On gel	w: 20–22 s: 25–>30	Outdoor	Mail	30	P Ca K ALT LDL GGT LD	8 11 13 6 10 32 87	3 2 5 25 21 48 74	11 13 18 31 31 80 161
B5	App. 4	On gel	Room temp. 20–26	Outdoor	Mail	30	P Triglyceride ALT K GGT LD	7 6 9 27 26 100	3 5 3 3 21 100	10 11 12 30 47 200
A2	3/4	On gel	w: 20–22 s: 25–>30	Outdoor	Coach	6	P LDL ALT GGT LD	2 4 14 25 20	6 6 20 26 32	8 10 34 51 52
A4	6	Whole blood	w: 20–22 s: 25–>30	Outdoor	Coach	6	Ca LDL LD K P	6 2 4 10 41	2 6 6 50 60	8 8 10 60 101

TSH, thyreotropin; P, phosphate; K, potassium; Ca, calcium. Results from winter (w), summer (s) and over-all (total) are shown. Number of results not fulfilling quality goals in transport forms A1–A7 and B2–B6 were found to be significantly different ($p < 0.00005$) from transport form B1.

defined and no standards seem to be available. Ideally, pre-analytical variation should be included in the goal for total analytical error.

This article differs from other papers investigating pre-analytical influence on laboratory results as this *is not a descriptive paper* listing various factors, e.g., manufacturer of sampling tubes, time, temperature and centrifugation and their influence on results.

Our present investigation was directed towards establishing practical, real-life circumstances for storage and transport preserving sample quality for *all* investigated components. It would be very confusing if blood samples for analysing some components could be sent in one transport form, but not in another. The other important point was that the impact of the single factor was not investigated. The potential effects of single factors were not compared. Only the number of results exceeding limits was interesting.

At first, we defined the acceptable error size as a deviation from the result obtained under optimal con-

Table 5 Deviations from 0-sample which will allow 95% of the results to fulfil goals (summer and winter).

Transport to		Centre A							Centre B					
Centrifugation at		GP		Hospital		GP	Hospital	GP	Hospital				GP	
Transport by		Courier	Coach	Courier	Coach	Courier		Mail	Courier				Mail	
Blood sample		On gel		Whole blood		On gel	Whole blood	On gel	Whole blood				On gel	Pipetted
		Limits at which 95% of the results will fulfil the goals												
Component	Allowable deviation, %	A1	A2	A3	A4	A5	A6	A7	B1	B2	B3	B4	B5	B6
Alanine transaminase	24	14	42	22	21	20	17	35	17	14	13	19	32	12
Albumin	17	5	5	6	7	5	5	5	4	5	5	5	5	5
Alkaline phosphates	20	4	6	5	6	6	6	10	5	5	6	6	9	6
Bilirubin	36	25	20	25	20	25	23	23	17	13	17	17	25	38
Calcium	7	3	4	3	7	5	5	7	3	3	3	4	6	6
Total cholesterol	8	5	6	4	8	6	6	6	5	5	6	6	6	5
Cobalamin (B_{12})	19								7	11	9	15	15	14
C-Reactive protein	36	8	11	8	7	11	6	6	1	5	3	1	2	5
Creatinine	11	5	8	6	7	7	8	8	4	5	5	6	7	7
Creatine kinase	16	6	11	8	10	9	7	11	6	6	7	7	10	8
Free thyroxine	12								8	8	8	8	8	8
γ-Glutamyltransferase	28	13	73	15	13	15	15	86	11	9	10	10	52	10
HDL-cholesterol	13	4	7	4	8	6	7	7	7	8	7	8	8	10
Lactate dehydrogenase	18	12	48	8	17	13	9	102	22	18	17	24	165	11
LDL-cholesterol	9	7	8	7	9	8	8	12						
Phosphate	14	6	11	21	29	8	25	14	13	21	21	29	16	17
Potassium	10	5	8	12	19	5	15	12	10	10	15	13	18	10
Sodium	6	2	2	3	3	2	2	2	2	2	1	2	2	2
Triglyceride	12	4	6	5	5	5	6	9	7	8	8	9	13	8
Thyreotropin	10								9	10	9	9	11	13
Urate	21	3	5	5	5	5	5	5	4	4	4	4	5	4

ditions. Secondly, we examined if the different ways in which the blood samples are transported influenced the results in relation to the pre-defined goals. Finally, we ranked the different transport models according to their capability of giving 95% results within the stated limits.

We investigated 13 combinations of transport, temperature and storage time. The common transport characteristics fulfilling the demanded criteria for all investigated components (except phosphate) were that in both centres the samples were stored at 20–25°C, transported by courier in a stable, upright position and centrifuged not later than 6 h after sampling (Tables 1 and 2).

All other transport forms demonstrate problems. Transport of plasma ''on gel'' by public coach or by mail (A2, A4, A7 and B5) and exposing the specimens to random positions and extreme temperatures invalidate results for some of the components.

The most sensitive components were potassium, phosphate, ALT, GGT and LD (Tables 1 and 2).

Potassium analyses are frequently requested because of its clinical applicability. Doctors are aware of its vulnerability and thus there is a focus on potassium (3, 4, 14). Tables 1 and 2 show, as expected, that temperature and duration of storage play a crucial role in its stability.

Deviations were observed both in winter and summer, depending on storage temperature.

But even if the samples were stored and transported at optimal and stable temperature, potassium concentrations increased when the samples were stored longer than 6 h (B3).

Plasma samples transferred to another tube can be sent by mail (B6), with the exception of phosphate (see below).

Phosphate represents, surprisingly, a serious problem for many combinations of time and temperature, even if the plasma is separated (Table 4, summer). Even the results for phosphate transported by mail ''on gel'' after centrifugation deviated modestly from the ''0'' value (A7 and B5).

Samples for phosphate estimation should be taken in a hospital.

ALT, GGT and LD should not be transported by coach or sent by mail ''on gel''. Additional experiments in Centre A demonstrated that shaking of tubes leads to gel fragmentation, which may be a reason for the lower values for ALT after transport with coach or by mail ''on gel'' (data not shown).

Regarding LD, haemolysis was observed in some samples, probably due to shaking during transport. As previously mentioned, we were not interested in the reason for the poor outcome but rather in which transport circumstances preserved sample quality best.

However, the percentage of results fulfilling or not fulfilling the defined criteria is not a crucial point. More interesting is the degree of deviation that could affect clinical conclusions.

The seriousness of the deviations can be observed in Table 5, which shows how much the ''acceptable maximal deviation'' defined in Table 3 should be extended to ensure that 95% of the results would fulfil the goals. It can be observed (Table 5) that for some components the extended limits would result in unacceptably large deviations from the 0-value and thus invalidate the utility of the involved transport form.

If the potassium limits were extended from 10% to 14%–16%, corresponding to 0.4–0.5 mmol/L, more results could be accepted for analysis according to the CLIA requirement of 0.5 mmol/L (8). But if the demand is that 95% of results should be within the limits, then the limits of acceptance should be extended to 16%–18%.

For ALT, the demand that 95% of results fall within the limits would be fulfilled if the new limits were to be extended from 24% to 30%–40%, depending on transport form.

For GGT, acceptable limits for change should be extended from 28% to 70%–90% if transported by coach or by mail ''on gel'' (A2, A7 and B5).

Similar changes should be introduced for LD (A7 and B5) and phosphate (A4, A6 and B4).

On the other hand, for some components (cholesterol, creatinine, urate) the limits could be narrowed if the criteria were based on the presented data.

The quality goals defined in this paper (Table 3) are based on actual analytical imprecision in Centres A and B and on a subjective estimation of clinical needs. It can be discussed if the limits are too strict or too loose, and if they should be moderated. If so, some transport forms could be acceptable for specimen transport from the GP to the hospital laboratory. For example, the US CLIA (8) criteria are based on biological variation and differ from ours regarding several components, some of them are even wider. If we redefine our criteria according to these wider limits, more flexible combinations of transport forms would be possible. However, for potassium we find that the CLIA rules are too wide, as hypokalaemia (2.7–3.0 mmol/L) can easily be missed if a deviation of 0.5 mmol/L is accepted.

Some practical aspects should be considered. The longest storage time possible for most components (excluding potassium) is found to be 6–8 h. The time of sample collection is therefore very important for both GPs and hospital laboratories. Doctors will probably not accept a courier service, if the pick-ups will not include samples taken from patients visiting doctors at the end of the day.

On the other hand, the acceptable storage period of 8 h will not leave enough time for the laboratory to carry out the analytical work. It should be emphasised that the time from blood sampling to analyses includes storage time at the general practitioners, collection by the courier, transport time, registration at the hospital laboratory and centrifugation. All these steps can prolong blood sample age and thus reduce its quality.

We have formulated quality goals for performance of 21 biochemical components and observed what happens when blood samples were exposed to different pre-analytical conditions. Using these goals, we

were able to investigate different combinations of storage and transport conditions and conclude that short storage time, controlled temperature and treating the samples gently under transport should be preferred.

We have decided in our region that blood samples should be picked up twice a day from GPs, which gives high patient safety and optimal user service, while assuring the quality of the blood samples to be analysed.

From a quality point of view, the best transport form for most components has traditionally been considered to be the transport of plasma centrifuged and separated shortly after sampling. This transport form is expensive (compensation to doctors' and for mailing), requires that all GPs have suitable centrifuges and also has labour requirements. The serious disadvantage is that transferring of plasma material to new tubes jeopardises the advantages of closed sampling systems: safety regarding patient identification and reduced risk for contamination and infection.

During the investigation period, we registered identity mismatch for four patient samples out of 20 in one doctor's office. It happened for plasma, which was transferred to another tube after centrifugation.

This investigation demonstrates that the degree of observed deviation from the defined goals depended markedly on chosen logistics, but also showed ways to solve the problems.

In Centre B, the transport form described as B1 was introduced 3 years ago. All GPs cooperating with the laboratory are equipped with thermostated boxes, which are able to keep a constant temperature of 21 ± 1°C. Whole blood samples are stored in these boxes until they are picked up by a courier car service using the same type of thermo boxes.

Conclusions

The investigated components, representing a broad repertoire of most frequently requested analyses from GP, fulfil the quality goals, if whole blood samples are transported to the hospital laboratory by a ''door-to-door'' courier service. This means that they are not exposed to temperatures beyond 20–25°C at any time, that they are protected from shaking and that the samples are centrifuged not later than 6 h after the sampling. Consequently, there is no need for centrifugation in the primary care sector if these conditions are satisfied.

If the storage/transport conditions are not optimised, biased results can be reported jeopardising safety, security and economy of patient management.

In our region it saves money, as centrifugation of blood samples at GPs' offices is twice as expensive as a courier service. Additionally, the recommended courier service allows for reporting patients' results to the GP the same day the blood samples were taken.

Samples sent via mail fulfil the quality goals only if plasma is separated. Samples mailed ''on gel'' do not fulfil the quality goals. Transferring of plasma is time consuming and expensive, can lead to sample mismatch and exposes the staff in primary care for contagious agents. Thus, we recommend that samples be collected by a courier service.

In summary, we recommend the following conditions assuring the best pre-analytical quality of blood samples transported from GPs' offices to the hospital laboratory:

- Primary tubes ensuring sample identity (reducing mismatch or infection risk).
- Temperature between 20°C and 25°C during both storage and transport. It can be achieved either by storage of samples in special thermostated boxes or by air conditioning in the GPs' offices.
- Collecting of whole blood samples twice a day eliminating the need for centrifugation of the samples in the primary sector.
- Transport by a reliable courier service (upright position and thermostated boxes).
- Time of storage without centrifugation: not longer than 6 h.

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