Clinical Chemistry 44:6
1325–1333 (1998)

General Clinical Chemistry

Effect of serum-clot contact time on clinical chemistry laboratory results

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The effect of serum-clot contact time on laboratory results was studied by dividing each blood specimen into four blood collection tubes. The control sera were separated from the clot within 30 min of the collection. The other tubes were incubated at 32 °C, and the sera were separated at 3, 6, and 24 h. The sera were stored at 4 °C and analyzed at the same time. The stability of the tests was determined by comparing the results of the 3-, 6-, and 24-h samples with the values from the 30-min samples. The acceptable limits around the 30-min values were derived from the analytical and intraindividual biological variation of the tests. A total of 63 analytes were studied. Potassium, phosphorous, and glucose were the least stable, and the serum should be separated from the clot within 3 h for these analytes. Albumin, bicarbonate, chloride, C-peptide, HDL-cholesterol, iron, LDL-cholesterol, and total protein should be separated within 6 h. The other analytes were stable for 24 h of serum-clot contact.

Changes in test results can be induced by preanalytical, analytical, or normal biological variations in addition to pathophysiologic processes. Preanalytical factors such as sample collection and handling, patient posture, venous stasis, time of sampling, diet, exercise, and drugs can all impact a test result. To detect real pathological changes in patients, the preanalytical and analytical variations must be reduced to acceptable levels at which they cause no impact on clinical interpretation of the results. As analytical variation decreases with development of new technologies, the relative contribution of preanalytical variation becomes a more dominant element of overall test variability.

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Received April 22, 1997; revision accepted February 13, 1998.

Prolonged contact of serum with the clot can cause preanalytical variation. The optimum time interval between sample collection and separation of serum from the clot should be long enough to allow complete clot formation but be shorter than the time in which a significant change in test result is induced by serum-clot contact. The minimum clotting time suggested by the *Tietz Textbook of Clinical Chemistry (1)* is 20–30 min. During a prolonged contact time between serum and clot, both biological activity of the cells and trans-membrane diffusion can change the concentrations of certain analytes in the serum. *NCCLS Procedures for the Handling and Processing of Blood Specimens (2)* recommends that serum or plasma should be physically separated from contact with cells as soon as possible, unless conclusive evidence indicates that longer contact times do not contribute to result inaccuracy. A maximum limit of 2 h from the time of collection to the time of separation was also recommended.

Each individual analyte has a different tolerance to a delay in separating serum from clot. Many analytes are stable for much longer than 2 h. In hospitals and outpatient clinics, transportation of specimens from a phlebotomy site to a laboratory sometimes takes longer than 2 h. If overly stringent transportation requirements are set for all tests, many acceptable specimens would be rejected unnecessarily. Ideally, a specific allowable transportation time should be applied for each analyte in a specimen. In practice, analytes are usually grouped into time blocks in which serum-clot contact cause no changes in analyte concentrations. Generally, specimens arrive in the laboratory at the Medical College of Virginia Hospitals <6 h after collection. Therefore, knowing the test stability within 6 h was critical to determine the cutoff times for acceptable specimens.

Information about test stability after prolonged contact of serum with clot is available in the literature for 41 chemistry tests *(3–7)*. All studies except Chu et al. *(5)* reported 24-h stability at room temperature (summarized in Table 1). Some reports also included data at different times and temperatures.

Table 1. Summary of literature reports for specimen stability at ≤24 h serum-clot contact.[a]

Analyte[b]	Laessig et al. (3)	Ono et al. (4)	Chu and MacLeod (5)	Rehak and Chiang (6)	Heins et al. (7)
Albumin	0	0	0	0	NA
ALP	0	0	0	0	0
ALT	0	+	0	+	0
Amylase	NA	0	0	0	0
AST	0	+	0	+	0
β-Lipoprotein	NA	0	NA	NA	NA
Calcium	0	0	−	0	0
Chloride	−	NA	−	0	−
Cholesterol	0	0	0	0	0
Cholinesterase	NA	0	NA	NA	0
CO_2	NA	NA	0	0	NA
Cortisol	NA	NA	0	NA	NA
CK	0	NA	0	0	0
Creatinine	0	0	+	+	0
Direct bilirubin	NA	0	NA	0	NA
Ferritin	NA	NA	+	NA	NA
Folate	NA	NA	−	NA	NA
GGT	NA	0	0	0	0
Glucose	−	−	−	−	NA
HDL	NA	NA	NA	NA	0
P	0	0	+	+	+
Iron	+	NA	NA	NA	NA
LD	+	+	+	0	+
LDL	NA	NA	NA	NA	0
Leucine aminopeptidase	NA	0	NA	NA	NA
Lipase	NA	NA	0	0	NA
Magnesium	0	NA	0	0	+
Potassium	+	0	+	+	+
Protein electrophoresis	0	NA	NA	NA	NA
Sodium	0	+	+	0	0
T_3	0	NA	NA	0	NA
T_3 uptake	NA	NA	+	NA	NA
T_4	0	NA	NA	0	NA
TBG	NA	NA	NA	0	NA
Total bilirubin	0	NA	NA	0	0
Total protein	0	0	NA	0	NA
Triglycerides	0	0	NA	NA	0
TSH	NA	NA	0	0	NA
Urea	NA	NA	−	NA	NA
Uric acid	0	0	0	0	0
Vitamin B_{12}	NA	NA	+	NA	NA

[a] Except the Chu and MacLeod study, which was at 3 days of serum-clot contact. Symbols are defined as: 0, no effect; +, increased; −, decreased; NA, analyte not tested.

[b] ALP, alkaline phosphatase; GGT, γ-glutamyltransferase; P, inorganic phosphorus; T_3, triiodothyronine; T_4, thyroxine; TBG, thyroxin-binding globulin; and TSH, thyroxin-stimulating hormone.

Laessig et al. *(3)* reported the serum-clot contact effect on 25 tests. Whole-blood samples were incubated at room temperature for 1, 2, 4, 8, 24, and 48 h before serum-clot separation. The sera were assayed along with quality-control (QC)[4] material immediately after separation from clot. The results of the 1-h sera and QC material were considered as target results and the percentage change in results at 2–48 h was calculated. The significance of the change was determined by comparing the percentage of change in results of the sera to that of the QC material. They reported that significant changes occurred for glucose, lactate dehydrogenase (LD), and potassium at 2 hand for iron and chloride at 8 h. The other tests were stable up to 48 h.

[4] Nonstandard abbreviations: QC, quality control; LD, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; apo, apolipoprotein; and FSH, follicle-stimulating hormone.

Ono et al. *(4)* studied the effect of serum-clot contact time at 4, 23, and 30 °C for 25 analytes. The contact time effect was tested at 2, 4, 6, 8, 24, and 48 h. All the sera were stored at −20 °C after separation from clots and thawed and assayed in the same run. The results of the 2–48 h samples were compared with that of the zero hour samples. Statistically significant changes (by Student's *t*-test) at 30 °C were observed for alanine aminotransferase (ALT, 6 h), aspartate aminotransferase (AST, 6 h), LD (8 h), glucose (2 h), sodium (6 h), potassium (4 h), calcium (48 h), and inorganic phosphorus (8 h). The stability of these analytes was sensitive to temperature.

Chu et al. *(5)* reported the changes in results after 3 days of serum-clot contact. The results from the stored samples were compared with those from sera separated from cells within 3 h of collection. The changes were classified as significant if the percentage of change was greater than the analytical variability of the method.

In the study by Rehak and Chiang *(6)*, whole blood specimens were stored at 3, 10, 15, 22, 25, 30, and 38 °C for 24 h before serum-clot separation. The results of these specimens were compared with the results from the fresh sera. They concluded that significant changes occurred for creatinine, glucose, inorganic phosphorus, potassium, AST, and ALT and that all of these changes were sensitive to temperature.

Heins et al. *(7)* reported the effect of storage temperature and time on 22 analytes. The whole-blood specimens were stored at 9 °C and room temperature for 1–7 days. The results from the stored specimens were compared with those from the corresponding fresh sera. The changes were classified as significant if they exceeded the maximum allowable inaccuracy according to the recommendation of the German Federal Medical Council.

Because of a variety of experimental designs, it is difficult to interpret information on specimen stability from the literature. First, only two of the previous reports *(3, 4)* presented the test results with contact times <24 h. Second, specimens from healthy donors were used, which had concentrations of some analytes so low that precise measurements were hard to achieve. Third, the control samples and the test samples were assayed in different analytical runs in all of these studies except Ono et al. *(4)*. Thus, run-to-run imprecision was introduced into the test results. Fourth, statistical significance of the change in test results was evaluated by analytical variations of QC results or the Student's *t*-test. However, a statistically significant change may or may not have any impact on the interpretation of the test result. The clinical relevance of the change was either not addressed or determined by the opinions of the authors.

In this study, we investigated the effect of serum-clot contact time on the laboratory results of 63 analytes. The specimen donors were selected to obtain analyte concentrations high enough to achieve precise measurement. The clinical impact of the change was evaluated with consideration of both analytical and biological variation of each test. The results of this study provided justification to make optimum decisions regarding specimen stability before reaching the laboratory.

Materials and Methods

SPECIMEN COLLECTION AND HANDLING

The clinical protocol was approved by the Committee on Conduct of Human Research. Each donor was informed of the purpose of this investigation and signed a consent form before blood was collected. To reduce the contribution of analytical imprecision, donors were specifically selected on the basis of their clinical conditions to obtain values for each analyte at which low analytical CV values could be achieved. Blood specimens were collected from a total of 56 donors. Specimens from some donors were used for multiple analytes. For each analyte, specimens from at least four donors were used. If the initial results were not congruent, additional donors were recruited. All results were used in the statistical analysis. The number of donors for each analyte is shown in Table 2. Each blood specimen was collected into four red-topped Vacutainer Tubes (Becton Dickinson), which were randomly labeled to eliminate the effects of the drawing sequence. The first tube was allowed to clot for 30 min at room temperature before the serum was separated from the clot. The rest of the tubes were incubated in a circulating water bath at 32 °C, to mimic a warm transportation condition, for 3, 6, and 24 h before serum-clot separation. To study the behavior of potassium at different incubation temperatures, two groups of specimens were incubated at room temperature or at 32 °C for 3, 6, and 24 h before serum-clot separation. All specimens were centrifuged for 10 min at 1500*g* to separate the serum from the clot. The sera from all serum-clot contact times were stored at 4 °C until assay. To eliminate the run-to-run analytical imprecision, the four specimens from each donor were assayed in the same run. Specimens from different donors for an analyte may have been run at different times. The specimens used for light-sensitive analytes, carotene, vitamin A, and bilirubin, were protected from light during the process.

The effect of specimen transportation conditions on the concentration of LD was evaluated using blood specimens collected from 12 donors. Three serum separation tubes were drawn on each individual. Two tubes were filled completely and one was half-filled. One full tube remained in the laboratory as a control sample. The other full tube and the half-filled tube were hand-carried to various pneumatic tube stations in our hospital, from which they were sent to our laboratory. The pneumatic tube system (PEVCO Systems International) used 6-inch carriers, and transport distances varied between 500 and 1750 feet. The average transport speed was ~5.2 m/s. When the samples arrived in the laboratory, the control sample and the two samples transported through the tube system were centrifuged and analyzed for LD in the same run. The percentage difference was calculated as $(LD_t - LD_c) \times 100/LD_c$, where LD_t was the LD result for the

Table 2. Statistical analysis of serum-clot contact effects.

Test[a]	Units	Number of donors	Analytical CV%	Biological CV%[b]	Analytically acceptable limits	Clinically acceptable limits	Mean at 0.5 h	Mean at 3 h	Mean at 6 h	Mean at 24 h
AFP	mg/L	4	4.5	3.4[c]	1272–1390	1258–1404	1331	1272	1277	1297
Albumin	g/L	4	1.4	2.7	42.7–43.9	42.0–44.6	43.3	44.5[d]	45.8[e]	47.0[e]
Aldosterone	ng/L	4	7	11.1[c]	335–384	313–406	359	340	356	363
ALP	U/L	4	2.6	7.7	82–86	78–91	84	83	84	83
ALT	U/L	6	1.9	19.4	152–156	129–178	154	152	149[d]	140[d]
Amylase	U/L	7	2.6	9.2	72–74	68–78	73	72	74	78[d]
Apo A	g/L	4	11.4	8	1.2–1.4	1.1–1.5	1.3	1.3	1.4	1.4
Apo B	g/L	4	7.1	9	1.1–1.2	1.0–1.3	1.1	1.1	1.2	1.2
AST	U/L	6	6.7	10.1	70–78	67–81	74	77	76	76
B_c	mg/L	4	3.3	19	52–56	44–64	54	59[d]	61[d]	56
Bicarbonate	mmol/L	4	3.7	5.6	26–28	25–29	27	26	26	23[e]
B_t	mg/L	4	2.2	19	85–88	70–103	86	96[d]	98[d]	102[d]
B_u	mg/L	4	2.1	19	82–85	68–99	84	88[d]	88[d]	72[d]
C-peptide	μg/L	4	8	9.3	3.0–3.5	2.8–3.6	3.2	3.4	3.1	2.2[e]
Calcium	mg/L	6	2.1	2.3	95–99	95–99	97	96	98	98
Carotene	μg/L	4	11	37.4[c]	2626–3260	1819–4067	2943	2848	2885	2893
Ceruloplasmin	mg/L	8	11	5.6	354–412	350–416	383	383	374	384
Chloride	mmol/L	4	0.9	1.2	102–103	101–104	102	102	101[d]	100[e]
Cholesterol	mg/L	11	1	5.9	1999–2023	1940–2082	2011	2017	2040[d]	2081[d]
CK	U/L	4	3.3	27.3	148–158	111–194	153	156	155	161[d]
CK-MB	μg/L	4	8.6	31.2	37–44	28–53	40	45[d]	47[d]	45[d]
Cortisol	μg/L	4	4.4	15.2	119–130	105–144	125	127	123	125
Creatinine	mg/L	4	1.4	4.6	56–57	54–59	56	56	57	54[d]
Estradiol	ng/L	4	12	41.6	69–87	45–111	78	74	75	74
Estriol	μg/L	4	6	69[c]	3.4–3.8	1.1–6.1	3.6	3.6	3.7	4.0[d]
Ferritin	μg/L	4	3.4	14.7	157–168	139–187	163	159	164	178[d]
Folate	μg/L	9	7.7	34[c]	10.5–11.6	8.5–13.6	11.1	11.1	10.3[d]	9.4[d]
Fructosamine	μmol/L	4	7.7	4.9	344–400	338–405	372	379	376	391
FSH	U/L	4	6	30.8	7.0–7.9	5.2–9.7	7.4	8.1[d]	8.1[d]	8.3[d]
GGT	U/L	5	1.4	13.5	275–281	245–311	278	276	279	271[d]
Glucose	mg/L	4	1.1	10.6	865–885	784–966	875	645[e]	548[e]	440[e]
Haptoglobin	mg/L	4	2.8	20.8	1366–1443	1116–1694	1405	1375	1403	1400
HCG	U/L	4	3.9		26027–28095	26027–28095	27061	26750	26724	27913
HDL	mg/L	7	3	7.2	511–535	493–553	523	534	549[d]	593[e]

IgA	mg/L	4	2.2	5.2	2290–2390	2210–2469	2340	2375	2408[d]	2455[d]
IgE	kIU/L	4	8	27.8[c]	83–97	64–116	90	92	90	96
IgG	g/L	4	2.7	4.7	11.57–12.20	11.25–12.52	11.89	11.93	12.15	12.40[d]
IgM	mg/L	4	2.9	6	1135–1201	1092–1244	1168	1205[d]	1192	1192
Iron	mg/L	9	3.81	19.8	1.03–1.08	0.92–1.20	1.06	1.11[d]	1.16[d]	1.36[e]
LD	U/L	11	2.3	9.5	173–178	166–186	175.6	186[d]	181[d]	185[d]
LDL	mg/L	7	5.1	7.8	1116–1204	1080–1240	1160	1111[d]	1124	940[e]
LH	U/L	4	6	13.5	21.5–24.1	19.5–26.1	22.8	21.3[d]	22.1	24.5[d]
Lipase	U/L	4	2.6	14.0	108–113	95–126	110	108	112	112
Magnesium	mmol/L	6	1.7	3.3	1.0–1.1	1.0–1.1	1.0	1.1	1.1	1.1
Phosphorus	mg/L	4	1.1	8.9	33–34	30–36	33	35[d]	41[e]	66[e]
Potassium	mmol/L	8	1.8	4.5	4.2–4.4	4.2–4.4	4.3	4.0[e]	3.7[e]	5.9[e]
Potassium (RT)	mmol/L	8	1.8	4.5	4.2–4.4	4.2–4.4	4.3	4.5[e]	4.6[e]	4.7[e]
Prealbumin	mg/L	4	2.4	20.9[c]	295–309	240–364	302	306	306	311[d]
Prolactin	μg/L	6	4.3	40.5	86.9–93.1	60.6–119.3	90.0	90.3	88.3	89.0
Sodium	mmol/L	4	0.9	0.7	142–144	141–144	143	143	143	144
T uptake	%	5	4	12[c]	32.0–34.3	29.5–36.8	33.2	33.2	32.7	32.3
T_4	μg/L	5	3.4	5.1	118–126	115–128	122	121	122	125
TBG	mg/L	4	5	4.4	18.2–20.1	17.9–20.4	19.2	20.1	18.7	19.5
Testosterone	μg/L	8	7	8.3	4.76–5.24	4.62–5.37	5.00	5.08	5.23	5.26[d]
Total protein	g/L	4	1.6	2.7	76–79	75–80	77	79	80[d]	81[e]
Transferrin	g/L	4	2.7	5.2	2.63–2.78	2.55–2.86	2.71	2.73	2.80[d]	2.81[d]
Triglycerides	mg/L	8	1.5	19.0	1319–1347	1157–1509	1333	1334	1340	1346
TSH	mU/L	5	4.8	17.8	3.7–4.0	3.2–4.5	3.8	3.8	3.8	4.1[d]
Urea	mg/L	8	1.8	11.3	291–299	272–318	295	303[d]	305[d]	285[d]
Uric acid	mg/L	4	1.1	8.2	52–54	49–57	53	51[d]	51[d]	49[d]
Vitamin A	μmol/L	4	9	22	0.45–0.53	0.37–0.61	0.49	0.49	0.49	0.51
Vitamin B_{12}	ng/L	10	5.3	35.8[c]	473–506	380–599	489	504	536[d]	564[d]
Vitamin C	mg/L	4	32	29.2[c]	8–16	7–18	12	11	10	10
Vitamin E_a	mg/L	4	12	11.9[c]	10.4–13.2	9.9–13.8	11.8	11.8	11.9	12.4
Vitamin E_b	mg/L	4	15	56.5[c]	1.9–2.6	0.9–3.6	2.2	2.2	2.3	2.4

[a] AFP, α-fetoprotein; ALP, alkaline phosphatase; B_c, conjugated bilirubin; B_t, total bilirubin; B_u, unconjugated bilirubin; CK-MB, creatine kinase isoenzyme MB; FSH, follicle-stimulating hormone; GGT, γ-glutamyltransferase; HCG, human chorionic gonadotropin; LH, luteinizing hormone; RT, room temperature (~24 °C); T_4, thyroxine; TBG, thyroxin-binding globulin; and TSH, thyroid-stimulating hormone.

[b] Biological CV from the literature generally reflects a reference range concentration for the analyte.

[c] For this analyte, the biological CV was estimated as one-quarter of the reference range.

[d] Exceeds the analytically acceptable limit.

[e] Exceeds the combined analytical and clinical acceptability limit.

sample transported through the tube system, and LD_c was the LD result for the control sample that remained in the laboratory.

METHODS FOR SPECIMEN ANALYSIS

Albumin, alkaline phosphatase, ALT, amylase, AST, bicarbonate, calcium, creatine kinase (CK), chloride, creatinine, γ-glutamyltransferase, glucose, iron, LD, lipase, magnesium, inorganic phosphorus, potassium, sodium, total protein, urea, and uric acid were measured with a Vitros 700 (Johnson & Johnson). Apolipoprotein A (apo A), apolipoprotein B (apo B), haptoglobin, IgA, IgG, IgM, prealbumin, and transferrin were measured with the Array 360 system (Beckman). Cholesterol, triglycerides, and the cholesterol component in HDL and LDL were measured on Hitachi 911. A heparin-manganese method was used for HDL pretreatment and a direct-LDL kit from Sigma was used for LDL separation. α-Fetoprotein, ferritin, β-human chorionic gonadotropin, IgE, prolactin, and thyroid-stimulating hormone were measured with IMX (Abbott Laboratories). Cortisol, thyroxine uptake, and thyroxine were measured with TDX (Abbott Laboratories). Folate and vitamin B12 were measured with RIA (Diagnostic Products). CK-MB was measured with the Opus Plus (Behring Diagnostics). All of the above tests were performed in the Department of Pathology clinical chemistry laboratory at the Medical College of Virginia Hospitals.

Estriol, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone were measured with Immulite (Diagnostic Products) in the Department of Obstetrics and Gynecology fertility laboratory at the Medical College of Virginia Hospitals.

The following tests were performed at the American Medical Laboratories (Chantilly, VA). Aldosterone (Diagnostic Products), C-peptide (INCSTAR), and vitamin D (Nichols Diagnostics) were measured by RIA. Thyroxin-binding globulin was measured with Immulite (Diagnostic Products). Testosterone was measured with ACS:180 (Chiron). Fructosamine was measured with a colorimetric method (Roche Diagnostics). Ceruloplasmin was measured with the Behring Nephelometer II (Behring Diagnostics). Vitamin C was measured with a colorimetric method. Carotene, vitamin A, and vitamin E were measured with HPLC methods.

STATISTICAL ANALYSIS

To estimate the average effect of prolonged serum-clot contact, the mean of the results from all specimens for each analyte at each serum-clot contact time was used to evaluate the change. The approach to the analysis was derived from a quality-control paradigm. The $\bar{X}$ chart, originally proposed by Shewhart *(8)*, tracks the mean of a process and, considering the noise in the system, determines when the process is out of control. In our case, the process was the measurement of analyte content after different contact times between serum and cells. $\bar{X}$ is the mean result of the 30-min samples. A variety of methods have been proposed for determining process limits, such as 2 or 3 SD from the mean or probability limits using the gaussian distribution *(9)*. Both approaches require an estimate of the SD, which can be estimated from the process or preferably, known from some external source. In this experimental design, each measurement under the same treatment condition was made once; thus an estimate of analytical variance could not be made from the data. Therefore, we used the well-established analytical SD from quality-control data for each method. Thus, the $\bar{X}$ chart with probability limits is:

$$\bar{X} \pm Z \times \sigma / N^{1/2} \quad (1)$$

where $\bar{X}$ is the mean of the 30-min samples; Z is the 0.975 percentile of the gaussian distribution; σ is the SD; and N is the sample size. The analytical CV of each test was estimated by the annual cumulative CV of quality-control results at an analyte value close to the mean of the 30-min samples. The goal of this statistical analysis was to determine which analytes were altered enough by serum-clot contact to exceed the total measurement error for a method. Thus, the within-run SD was not used as an estimate of analytical imprecision.

It was necessary to assume that the analytical CV was constant over the concentration ranges tested. To ensure validity of this assumption, we selected donors to give increased concentrations of those analytes that would have had changes in analytical CV at low reference range concentrations. Therefore, the imprecision contributed by the analytical variation at the 30-min value can be expressed as $CV_a \times \bar{X}$, which can be placed in Eq. 1. The 95% confidence interval based on the measurement variation defines the analytically acceptable process limits as:

$$\bar{X} \pm Z \times CV_a \times \bar{X}/N^{1/2} \quad (2)$$

where $\bar{X}$ is the mean of the results of the 30 min samples; Z is the 97.5% point of the gaussian distribution; CV_a is the analytical CV of the test, and N is the number of donors used for each test.

Because of the existence of intraindividual biological variation, a mean result at a contact time that exceeds the analytical imprecision-based process limits defined by Eq. 2 may not be clinically relevant. We combined analytical and intraindividual biological variations of each test as:

$$CV_c = (CV_b^2 + CV_a^2)^{1/2} \quad (3)$$

Substituting this combined CV_c for CV_a in Eq. 2 provided clinically acceptable process limits as:

$$\bar{X} \pm Z \times CV_c \times \bar{X}/N^{1/2} \quad (4)$$

An analyte was considered to have unacceptable stability at a serum-clot contact time with a mean of the results that exceeded these process limits.

In this study, the experimental design did not allow

estimation of biological variation from the data because the specimens from each patient were collected via a single phlebotomy and then divided for different incubation times. The intraindividual biological CV of each analyte (CV_b) was obtained from Fraser *(10, 11)*, in which he summarized the intraindividual biological variation of several tests reported in the literature. In those reports, the data were obtained by collecting specimens from cohorts of subjects for certain time spans and analyzing them in duplicate. The analytical, within-subject, and between-subject components of variation were derived by nested analysis of variance after exclusion of outliers. We took the median intraindividual biological variations of healthy individuals from the literature as CV_b after excluding the data collected in a time span of <5 days. For FSH and prolactin, where the biological variation is much higher in females than in males, the higher female intraindividual biological variations were used. When no data about intraindividual biological variation could be found in the literature, one-quarter of the reference range was used as an estimation of the intraindividual biological SD *(12)*. Ricos et al. *(13)* reported a similar biological variation of total and conjugated bilirubin. Therefore, the median biological variation of total bilirubin was adopted as the CV_b for total bilirubin, conjugated bilirubin, and unconjugated bilirubin.

Results

The statistical analysis of serum-clot contact time effects is presented in Table 2. The tests that were suitable for analysis after 24 h of serum-clot contact, based on clinically acceptable limits, were α-fetoprotein, aldosterone, ALT, alkaline phosphatase, amylase, apo A, apo B, AST, bilirubin, calcium, carotene, ceruloplasmin, chloride, cholesterol, creatinine, CK, CK-MB, cortisol, estradiol, estriol, ferritin, folate, fructosamine, FSH, γ-glutamyltransferase, human chorionic gonadotropin, haptoglobin, IgA, IgE, IgG, IgM, LD, luteinizing hormone, lipase, magnesium, pre-albumin, prolactin, sodium, thyroxine, thyroxin-binding globulin, testosterone, transferrin, thyroid-stimulating hormone, thyroxine uptake, urea, uric acid, vitamin A, vitamin B_{12}, vitamin C, and vitamin E. Tests that were suitable for analysis at a 6-h incubation but not suitable for analysis at a 24-h incubation were bicarbonate, chloride, C-peptide, iron, HDL, LDL, and total protein. Albumin and inorganic phosphorus were suitable for analysis at a 3-h incubation but not suitable for analysis at 6 h. Glucose and potassium had clinically relevant changes at 3 h.

Time-course plots for selected analytes that illustrate various stability situations are presented in Fig. 1. Glucose, potassium, and inorganic phosphorus were the most unstable tests. Note that potassium changes were temperature-dependent. Calcium was a stable analyte. Albumin exceeded acceptability criteria over time. Changes in LD exceeded the process limit determined by analytical imprecision but were within the clinically acceptable limits

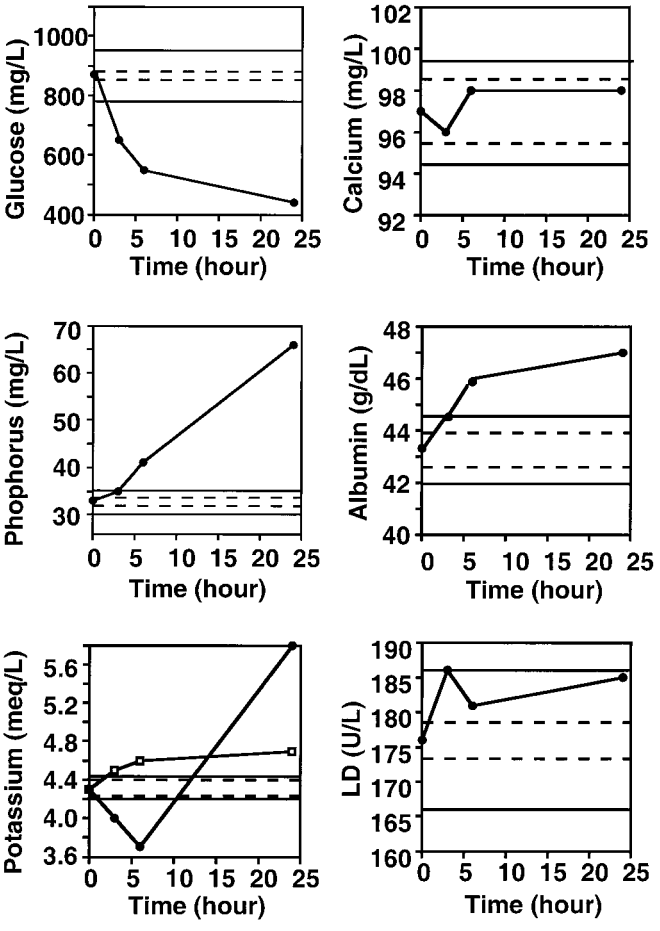

Fig. 1. Effect of serum-clot contact time on test results for selected analytes.

(●) indicates incubation at 32 °C; (□) indicates incubation at 24 °C; ([- - -]); indicates acceptable limits based on analytical variation; ([——]) indicates acceptable limits based on combined analytical imprecision and clinical variation.

determined by both analytical imprecision and intraindividual biological variations.

The differences in LD results of the samples before and after pneumatic tube transportation are presented as percentages in Fig. 2. LD value changes ranged from −1.0% to 13.9% for the samples in full Vacutainer Tubes and from 8.6% to 30.7% for the samples in half-filled Vacutainer Tubes.

Discussion

Because of practical and economic constraints, only a relatively small number of patient specimens could be tested per analyte. Replication was not used for each assay; therefore, the analytical imprecision for each method required a known estimate of SD or CV from routine QC data. To circumvent the problem of variable CV values at different analyte concentrations, we selected patients with increased concentrations for analytes that were known to have larger CV values at low reference range concentrations. This allowed all specimens to have concentrations that could be measured with a nearly

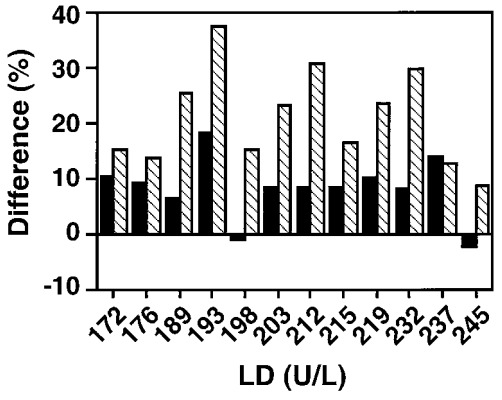

Fig. 2. Effect of transportation conditions on LD results.

Difference (%) was calculated as (LD_t - LDc) × 100/LD_c, where LD_t was the LD result for the specimen transported by pneumatic tube and LD_c was the result for the control specimen that remained in the laboratory. (■) indicates blood specimens transported in full collection tubes, and (▨) indicates blood specimens transported in half-filled collection tubes.

constant CV. Acceptable limits derived solely from analytical imprecision can lead to unnecessary specimen rejection for analytes with very precise methods. Thus, we developed a pragmatic procedure to introduce biological variation based on consensus literature estimates. In this way, we established clinically acceptable limits that permitted a practical evaluation of specimen stability.

The statistical evaluation developed here was based on a QC paradigm. The critical question was if the result from a specimen was altered by serum-clot contact enough to exceed the variability expected for physiological processes. A QC model performs exactly that evaluation for each sequential data point in a process by evaluating that point vs fixed limits based on expected variability in the process. In this case, each serum-clot contact time represented a unique preanalytical condition that could change the analyte stability independently of any physiologic condition in the patient. To establish clinically meaningful evaluation limits within the technical limitations of the measurement process, we combined literature estimates of biological variation, which do not include an analytical component, with the known imprecision for the measurement process.

Glucose concentrations decreased with increasing serum-clot contact time. It is well-documented that glycolysis of cells consumes glucose and causes a decrease in glucose concentration in blood during transportation and that the rate of decrease is sensitive to temperature. Serum glucose decreases 100–200 mg · L · h at 37 °C *(14)* and 50–100 mg · L · h at room temperature *(15)*. The rate of glycolysis is markedly increased in the presence of leukocytosis *(15)*. Glycolysis can be inhibited and glucose concentration can be stabilized by using sample collection tubes with sodium fluoride as an additive *(16)*.

Inorganic phosphorus increased after three hours serum-clot contact. The concentration of organic phosphates is about seven times higher in erythrocytes than that in serum *(17)*. These organic phosphates are susceptible to hydrolysis to produce inorganic phosphorus, which leaks from the cells and consequently increases the concentration in serum *(18)*.

The effect of serum-clot contact on serum potassium depended on incubation temperature (Fig. 1). At room temperature, the serum potassium values increased with contact time; the change became clinically significant by 3 h. At 32 °C, the potassium values fluctuated with an initial decrease followed by a large increase after 6 h. The change of potassium is the net effect of glycolysis, which moves potassium into cells, and passive diffusion, which allows potassium to diffuse out of the cells *(19, 20)*. At 32 °C, glycolysis was dominant initially; thus serum potassium concentrations decreased. As time went on, the glucose in serum was depleted, and passive diffusion of potassium from cells became dominant, producing an increase in potassium after longer serum-clot contact. At room temperature, glycolysis was slower; thus passive diffusion was dominant, allowing potassium to increase.

A small increase in concentration was observed for albumin at 6 h (Fig. 1) and total protein at 24 h, which was outside the clinically acceptable limits. These limits are very small because of the small analytical and biological variations. Although these analytes exceeded the clinically acceptable limits defined by Eq. 4, such a small increase generally will not cause changes in clinical treatment or diagnosis. Because the small increases occurred with both albumin and total protein, they were possibly due to water shifting into the cells.

Previous studies are contradictory regarding the stability of LD during serum clot contact. Rehak and Chiang *(6)* found no significant change of LD up to 24 h, whereas Laessig et al. reported that LD concentration increased after 2 h of contact between serum and cells *(3)*. Fig. 1 shows an increasing trend in LD concentration over time, with all values remaining within the clinically acceptable limits. We observed substantial increases for samples transported through the pneumatic tube (Fig. 2). The effect of transportation on half-filled Vacutainer Tubes was more pronounced than the full Vacutainer Tubes, presumably because of greater agitation of cells. We concluded that prolonged contact between serum and cells without mechanical agitation did not cause a substantial increase in LD results. Agitation of the sample before serum/cell separation was the major contributor to the preanalytical variation of LD results. In this study, except for the samples in the transportation study, blood specimens were handled with care to avoid any agitation.

In conclusion, only a few routine analytes required stringent control of delivery times before serum-clot separation. Most routine tests can tolerate fairly long delays in transportation without changes in analyte content. Samples for glucose, potassium, and inorganic phosphorus should be spun and separated from the clot within 3 h after collection. Samples for albumin, bicarbonate, chloride, C-protein, HDL, iron, LDL, and total protein should

be processed within 6 h. The remaining analytes evaluated were stable for 24 h.

We thank the Department of Obstetrics and Gynecology fertility laboratory at the Medical College of Virginia Hospitals for measuring some of the analytes and Carol Rodgers for performing the stability test of LD on the pneumatic tube system.

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