The Impact of the Packaging System on the Stability of Blood Samples Used for Safety Assessments in Clinical Trials

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ABSTRACT

Reliable shipment of clinical blood, serum and plasma samples from remote sites to central labs for standardized analyses is increasingly important in medicnal product trials. This study investigates the benefits of dedicated packaging for protecting such samples from extreme seasonal temperature fluctuations during shipment. The new packaging system, MLM Safeguard Box®[1)], and a conventional cardboard transport box were compared in terms of their protective performance under thermal stress conditions. These boxes and their contents were subjected to cold stress (–30 °C) and heat stress (50 °C) for 3 h within a 24 h period. These are conditions that could be encountered during overnight transport with commercial logistics providers. The temperature profiles showed that the custom-engineered blood transport system protects clinical samples against rapid temperature changes, whereas the cardboard box completely equilibrated to the external temperature after a short time. This was shown to have a direct impact on the hematology, clinical chemistry and coagulation of the study samples. While the custom-engineered blood transport system preserved all parameters of the differential blood count, the samples in the cardboard box displayed strongly altered differential blood counts after heat stress and a complete eradication of subpopulations after cold stress. The activity of liver (alanine aminotransferase – ALT, alkaline phosphatase – ALP), pancreatic (lipase) and cardiac (creatine kinase – CK, creatine kinase isotype MB – CK-MB) enzymes was maintained in the samples in the custom-engineered blood transport system, but strongly reduced (ALT, ALP, lipase) or absent (CK, CK-MB) in the samples in the cardboard box after heat stress. Coagulation (activated partial thromboplastin time – aPTT, prothrombin time – PT or international normalized ratio – INR) was not affected after storage in the custom-engineered blood transport system under heat stress conditions, but in a standard cardboard box aPTT and PT were prolonged after heat stress. Temperature stress can lead to significant misinterpretations of clinical safety data, e.g., in the context of drug-induced liver injury, myocardial infarction or coagulation disorders. Therefore, the novel custom-engineered blood transport system is a significant improvement in the safe transport of samples for clinical studies.

ZUSAMMENFASSUNG

Der Einfluss des Verpackungssystems auf die Stabilität von Blutproben im Rahmen klinischer Studien

In der klinischen Prüfung von Arzneimitteln im Rahmen multizentrischer Studien spielt der Übernachtversand klinischer Blut-, Serum- und Plasmaproben zwischen Studienzentren und einem Zentrallabor eine wichtige Rolle für die Erhebung zuverlässiger Sicherheits- und Wirksamkeitsdaten. In der vorliegenden Studie wurden 2 verschiedene Verpackungssysteme, eine einfache Versandbox aus Karton und eine neuartige Spezialverpackung, die MLM Safeguard Box, unter thermischen Stressbedingungen erprobt. Letztere ist in der Lage, die Proben über einen Zeitraum von mehreren Tagen vor extremen Temperaturschwankungen auf dem Transportweg zu schützen. Die physikalischen Eigenschaften der beiden Verpackungssysteme wurden mit Vollblut-, Serum- und Plasmaproben mit Parametern aus der Hämatologie, Klinischen Chemie und Gerinnung überprüft. Dabei zeigte sich, dass die Spezialverpackung insbesondere die temperaturempfindlichen Parameter, darunter wichtige kardiale und hepatische Parameter, wirksam vor Temperaturstress schützt. Damit trägt die Verpackung wesentlich zur Erhebung zuverlässiger Sicherheits- und Wirksamkeitsdaten in klinischen Multizenterstudien bei.

KEY WORDS

- Clinical trials
- Sample logistics
- Thermal stress
- Central laboratory
- Safety parameters

[1)] Henceforth referred to as 'custom-engineered blood transport system.'

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1. Introduction

Accurate screening of potential study subjects for clinical trials and safety and efficacy assessments of drug candidates are well-known requirements by medicinal product authorities such as the European Medicines Agency and the U.S. Food and Drug Administration. The overall health of clinical trial participants and any physiological effects of the drug candidates are monitored through regular analyses of blood, serum and plasma samples, among other tests.

The complete blood count (CBC) is used to test for a wide range of pathological conditions, including anemia, leukemia or erythropoietic disorders, when screening potential study subjects. Combined with the differential blood count, these hematological parameters serve as a primary indication of inflammation and infection, and are used to monitor the health status of the study subjects [1, 2]. Coagulation tests are widely used to exclude coagulation disorders during the screening of potential study subjects [2, 3].

A variety of standard biomarkers, such as enzymes, hormones and electrolytes, and metabolic activity markers are significantly changed in certain disease states or under the influence of pharmaceutically active compounds. Therefore, several clinical chemistry parameters including electrolytes, lipids, metabolites and enzymes are measured to test for multiple pathological conditions, such as liver and kidney diseases, disturbances in carbohydrate or lipid metabolism and even myocardial infarction, as well as for pharmacological side effects during drug trials [4].

For many years, these health and drug safety and efficacy parameters were measured in medical laboratories that were close to the clinical study centers involved in the clinical trials of new drug candidates. However, increasing demands from regulatory authorities for standardization of analytical methods and more stringent quality requirements have forced a change toward the use of laboratories specialized in clinical studies for the analysis of safety samples. This means that blood, serum and plasma samples have to be shipped from the clinical study centers to such labs.

While most parameters are stable under normal ambient temperatures (i. e., 18–28 °C), extreme seasonal temperature fluctuations or extreme cooling, such as those that can be experienced during transport, do affect these parameters. This can have a considerable impact on the outcome of analyses, making the diagnosis of subject health and monitoring of drug effects false or impossible. Therefore, it is of great importance to determine the stability of the samples in the packaging system used.

Most laboratories use a simple cardboard box, which provides no special protection against thermal stress. Additional protective measures are necessary to protect samples from the effects of temperature fluctuations.

The newly developed custom-engineered blood transport system consists of an outer cardboard box with an additional polystyrol box inlay and a winged gel pack (Fig. 1). The combination of polystyrol box and gel pack was selected because of its potential to protect samples against variations in environmental temperatures during transport. While the effects of extreme temperatures on parameters in hematology, clinical chemistry and coagulation have been studied previously [3, 5–7], the impact of packaging and oscillating temperatures has been neglected, despite being so critical for sample stability. In addition, the time intervals and temperature conditions chosen in previous studies did not simulate sample transportation involving real-life overnight and overseas transport conditions.

Therefore, the authors undertook a comparative analysis of the new custom-engineered blood transport system and a conventional cardboard transport box to determine its protective performance against temperature fluctuations.

2. Materials and Methods

2.1 Boxes

The standard cardboard transport box had the dimensions 250 x 172 x 82 mm (L x W x H) and a wall thickness of 1.5 mm. The custom-engineered blood transport system consists of a cardboard box of the same size and wall thickness with a polystyrol inlay (15 mm thick) and a winged gel pack containing 780 g aqueous gel. These materials were custom manufactured for MLM Medical Labs GmbH by qualified vendors.

2.2 Assessment of Temperature Profiles

Hamster EHT1 temperature and humidity recording units (ELPRO GmbH, Schorndorf, Germany) were placed in the boxes to measure the temperature during the experiments. The boxes were first held at room temperature (23 ±5 °C) for 2 h, then stressed inside an INCU-Line IL-23 microbiological incubator (VWR International GmbH, Langenfeld, Germany) at 50 ±5 °C for 3 h before being kept overnight at room temperature. After 24 h, a time-dependent temperature profile was generated.

Figure 1: The custom-engineered blood transport system consists of an outer cardboard box with an additional polystyrol inlay box and a winged gel pack (Source of all figures: MLM Medical Labs GmbH).

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The thermal stability in a cold environment was tested with the same experimental setup, but instead of an incubation at 50 °C, the boxes were put into a Freezer-720 at –30 ±5 °C (Philipp Kirsch GmbH, Offenburg, Germany) for 3 h.

In addition, the thermoprotective performance of the custom-engineered blood transport system under real-life conditions was assessed by shipping it on a summer day (maximum temperatures around 30 °C) from Mönchengladbach in Germany to Raeren in Belgium. In this case, a TempTale 4 Logger (Sensitech Inc. Beverly, MA, USA) was used for temperature readings because it enables simultaneous recording of the environmental and box interior temperatures.

2.3 Parameter Analysis after Temperature Treatments

Sample Preparation

For each temperature stress experiment, whole blood from three (one female, two male, age: 24–53) healthy donors was drawn by venipuncture into tripotassium ethylene diamine tetraacetate (K_3EDTA; S-Monovette 2.6 ml K3E), serum (S-Monovette 2.6 ml Z) and citrate (S-Monovette 3 ml 9NC) blood collection tubes (Sarstedt AG & Co, Nürnbrecht, Germany). The whole blood in the serum and citrate tubes was centrifuged using a Rotixa 50RS (Andreas Hettich GmbH & *Co.KG*, Tuttlingen, Germany) as per the manufacturer's protocol to obtain serum and plasma, respectively.

Assessment of Parameters before Starting of the Treatment Period

Hematology: K_3EDTA blood samples were loaded onto a Sysmex XT-2000*i*™ automated hematology analyzer (Sysmex Deutschland GmbH, Norderstedt, Germany) to determine the hematocrit and red cell indices and the contents of leucocytes, lymphocytes, monocytes, granulocytes, erythrocytes, hemoglobin and platelets.

Clinical chemistry: Serum samples were analyzed using a Roche-Hitachi EVO Modular Analyzer (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). The analysis focused on electrolytes, lipids, metabolites and enzymes.

Coagulation: Citrate plasma samples were loaded onto an STA Compact© coagulation analyzer (Diagnostica Stago Deutschland GmbH, Düsseldorf, Germany) to measure prothrombin time (Quick), aPTT and the INR.

Temperature Challenge

After the base parameter measurements (t = 0 h), the samples were subjected to the temperature treatments. For heat stress, one set of K_3EDTA blood, citrate plasma and serum tubes from each donor was kept at room temperature for 24 h in the dark (control box). The second set from each donor was placed into the cardboard box and the third into the custom-engineered blood transport system. Both boxes were held at room temperature for 2 h, then stressed for 3 h at 50 ±5 °C and finally kept at room temperature overnight. For cold stress, the corresponding experiment was performed with three sets of samples from each donor in corresponding boxes, but with the 3 hours' stress at –30 ±5 °C.

Assessment of Parameters at the End of the Treatment Period

Parameters for K_3EDTA blood, citrate and serum were assessed after this treatment (t = 24 h) as described above. All values obtained from the samples from the control box, cardboard box and custom-engineered blood transport system were compared according to their deviation from t = 0 h to t = 24 h. The safety parameters were defined to be sufficiently stable if they did not deviate more than 20 % compared to the original concentration. This arbitrary definition of stability reflects the generally relatively broad reference ranges of such safety parameters. The control measurement of t = 24 h was compared to the concentrations after storage in the cardboard box and the custom-engineered blood transport system at t = 24 h, to reveal which parameters of which box showed similar concentrations to the control box and were thus stable when thermally stressed.

3. Results and Discussion

3.1 Protection from Extreme Thermal Oscillations

Clinical samples are generally transported in simple cardboard boxes. These boxes have no thermal protection against the extreme seasonal temperature fluctuations experienced during transport. The custom-engineered blood transport system is a cardboard box with an inner polystyrol box and a gel pack as insulating components. A comparative analysis of temperature profiles for the cardboard box and custom-engineered blood transport system shows that the latter has superior performance in terms of modulating thermal transitions from room temperature to 50 °C or –30 °C and back (Fig. 2).

The custom-engineered blood transport system showed a much lower rate of temperature equilibration than the simple cardboard box. For example, when incubated for 3 h at 50 °C (Fig. 2 A), the temperature inside the cardboard box equilibrated within less than an hour, whereas the custom-engineered blood transport system resisted this thermal increase for a longer period, only reaching a maximum of around 30 °C by the end of the period of thermal stress.

After exposure to 50 °C heat stress, the boxes rested overnight at room temperature. The cardboard box adapted to the room temperature within an hour, whereas the custom-engineered blood transport system displayed a delayed adjustment, with a gradual decline of 1–2 °C per hour until complete equilibration to 21–22 °C.

When placed in a freezer at –30 ±5 °C, each box yielded a similar profile with inverted temperature curves

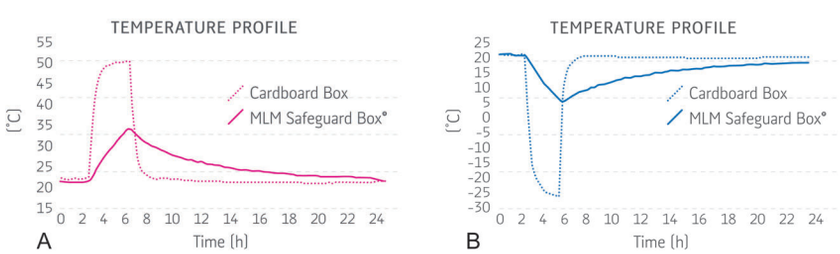

Figure 2: The custom-engineered blood transport system protects against the effects of extreme temperatures. Both packaging systems were left at room temperature for 2 h, stressed with extreme temperatures (50 °C (A) and –30 °C (B)) for 3 h, and then transferred to room temperature and left overnight.

(Fig. 2 B). The temperature inside the cardboard box decreased rapidly to –20.5 °C after one hour, reaching a minimum of –26.6 °C after 3 h of incubation, whereas the custom-engineered blood transport system reduced in temperature more moderately: to 14.6 °C after one hour and reaching a minimum of 4.9 °C after 3 h. The cardboard box reached a temperature of 20 °C just 2 h after being returned to room temperature, whereas the custom-engineered blood transport system was able to sustain the low temperature for a longer period, steadily increasing to 18.1 °C after 24 h.

This more gradual temperature adaption exhibited by the custom-engineered blood transport system is attributed to its additional components compared to the standard cardboard box. The implemented polystyrol inlay functions as a resistor, impeding heat transfer and reducing the rate at which thermal energy enters or leaves the package. The gel pack increases the thermal capacity inside the box, making it more challenging to change the temperature within the box. Because the gel contains ca. 780 ml water, which has a specific heat capacity of 4182 J/(kg·K) at 20 °C and 0.1 MPa, the specific heat capacity of the gel is about 3260 J/K under normal environmental conditions. This combination of thermal resistance conferred by the polystyrol and improved thermal capacity conferred by the water provides the desired protection from extreme temperature fluctuations.

3.2 Thermal Effect on Parameter Stability of Clinical Samples

The capability of the two boxes to stabilize clinical parameters when transported within these thermal oscillations was tested by assessing hematology, clinical chemistry and coagulation parameters before and after both temperature treatments. The concentration of unstable parameters (deviation above 20 %) after 24 h was compared with the parameter concentration of samples kept within an untreated control box stored at room temperature for 24 h.

Heat stress test

The results for hematologic parameter stability are shown in Fig. 3. The number of leucocytes (Fig. 3 A) and their relative cell densities for the samples in the custom-engineered blood transport system remained almost the same as those for the samples kept in the untreated control box, indicating their preservation during thermal stress. By contrast, the assimilated extreme temperatures in the cardboard box distorted the cell shape, leading to a different appearance in the flow cytometric analysis. The analyzer incorrectly detected a higher cell density and was not able to effectively differentiate the leucocytes, making quantification of lymphocytes (Fig. 3 B), monocytes and granulocytes (data not shown) impossible.

The number of platelets (Fig. 3 C) in the custom-engineered blood transport system samples were unaffected by the thermal increase. In the cardboard box, extreme temperatures had deformed the cells, causing them to be erroneously recorded as an increased cell number.

These observations suggest that the assessment of general health and the exclusion of inflammatory processes, leukemia, lymphomas or drug-induced modifications of the differential blood count may be compromised when samples are transported with a conventional cardboard box.

The assessments of clinical chemistry (Fig. 4) and coagulation parameters (Fig. 5) further highlight the benefits of the custom-engineered blood transport system. The activities of crucial temperature-sensitive enzymes in the serum samples stored in the custom-engineered blood transport system were very similar to those for samples stored in the control box.

However, the samples in the cardboard box subjected to thermal stress showed reduced or absent activity for several relevant enzymes. A significant reduction was observed in activities of lipase, ALT, ALP and CK-MB (Fig. 4 A–D). In the sample from one donor, the activity of CK-MB was completely absent. CK activity was also remarkably reduced in all subject samples (Fig. 4 E).

Because protein stability is temperature-sensitive, the elevated temperatures inside the cardboard box resulted in protein denaturation, rendering several enzymes irreversibly inactive or significantly reducing enzyme activity. The inability to quantify relevant enzyme activities could have immense adverse effects on the diagnosis of diseases or monitoring of treatment. For example, CK-MB is found almost exclusively in the myocardium. Elevated levels in

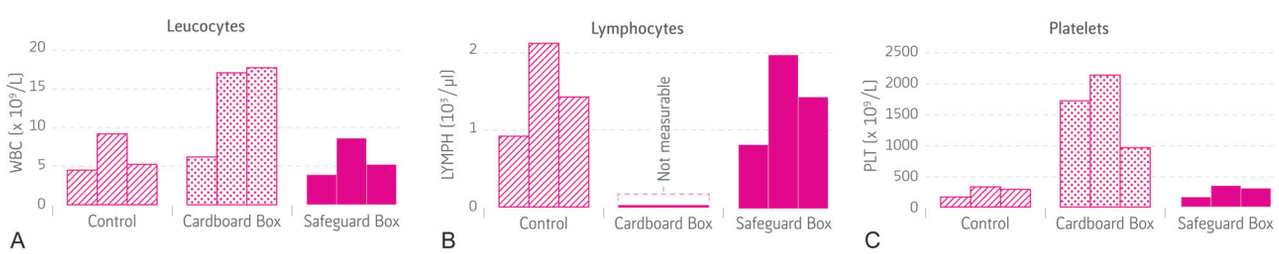

Figure 3: The custom-engineered blood transport system protects samples from heat stress as shown by a study of hematological parameters. The levels are shown after 24 h within the control box, the heat-stressed cardboard box and the heat-stressed custom-engineered blood transport system. The left column represents blood donor A, the middle donor B and the right column donor C.

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the serum may indicate an acute myocardial infarction. Such a potentially life-threatening condition would not be diagnosed if proteins were degraded through thermal stress and such elevations in serum were not measurable because of transport conditions. Another example is the potential for an increase of the activity of the liver enzymes ALP and ALT to be masked. This potential indicator of chronic liver disease, hepatitis infection or drug-induced liver injury could be lost through improper sample transport under thermal stress conditions.

The same thermal effect was recorded for coagulation parameters (Fig. 5). The activation of enzymes in the coagulation cascade leads to the conversion of prothrombin into thrombin, which promotes clot formation via conversion of soluble fibrinogen to fibrin. The PT, activated aPTT and INR are measured to monitor the risk of bleeding complications and the function of the liver, and to detect hemostatic disorders. However, these parameters are significantly affected by thermal stress. The activated partial thromboplastin time (Fig. 5 A) was prolonged and the prothrombin time (Fig. 5 B) was not measurable in those samples that were stored in the cardboard box subjected to thermal stress. By comparison, the custom-engineered blood transport system protected plasma samples from thermal stress: All the coagulation parameters were comparable with those obtained from samples stored in the control box at ambient temperature for 24 h.

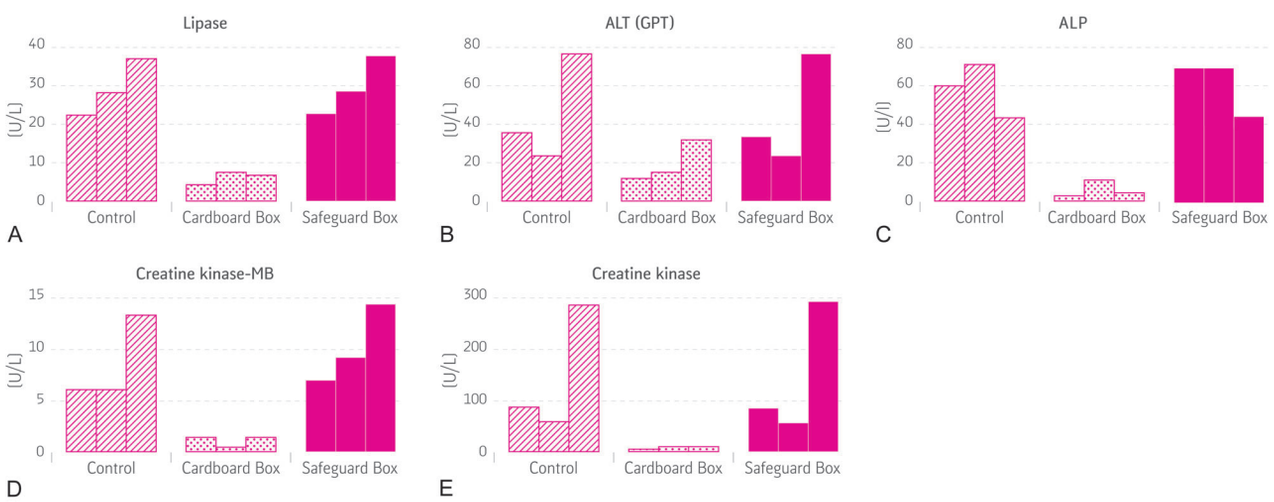

Figure 4: The custom-engineered blood transport system preserves the enzyme activity against heat stress. The parameter concentrations after 24 h within the control box, the heat-stressed cardboard box and the heat-stressed custom-engineered blood transport system. The left column represents blood donor A, the middle donor B and the right column donor C.

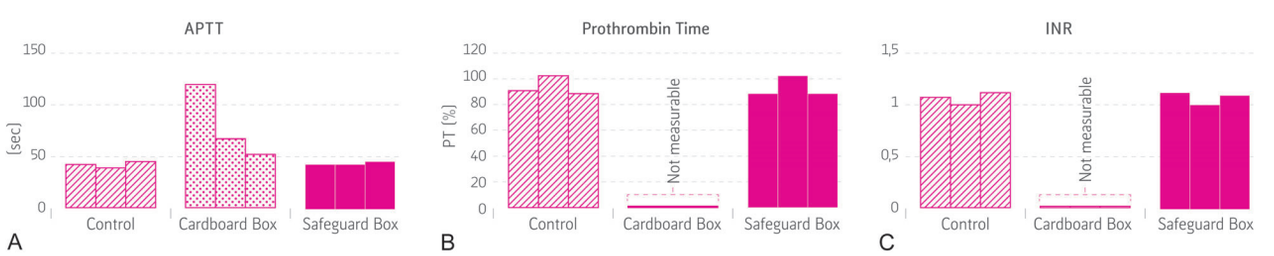

Figure 5: The custom-engineered blood transport system protects samples from heat stress as shown by a study of coagulation parameters. The levels are shown after 24 h within the control box, the heat-stressed cardboard box and the heat-stressed custom-engineered blood transport system. The left column represents blood donor A, the middle donor B and the right column donor C.

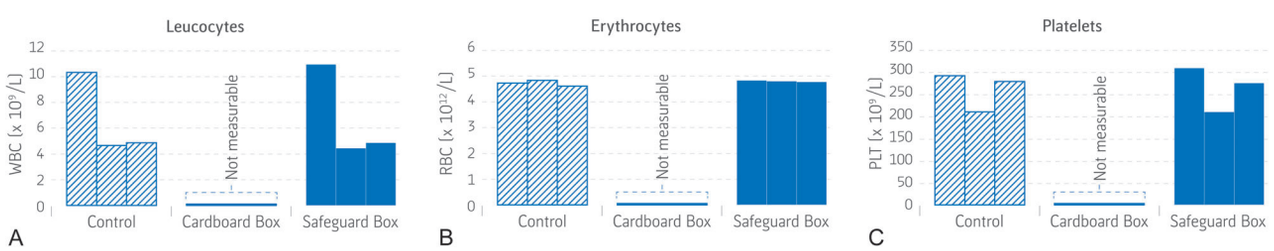

Figure 6: The custom-engineered blood transport system protects samples from cold stress test as shown by a study of hematological parameters. The levels are shown after 24 h within the control box, the cold-stressed cardboard box and the cold-stressed custom-engineered blood transport system. The left column represents blood donor A, the middle donor B and the right column donor C.

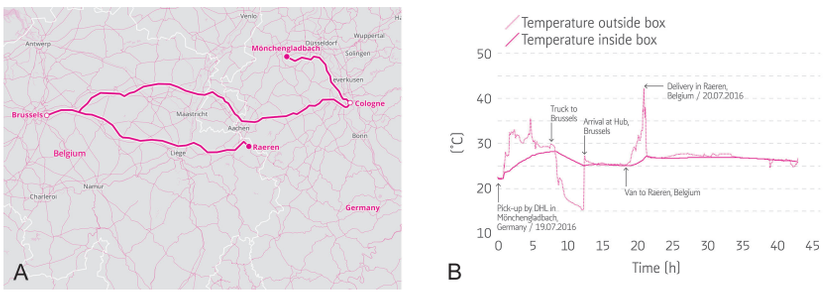

Figure 7: Test of the custom-engineered blood transport system under real-life conditions. The aforementioned box was equipped with a TempTale 4 Recorder (see insert), which recorded the environmental temperature (red graph) and the temperature inside the box (blue graph) while the box was shipped with DHL from MLM headquarters in Mönchengladbach, Germany to Raeren, Belgium (see map) on July 19, 2016. The minimum and maximum environmental temperatures were 15.3 and 42.2 °C, respectively. The maximum temperature inside the box was 28.2 °C, and the minimum temperature was 22.1 °C.

Cold Stress Test

The clinical chemistry parameters determined from serum and the coagulation parameters determined from the plasma were not affected by the cold stress conditions. This was expected because most enzymes and metabolites are not sensitive to a single freeze–thaw cycle, as shown in a series of method validation studies conducted at MLM Medical Labs GmbH (data not shown).

However, based on the results of the differential blood counts, the exposure to sub-zero temperatures and the inability of the cardboard box to protect against these extreme conditions most likely resulted in total cell membrane disruption through the freeze–thaw process. Without exception, the parameters of the differential blood count were not measurable (Fig. 6) in those samples, so relevant information about the subjects would have been lost if these samples were part of a clinical study.

The custom-engineered blood transport system efficiently protected the whole blood samples used for the complete and differential blood counts against cold stress. This is an important contribution for safe transport of blood samples and the interpretation of clinical data.

Real-life Test

The custom-engineered blood transport system was shipped on a summer day with maximum temperatures around 30 °C from Mönchengladbach in Germany to Raeren in Belgium. As shown in Fig. 7, the box was exposed to significant environmental temperature changes: the maximum was 42.2 °C and the minimum was 15.3 °C. Despite these strong temperature fluctuations, the average temperature inside the box remained around 26.3 °C and only minor and gradual changes were seen.

These data confirm that the custom-engineered blood transport system exerts its thermoprotective properties under real-life conditions. Additional test shipments were performed to Lisbon in Portugal and again to Raeren in Belgium using a different courier service. The same outcome occurred (data not shown).

4. Conclusion

The custom-engineered blood transport system clearly outcompetes the simple cardboard box for efficient protection of clinical samples against thermal fluctuations. The temperature profile shows superior performance in hindering content heating and cooling and the parameter measurements substantiate the benefits for clinical sample transport.

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