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Determination of Hemolysis Thresholds by the Use of Data Loggers in Pneumatic Tube Systems

Thomas Streichert,[1†*] Benjamin Otto,[1†] Claudia Schnabel,[1†] Gerhard Nordholt,[1]
Munif Haddad,[2] Mario Maric,[2] Astrid Petersmann,[3]
Roman Jung,[1] and Christoph Wagener[1]

BACKGROUND: Pneumatic tube systems (PTSs) for the transport of blood samples are regaining popularity in medical centers after earlier reports that their use could introduce preanalytical distortions such as hemolysis and changes in blood gases.

METHODS: We drew duplicate blood samples from 30 volunteers. One sample was hand transported, and the other sample was transported through a PTS together with a mini–data logger that provided continuous measurements of temperature, humidity, pressure, and acceleration. After transport the samples were analyzed at the same time. We looked for possible relationships of the transport method and the parameters measured by the data loggers with differences in hematological parameters, standard clinical chemistry analyses, blood coagulation, erythrocyte sedimentation rate, and blood gas analysis.

RESULTS: There were no significant differences in temperature, humidity, and pressure between the methods of transport, but we observed significant differences in 3-axis accelerations. The combined effect of these forces could be described by the right-tailed area under the vector sum acceleration distribution. Our data show that this area correlated with PTS speed and that PTS speed and the area under the curve exhibited a direct relation to the degree of hemolysis.

CONCLUSIONS: Assessment of 3-axis acceleration by use of data loggers can be used to identify preanalytical deviations that result from the transportation of blood samples in PTSs. Our approach could be used for the evaluation and regular control of PTSs without the need for repeated blood drawing and laboratory analyses.

Modern pneumatic tube systems (PTSs)[4] with soft-start and variation of speed for different transports offer fast, reliable, and efficient transportation of blood samples to the laboratory.

The effects of PTSs on laboratory results have been described previously; these effects manifest primarily as hemolysis *(1)* leading to increases in potassium concentration and lactate dehydrogenase (LDH) activity *(2, 3)* and other changes. For certified laboratories, it is essential to control preanalytical factors caused by different means of transportation. Each installation of a PTS is dependent on architecture, technical considerations, and length differences, thus necessitating evaluation of every single system as a unique entity, as previously recommended *(2)*. Here we describe an approach to the evaluation of the PTS by use of mini–data loggers to measure temperature, humidity, pressure, and acceleration, in combination with the assessment of hematological parameters, standard clinical chemistry variables, blood coagulation, erythrocyte sedimentation rate, and blood gas analysis. The present study was carried out to investigate the influence of transportation via a PTS at different speeds compared with hand transport on blood samples and to establish a method for evaluation by use of data loggers without the need for repeated blood drawing.

[1] Institut für Klinische Chemie/Zentrallaboratorien, Universitätsklinikum Eppendorf, Hamburg, Germany; [2] Labor Lademannbogen, Hamburg, Germany; [3] Institut für Klinische Chemie und Laboratoriumsmedizin, Universitätsklinikum, Greifswald, Germany.
* Address correspondence to this author at: Institut für Klinische Chemie/Zentrallaboratorien, Universitätsklinikum Eppendorf-Hamburg, Martinistrasse 52, 20246 Hamburg, Germany. Fax +49-40-741054621; e-mail streichert@uke.de.
† Thomas Streichert, Benjamin Otto, and Claudia Schnabel contributed equally to the work and should all be considered as first authors.
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[4] Nonstandard abbreviations: PTS, pneumatic tube system; LDH, lactate dehydrogenase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; AUC, area under the curve.

Methods

DESCRIPTION OF THE PTS

The system was installed by Sumetzberger with tubes made of polyvinyl chloride (diameter 160 mm, bend radius 1200 mm) by using carriers of the type NW 160 400 × 115 with a loading capacity of 400 × 115 mm and a swivel lid. Carriers are sent from automated stations at the nursing units with a soft-start technique. When a carrier arrives at the central laboratories, an air cushion slows it down and places it on a conveyer belt for a soft stop. The arrival of an emergency sample is announced by an automatic acoustic signal. The speed of transit was set to 3 levels, 100%, 80%, and 62%, corresponding to approximately 2.5 m/s, 2 m/s, and 1.5 m/s.

ANALYTES

The parameters were chosen with respect to literature on the evaluation of similar systems *(4, 5)*. We decided to analyze an expanded panel including differential blood count (LH 750, Beckman Coulter), coagulation tests (BCS XP, Siemens), erythrocyte sedimentation rate (S2000, Sarstedt), sodium, potassium, chloride, phosphate, glucose, magnesium, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lipase, LDH, troponin T, IgG, free thyroxine (Modular-System, Roche), procalcitonin (Kryptor, Brahms), and blood gas analysis (ABL 700, Radiometer). The analytical methods were all standard procedures in use in our central laboratories; all were measured in between clinical samples.

VOLUNTEERS

For each condition, volunteers (n = 10) were recruited for this study. According to the requirements of our ethics committee, all participants provided signed informed consent. A total sample group of 30 healthy volunteers was recruited. This corresponded to 60 different transports. Duplicate blood samples were drawn by venipuncture with Safety-Multifly sets (Sarstedt) and collected into EDTA K, coagulation, NH_4-heparin, and serum Monovettes (Sarstedt). For blood gas analysis PICO samplers (Radiometer) and for erythrocyte sedimentation rates Sedivettes (Sarstedt) were used. All blood samples were obtained by a team of medical doctors. To minimize variation, blood pairs were drawn by the same individual, an MD, with a randomized order (hand-carried transport vs PTS); all tubes were filled to capacity and sent to the central laboratories either by being hand carried in a transport box (Sarstedt) or by PTS.

DATA LOGGER

To get an impression of what happens to a sample during the hand-carried transport or by PTS we used MSR 145W miniaturized (18 × 14 × 62 mm, 18 g) data loggers for measuring and recording temperature, humidity with integrated temperature, pressure, and 3-axis acceleration (MSR Electronics). The loggers have an accuracy of ±0.1 °C (5–45 °C) for temperature, ±2% for relative humidity (10%–85% relative humidity; 0–40 °C), ±2.5 mbar (750–1100 mbar absolute) for pressure, and ±0.15 g (25 °C) for acceleration. The measured parameters were transferred to a PC after data logging was completed by use of the software tool MSR-reader (version 3.74), visualized, and converted by an MSR-viewer (version 1.58) and MSR-csv (version 1.44).

DATA ANALYSIS

Data analysis was carried out by use of R-software (version 2.8.0, www.r-project.org). Each sample profile was processed as follows (see Fig. 1): To determine the forces acting on the samples during transport, we calculated the absolute vector sum of the *x*, *y*, and *z* acceleration. A signal distribution was generated by partitioning the signal peak range from 0 to 15 into 256 bins, and the number of signal peaks in the profile corresponding to each bin was counted.

Finally, we calculated the mean distribution of each group by summing up the bin counts and dividing the sum by the group size. Given a vector sum lower cutoff, the right-tailed areas under the curves (AUCs) above the cutoff were calculated with a weight (counts multiplied by half of the corresponding acceleration). Different cutoffs were investigated, and a cutoff of 2 was chosen (see Fig. 1C).

The blood parameters measured after transport by hand or by the PTS were analyzed and compared between groups by using a paired and 2-sided Welch *t*-test. In addition, a relative change, $100 \times (x_t\text{-}x_f)/x_f$, was calculated for each sample, where x_f is the value measured after hand-carried transport and x_t is the value measured after transport by the PTS.

VALIDATION

To show that this approach also works in other hospitals with different PTSs, we used the same experimental design at the university hospital in Greifswald. The PTS in Greifswald, installed by Aerocom, has stainless steel tubes with an external tube diameter of 160 mm and a bend radius of 800 mm and uses a carrier of type DS-2A-MA-KL 160-154/115-330, with a loading capacity of 330 mm and a swivel lid.

The analytical methods were all standard procedures currently in use in the laboratories in Greifswald.

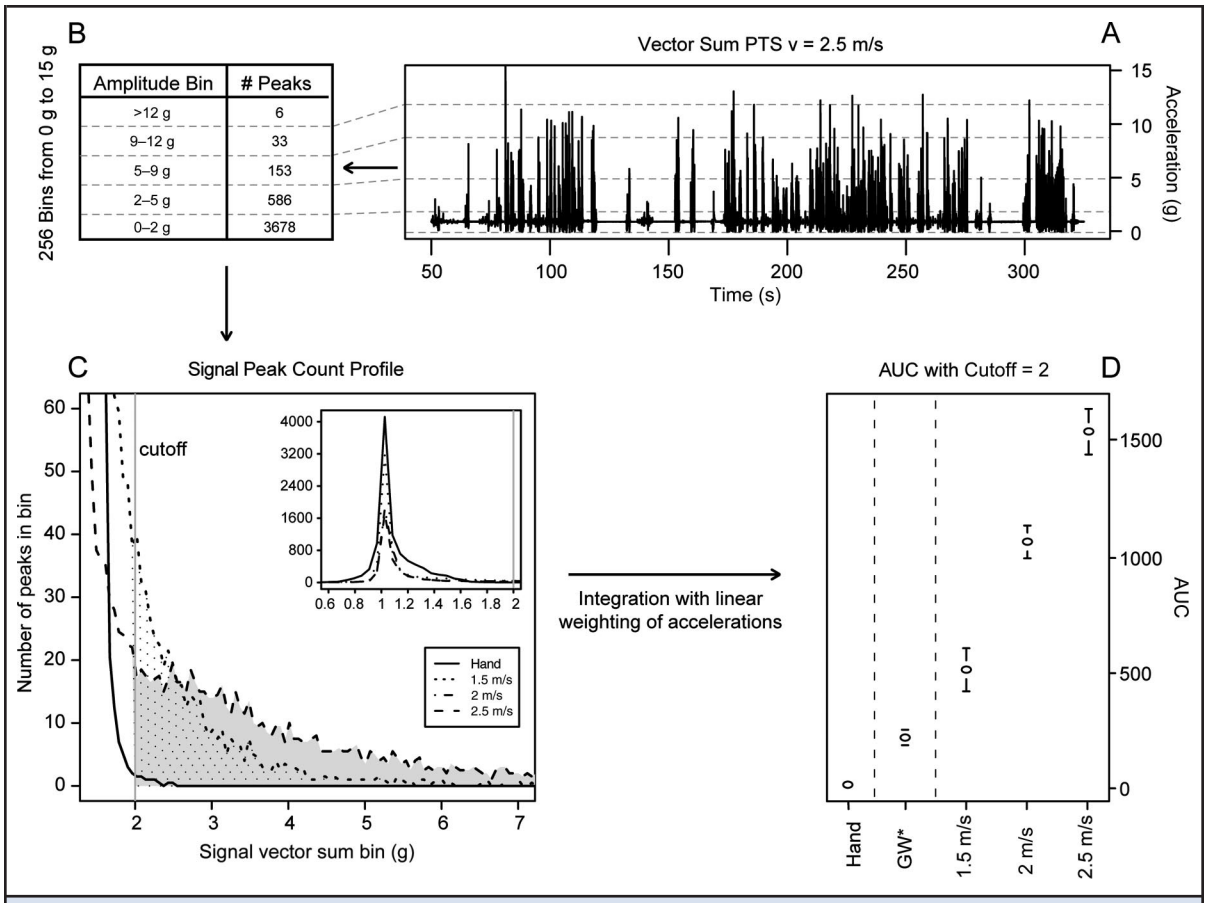

Fig. 1. Analysis of the acceleration profiles measured with the data logger.

(A), Acceleration profile. (B), The number of observed peaks was determined and classified in 256 bins according to their amplitudes. (C), This distribution was then used for the calculation of the weighted, right-tailed AUC with a cutoff of 2 g. (D), Box plots of the AUC for hand transport (Hand) and transports by PTS with different speed settings, 1.5 m/s, 2 m/s, and 2.5 m/s. GW* is the validation of the approach with an independent PTS at the University Hospital Greifswald. The box plots show a positive correlation between speed and the AUCs; v, velocity.

Data analysis was carried out as described in the section above. We applied the same vector sum cutoff (=2) for the calculation of the AUC.

Results

For the evaluation of the PTS, a nursing unit with a maximum distance to the laboratory was chosen. The average duration of the sample transport via PTS was 5–6.5 min depending on PTS speed and 7.5 min for transport by hand. To exclude further variables in the preanalytic conditions, all samples (PTS and hand transported) were processed and analyzed at the same time point.

Data loggers that monitored temperature, humidity, pressure, and 3-axis acceleration were sent along with each sample to the laboratory. Differences in temperature, humidity, and pressure between hand transport and PTS transport were found to be negligible (data not shown). However, there were major differences in 3-axis acceleration. To get an overall impression of the stresses caused by the different accelerations, the vector sum of the 3 axes was calculated. Fig. 2 shows the accelerations plotted over transport time for the different conditions (hand-carried transport, and 3 transit speeds of the PTS). The peak accelerations expressed in multiples of gravitational force in the PTS were up to 15 g for 2.5 m/s, 14 g for 2 m/s, 13 g for 1.5 m/s, and 9 g for hand-carried transport (only once, see last peak). The profiles reveal an increasing number of peaks with high accelerations. The cumulative effect of these forces was analyzed by using the distributions of the accelerations (see Fig. 1). The accelerations caused by hand-carried transport were assumed to be minor. We found

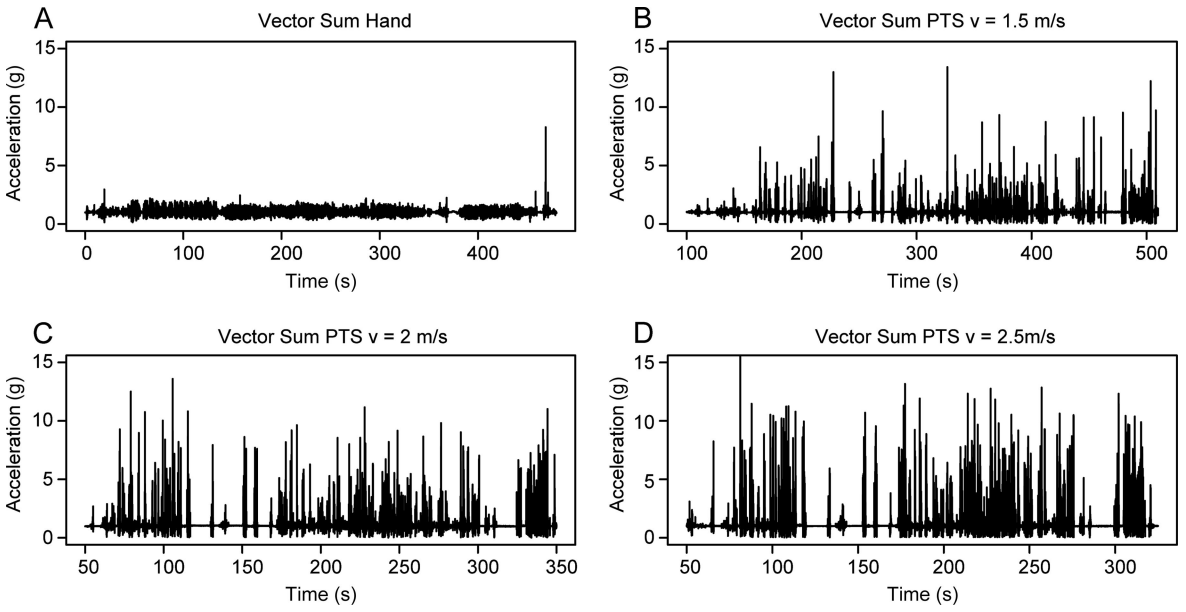

Fig. 2. Acceleration profiles after calculation of the vector sum for the *x*, *y*, and *z* axes.

The profiles were recorded with data loggers that were sent together with blood samples to the laboratory. The number of peaks observed in the profiles and their amplitudes increased with transportation speed. (A), Acceleration profile of hand transport, which served as control. (B–D), Acceleration profiles of transports by PTS with different speed settings, 100%, 80%, and 62%; v, velocity.

the lowest discriminating cutoff at 2 g, so we set the lower limit to this value. Then the AUC of the acceleration distribution above this cutoff was calculated (see Fig. 1C). The AUCs of the 4 groups are shown in the box plots in Fig. 1D. As expected, there is a clear positive correlation between PTS speed and the accelerations exerted on the blood sample.

All samples were analyzed in the central laboratories, and the hand-transported samples served as controls.

Reproducibility of the methods used was shown by QC materials analyzed in the clinical routine according to legal regulations and our QC guidelines [DAR (Deutscher Akkreditierungs Rat)-certified laboratory; DAC-ML-0133-01-10, data not shown].

The results are presented in Table 1 and Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue10. At 2.5 m/s the Welch *t*-test identified significant changes for platelets, monocytes, batroxobin test (Reptilase time), D-dimer fragments, antithrombin, potassium, phosphorus, magnesium, ASAT, ALAT, lipase, and LDH. Although these changes had *P* values below 0.05 they were critical only for potassium, phosphate, ASAT, and LDH, for which the relative changes exceeded the allowed relative deviation of QCs as specified in the guidelines (RiliBäK) of the German Federal Medical Council. The RiliBäK defines minimum requirements similar to the CLIA proficiency limits in the US for the quality of quantitative test results for laboratories in Germany. According to these guidelines the root mean square deviation must not exceed 4.5% for potassium, 9% for phosphate, 11.5% for ASAT, and 9% for LDH *(6)*.

These changes were seen clearly to decrease with reductions in transportation speed and with decreasing AUCs obtained from the logger data (see Table 1, Fig. 3, and online Supplemental Fig. 1). At 1.5 m/s only LDH and ASAT showed changes that were significant and were above the limit of the RiliBäK 2010 *(6)*. For ASAT activities it is questionable whether an absolute deviation of 1.5 U/L (0.03 μkat/L) is relevant in a clinical setting. As displayed in Fig. 3 and online Supplemental Fig. 1, a clear positive relation between PTS speed, AUC, and increased concentrations of potassium and increased activities of ASAT, and LDH were observed. These levels decreased with reduced speed of the PTS.

We did not find any significant changes in the blood gas analysis results at any speed.

The validation of the PTS at the University Hospital in Greifswald showed AUCs below the ones ob-

Table 1. Comparison of results achieved from normal samples hand transported (control) or PTS transported at different speed settings, 100% (2.5 m/s), 80% (2 m/s), and 62% (1.5 m/s).[a]

	Maximum deviation (absolute)			Mean deviation (absolute)			Maximum deviation, %		
Analyte	1.5 m/s	2 m/s	2.5 m/s	1.5 m/s	2 m/s	2.5 m/s	1.5 m/s	2 m/s	2.5 m/s
Hemoglobin, g/dL	0.20	0.10	0.20	0.04	0.04	0.05	1.52	0.76	1.31
Hematocrit, %	0.00	1.20	0.60	−0.27	−0.12	−0.04	0.00	2.97	1.53
Erythrocytes, 10^9/mL	0.01	0.07	0.02	−0.01	−0.01	0.00	0.23	1.51	0.51
MCV, fl[b]	0.20	1.00	0.80	−0.39[c]	−0.09	−0.13	0.23	1.15	0.89
MCH, pg	0.50	0.50	0.50	0.20[c]	0.16	0.07	1.88	1.59	1.64
MCHC, g/dL	1.10	0.80	0.50	0.40[c]	0.19	0.12	3.26	2.33	1.42
RDW, %	0.20	0.60	0.20	−0.02	0.01	0.01	1.46	4.62	1.68
Leukocytes, 10^9/L	0.40	0.30	0.20	0.03	0.09	0.03	6.98	3.80	3.12
Platelets, 10^9/L	46.00	38.00	37.00	10.00	10.10[c]	15.80[c]	20.09	12.30	19.46
Neutrophils, 10^9/L	0.30	0.20	0.40	0.04	0.09	0.08	8.33	3.77	5.88
Lymphocytes, 10^9/L	0.10	0.10	0.10	−0.02	−0.03	−0.02	7.14	4.55	5.00
Monocytes, 10^9/L	0.10	0.10	0.00	0.02	0.00	−0.04[c]	33.33	33.33	0.00
Eosinophils, 10^9/L	0.00	0.00	0.10	0.00	0.00	−0.01	0.00	0.00	100.00
Basophils, 10^9/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Quick test, %	2.03	1.05	4.62	−0.05	−0.52	0.23	2.20	0.90	4.33
INR, s	0.01	0.02	0.02	0.00	0.00	0.00	1.00	2.08	2.06
aPTT, s	1.41	0.83	1.16	0.34	−0.09	−0.38	4.36	2.65	4.64
Thrombin time, s	0.48	0.72	0.27	0.05	0.11	−0.12	2.88	4.01	1.66
FibC, g/L	0.04	0.30	0.29	−0.11	−0.02	0.06	1.60	5.69	10.58
Batroxobin test, s	0.67	0.54	0.62	0.10	0.06	0.24[c]	3.75	3.01	3.69
DD, mg/L FEU	0.18	0.04	0.03	0.03	0.01	0.01[c]	20.00	10.53	15.79
Antithrombin, %	2.08	17.27	0.13	−0.17	−2.00	−1.36[c]	2.23	16.53	0.14
F05.k, %	4.26	12.21	13.12	0.81	3.05	2.31	3.87	11.66	10.92
ESR, mm/h	2.00	115.12	1.00	−0.11	11.21	−0.10	25.00	2302.40[d]	16.67
Na, mmol/L	1.00	2.00	2.00	−0.50	0.10	−0.70	0.71	1.43	1.43
K, mmol/L	0.09	0.44	0.82	0.04	0.15[c,e]	0.47[c,e]	2.49	12.43[e,f]	22.07[e,f]
Cl, mmol/L	2.00	3.00	3.00	0.25	0.60	0.00	1.89	2.94	2.88
Phosphorus, mmol/L	0.02	0.04	0.11	0.00	0.02	0.04[c,e]	2.00	3.96	11.70[e,f]
Glucose, mg/dL	2.00	2.00	1.00	0.63	0.70	−0.30	2.27	2.35	1.19
Mg, mmol/L	0.00	0.02	0.03	0.00	0.00	0.02	0.00	2.17	3.57
ASAT, U/L	4.00	8.00	28.00	1.50[c,e]	4.10[c,e]	14.60[c,e]	22.22[e,f]	53.33[e,f]	107.69[e,f]
ALAT, U/L	1.00	3.00	3.00	−0.25	−0.10	1.40[c]	5.56	20.00	20.00
Lipase, U/L	1.00	4.00	8.00	−0.13	0.50	2.90[c]	3.85	8.70	40.00
LDH, U/L	35.00	88.00	305.00	21.00[c,e]	47.20[c,e]	168.40[c,e]	26.32[e,f]	60.27[e,f]	211.81[e,f]
Troponin T, ng/mL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pH	0.01	0.01	0.01	0.00	0.00	0.00	0.14	0.14	0.14
PO_2, mmHg	7.40	6.30	2.90	1.54	0.59	−0.22	9.37	27.75	10.47
PCO_2, mmHg	0.30	2.00	3.80	−0.19	0.10	0.31	0.53	3.51	9.77
HCO_3, mmHg	0.30	0.50	1.80	0.04	0.08	0.21	1.29	1.92	7.59
Base excess, mmol/L	0.30	0.50	1.20	0.10	0.05	0.16	37.50	200.00	50.00
Oxygen saturation, %	0.30	0.40	0.60	0.10	0.09	0.09	1.38	1.75	2.53

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Table 1. Comparison of results achieved from normal samples hand transported (control) or PTS transported at different speed settings, 100% (2.5 m/s), 80% (2 m/s), and 62% (1.5 m/s).[a] *(Continued from page 1394)*

	Maximum deviation (absolute)			Mean deviation (absolute)			Maximum deviation, %		
Analyte	1.5 m/s	2 m/s	2.5 m/s	1.5 m/s	2 m/s	2.5 m/s	1.5 m/s	2 m/s	2.5 m/s
Methemoglobin, %	4.60	15.90	7.30	1.20	1.52	−1.46	8.27	45.69	15.87
Carboxyhemoglobin, %	0.30	0.10	0.10	0.10	−0.02	0.01	100.00	20.00	25.00
IgG, g/L	0.20	0.10	0.20	0.04	0.03	0.00	66.67	25.00	22.22
Procalcitonin, μg/L	0.18	0.16	0.06	0.02	0.00	−0.07	1.49	1.69	0.58
Free thyroxine, pmol/L	0.05	0.00	0.00	0.01	0.00	0.00	50.00	0.00	0.00

[a] For each parameter *P* values were calculated by using Welch's *t*-test. To get an impression of the alteration caused by different means of transport, the mean difference and the maximum difference were calculated.
[b] MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; INR, international normalized ratio; aPTT, activated partial thromboplastin time; FibC, fibrinogen (Clauss method); DD, dimeric D-fragments; F05.k, factor 5 (coagulometric); ESR, erythrocyte sedimentation rate; PO_2, partial pressure of oxygen; pCO2, partial pressure of carbon dioxide.
[c] *P*-value < 0.05.
[d] Erythrocyte sedimentation rate value caused by outliers.
[e] Meets the criteria specified in footnotes c and f.
[f] Relative changes exceed the allowed relative deviation of QCs specified in the RiliBÄK 2010, part B1a.

served at 1.5 m/s in Hamburg (see Fig. 1D). As expected, the relative changes of LDH, ASAT, and potassium were between the results for hand-carried transport and the PTS at 1.5 m/s (see Fig. 3).

PTSs are gaining popularity in medical centers. However, blood samples are particularly sensitive to strong forces or vibrations with high frequencies and require special attention. It has been shown that PTS transport could affect samples *(7–9)* and lead to hemolysis or altered blood gases *(5)*. On the other hand, there are hospitals working with PTSs constructed in such a way that they do not affect samples or do so only minimally *(1, 10–12)*. Possible explanations for such differences may be related to the different manufacturers of these systems but also could be related to different methods of installation. Each installation of a PTS is uniquely characterized by architecture, technical specifications, and differences in speed and length, thus demanding individual evaluation of every single system *(2)*. In addition, each modification or malfunction of the PTS could change the forces acting on samples to high enough levels to produce undesirable effects such as excessive hemolysis *(8)*. These factors suggest that each PTS should be evaluated not only at

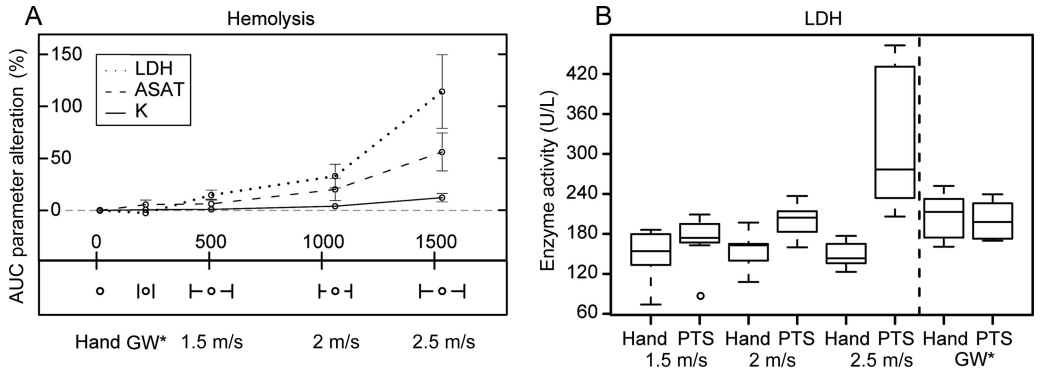

Fig. 3. (A), Relation between calculated AUCs and selected analytes indicating hemolysis [$(X_{tube} - X_{hand})/X_{hand} \times 100$]. A clear correlation between LDH, ASAT, K, and the AUC can be observed. (B), Box plots of LDH activities found after hand transport (Hand) and PTS transport with different speed settings, 100%, 80%, and 62%. GW* are the results from the validation with an independent PTS at the University Hospital Greifswald.

the time of installation but also at regular intervals thereafter.

With this work we have established a method for the evaluation, determination of appropriate speed, and periodic assessment of installed PTSs without the need for repeated blood drawing. This new approach of measuring 3-axis acceleration in relation to laboratory results identified significant preanalytical influences in PTS that could lead to speed-dependent hemolysis.

Blood samples can be centrifuged with a force of up to 1500*g* for 10 min without exhibiting significant hemolysis *(13)*, so we assume that it is not the high acceleration alone that leads to cell destruction. Hemolysis is more likely to be caused by rapid and large acceleration changes. The deleterious effects of blood pumps, cardiac valves, and even needles on erythrocytes are well characterized. The cell damage is known to be a function of several parameters, among them shear stress, exposure time, blood contact material, and blood composition *(14)*. For a cannula it has been shown that most of the hemolysis is produced on the external edge of the tip of the cannula, as the blood makes a 180-degree turn *(15)*. In contrast, the hemolytic effects of transportation within a tube designed for blood collection are largely unknown. To get further insight into the conditions in a PTS that could cause preanalytical interference, we measured temperature, humidity, pressure, and 3-axis acceleration. Although there were no considerable changes in temperature, humidity, and pressure compared with hand-transported samples, we observed significant differences of 3-axis accelerations. Two relevant stress parameters are given by the number and the magnitude of acceleration changes in the observed profiles (see Fig. 2). The exposure time is represented by the number of observed peaks. Taken together, the overall effect of these forces could be described by measuring the right-tailed area above a given threshold under the acceleration distribution. Our data show that this area correlates with PTS speed and that PTS speed and the AUC exhibit a direct relation to hemolysis (see Fig. 3 and online Supplemental Fig. 1).

To test whether our approach is widely applicable independently of the nature of the PTS installation, we evaluated a PTS at the university hospital in Greifswald, where we found similar results. The cumulative accelerations of the PTS at Greifwald did not reach the critical limit found in Hamburg and, as predicted, the hemolysis was clinically not significant.

The critical limit has to be determined at the initial evaluation and is of course dependent on the patient population. Patients suffering from hematological disorders such as chronic lymphocytic leukemia are more likely to show increased levels of potassium or LDH because of the mechanical fragility of the leukocytes *(16, 17)*. Thus transport of a sample by PTS could lead to the misdiagnosis of a tumor lysis syndrome *(9)*. In addition, it will be important to test the use of this device for transport of samples from patients with hematological disorders that affect the erythrocyte, such as hereditary elliptocytosis *(18)*, sickle cell anemia, hypochromic anemia, and hereditary spherocytosis *(19)*. In this way, the upper limits of acceleration may be defined to avoid damage of erythrocytes in these critical samples.

We have described the use of data loggers for the identification of preanalytical interferences introduced by the transportation of blood samples in PTSs. Our approach will be helpful to determine the critical threshold for hemolysis in a PTS and could be applied to different scenarios: (*a*) tuning of the PTS so that the maximum speed and hence the maximum capacity of the PTS can be determined; (*b*) determining the effectiveness of cushioning; (*c*) conducting the initial evaluation and subsequent regular control of PTSs without the need for repeated blood drawing and laboratory analyses. This process would offer an easy and cost-effective strategy for quality assurance.

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