1 Dream and reality

A new patient with diabetes mellitus is encountered

Mrs Haseltine is a 56-year-old lady who lives in a remote area. She consults her nearby practitioner and reports that over the last 2 weeks she has urinated more frequently than usual. Also, her body weight has decreased, although she "drinks more soft drinks than ever before". The practitioner finds a positive dipstick result for glucose in her urine. Using a glucometer, he measures glucose from fingertip blood obtained by pricking with a fine lancet. The first drop of blood is washed away with a swab of gauze. In the following drop, glucose is measured by the meter, a process that takes about 30 seconds. The result is 280 mg/dL (15.56 mmol/L), far above the upper limit of the normal range. Mrs Haseltine is informed that she may have diabetes mellitus and is referred to a diabetologist the next day.

The right sample for the right test at the right time

The diabetologist confirms the result obtained by the practitioner using a capillary blood sample taken 1 hour after breakfast.

Fig. 1-1

Two blood samples are drawn from the patient the following morning (after she has fasted for 12 hours), from the antecubital vein into closed tubes, one, with a lavender-coloured stopper, containing EDTA, the other, with a green cap, containing heparin. Mrs Haseltine is informed that she has type II diabetes mellitus. She is asked to phone the next day to obtain information on her laboratory results and for further advice.

In the meantime, the heparin blood sample has been centrifuged to separate plasma from the cellular elements. Both tubes are sent to the laboratory by courier in a container especially designed to keep samples at constant temperature. The laboratory receives the samples together with the patient's data and requests for determinations: glycated haemoglobin and blood cell counts from the EDTA blood; potassium and creatinine from the plasma, which has been separated from blood cells, in the closed heparin tube.

The laboratory technician identifies all the samples by comparing the name and bar code number with those on the request sheet. He then enters the request into the lab computer. The samples are put into bar code-reading analyzers for identification and performance of the requested tests. A subsample is taken from the EDTA blood – after slowly mixing it for 3 minutes on a roller mixer – for the determination of haemoglobin A_{1c} by chromatography. The laboratory report, shown in Tab. 1-1, is sent to the diabetologist the next morning.

Mrs Haseltine is taken into the neighbouring county hospital together with a letter from the diabetologist informing the clinician about all tests performed and the results obtained. After 2 weeks of treatment, Mrs Haseltine has learned to control her blood sugar using a small glucometer. No further treatment is needed for the next few years.

Tab. 1-1 Laboratory report
Haseltine, Elsa – July 13, 2008, 10 a.m.

	Units	Patient's result	Reference interval
Haemoglobin A_{1C}	%	6.5	2.9–3.9
Haemoglobin	g/dL	14.4	12–15
Potassium	mmol/L	3.6	3.5–4.5
Creatinine	mg/dL	1.0	Below 1.1

This is what might happen in reality

Mrs Haseltine goes to the practitioner with the same symptoms for the same condition. In contrast to the positive urine dipstick result for glucose, the blood sugar is nearly normal (120 mg/dL). The practitioner, to play it safe, again refers the patient to a diabetologist.

One week later, Mrs Haseltine is called in for a glucose tolerance test. The only advice she is given is to fast the night before the test. Mrs Haseltine wakes up late, however, and misses her morning appointment. She arrives at the doctor's office at noon, having had a snack on the way. She is stressed when the nurse offers her a glucose-containing drink after taking a "fasting" blood specimen.

She feels nauseated while slowly consuming the drink. Whilst waiting for the nurse, she decides not to drink it all and empties the remaining drink down the bathroom sink. Of course, she doesn't report this incident to the nurse when she returns to take a capillary blood sample at 1 and 2 hours after the first sample.

When the results are shown to the doctor (Tab. 1-2), he realizes that the glucose concentrations after the first and second hour are not that much different. The diabetologist, unable to arrive at a diagnosis, asks the patient to report the following day at which time two venous blood samples are collected, one with a lavender-coloured stopper

Fig. 1-2

Tab. 1-2 Results in doctor's office: glucose tolerance test
Haseltine, Elsa – July 12, 2008, 2 p.m.

Fasting	glucose 160 mg/dL
1-Hour	glucose 110 mg/dL
2-Hour	glucose 120 mg/dL

and the other with a green cap. The tubes are sent to a private laboratory by car. Next day, the results shown in Tab. 1-3 are received by E-mail together with the reference values for each test. The glucose value is now normal, potassium elevated and haemoglobin A_{1c}, an indicator of mean blood glucose, elevated to diabetic levels. The diabetologist, concerned by the high potassium level, refers the patient to a clinic. This institution diagnoses that the patient has type II diabetes mellitus, based on their laboratory results.

Tab. 1-3 Report from private laboratory
Haseltine, Elsa – July 13,2008, 3 p.m.

	Units	Patient's result	Reference range
Haemaglobin A_{1c}	%	6.5	2.9–3.9
Haemoglobin	g/dL	13.5	12–15
Potassium	mmol/L	5.8	3.5–4.5
Creatinine	mg/dL	1.0	Below 1.1
Glucose	mg/dL	105	70–110

Fig. 1-3

What happened to Mrs Haseltine's samples?

Undoubtedly, Mrs. Haseltine was in a diabetic state. Why was the fasting blood sugar nearly normal?

Answer: Fasting may result in near-normal values in type II diabetics. In this case, the nurse took the first drop of blood from a fingerprick after "milking" the finger to obtain sufficient blood.

Why was the result of the glucose tolerance test inconclusive?

Answer: The first result was related to patient stress, which leads to increased amounts of glucose being released from liver glycogen stores. Moreover, Mrs Haseltine had a snack on her way to the doctor because she was hungry.

She did not report this to the doctor or the nurse, because she wasn't aware of the possible influence of this snack. For the same reason, she did not report not consuming all of the glucose drink, which had led to a decrease rather than an increase of blood glucose after 1 hour. The "increase" at the second hour may have been due either to method variation or to a reactive increase brought about by metabolic reactions in the late afternoon. Normally, a glucose tolerance test is performed in the morning, the reference values being valid only for the morning. It should be carried out under standard conditions, as recommended by national and international expert panels.

Why was potassium elevated and glucose normal in the venous specimen?

Answer: The sample was transported in contact with the cells for over 2 hours in a non-air-conditioned car on a hot day. This caused the blood cells to metabolize glucose and release potassium, the concentration of which is approximately 40 times higher in cells than in plasma. This *in vitro* influence makes unstabilized blood unsuitable for glucose determination. Potassium can be reliably measured only if plasma is promptly separated from the cells.

All these errors could have been prevented had the preanalytical phase been strictly controlled. Mrs Haseltine would have been diagnosed earlier with less stress and fewer costs would have been incurred.

This book is intended to increase awareness of the importance of all steps of the preanalytical phase, including patient preparation, sampling, transport and storage of patient samples.

In each chapter, possible preanalytical variables are explained with regard to mechanisms, effects and preventive actions intended to prevent misinterpretation of laboratory results. In the respective chapters, warnings are given in red and recommendations in green. Like disease mechanisms, biological influences can change the concentration of measured analytes *in vivo*, whereas *in vitro* changes have to be separated into changes undergone by the measured analyte and interference of the method used to measure the analyte. These definitions are important, because only the latter can be avoided by using a

more specific method. The interested reader is referred to the References (p. 90) as well as the Glossary which defines all the special terms used in this book (p. 102). Detailed information on preanalytical variables of all analytes together with the recommendations on the choice of anticoagulant, the optimal sample volume and the stability of analytes in sample matrix is referred to the website www.diagnosticsample.com (112). It is to be hoped that the new edition helps to improve sample quality and decrease preanalytical errors (25, 171).

Optimal treatment of the patient and their samples is defined as the gold standard