Use of instant glucose measurements for detection of acute hypoglycaemia in rats

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INTRODUCTION
Direct glucose measurement in a small sample of whole blood provides clear benefits in studies where the risk of hypoglycaemia is high (e.g. safety tests using insulin), so that any necessary immediate actions can be taken. This is particularly relevant in rats, as clinical signs of hypoglycaemia appear at very low glucose levels and are not easily distinguishable from clinical signs of other conditions.

One Touch Ultra glucometers (referred to as glucometer, LifeScan, Johnson & Johnson) are used successfully in humans, but experience for use in animals is rather limited. We have earlier (Ref. 1) validated the use of the glucometer for early detection of hypoglycaemia in dogs. The present study addresses instant detection/confirmation of hypoglycaemia in rats.

The objectives of this study were:
(1) to validate the glucometer for detection/confirmation of hypoglycaemia in rats;
(2) to define the conversion equations for re-calculation of glucose measurements (rat) in whole blood or serum to plasma values (as required for reporting (Ref.2)).

MATERIALS AND METHODS
Animals and treatment. The study was performed in 20 male and 10 female SPF Sprague Dawley rats from Taconic Europe A/S, Ejby, Denmark. In order to reliably validate the method across a broad range of glucose levels, blood sampling was performed in normoglycaemic animals and hypoglycaemic animals (treated with human insulin (Actrapid®, Novo Nordisk A/S)). The range of measured plasma glucose levels in the study was 1.1-11.6 mmol/L.

Blood sampling. Whole blood glucose measurements were performed on blood collected from the tail vein (tail) and sublingually (tongue), while serum and plasma glucose measurements were performed on blood collected only sublingually. In Phase I (10 male and 10 female rats); at each blood sampling, two measurements using the glucometer (blood drop from the tail vein), and blood drop of sublingual blood taken with a pipette from the plain tube for serum (see below) immediately after collection) were compared with measurements of glucose in plasma and serum (using Hitachi 917). In Phase 2 (10 male rats), whole blood for glucometer measurements was taken both from the tail vein and sublingually. Blood from the tail was measured both directly and immediately after collection of a small volume of blood (approx. 50 μL) in the plain tube, while sublingual blood was measured only from the sample collected in the plain tube, that was further used to obtain the serum sample. For glucometer measurements, only one drop of full blood was required, and the sample was measured instantly.

Plasma and serum (required blood sample volume 0.3 ml) glucose analysis was performed using a Hitachi 917 clinical chemistry analyser.

RESULTS
Comparison of glucose measurements in whole blood and in plasma/serum
There was a good linear correlation between glucose measurements performed in (sublingual) plasma and serum (Figure 1A, r2=0.96) but, as expected, the values were identical. Thus, care has to be taken to use the correct conversion equation (for serum or for plasma, study specific) when comparing measurements using glucometer with the other data (e.g. measurements performed using Hitachi 917). Glucose levels measured in whole blood samples collected sublingually showed a good correlation to glucose levels measured in (sublingual) plasma and serum (Figure 1B-C, R2=0.85 (plasma) and R2=0.84 (serum)), while for the 20 whole blood samples collected from the tail vein the correlation was rather poor (Figure 1B, B-C, R2=0.51 (plasma) and R2=0.47 (serum)).

Clarification of the discrepancy of the whole blood glucose measurements in blood collected at different sites
The difference between the results obtained in blood collected from different sites was unexpected. Therefore, the contribution of the method of blood sampling to the observed inaccuracy of measurements was evaluated. Measurements performed «directly» from the tail were compared to the measurements after collection in the tube (as with sublingual sampling). The obtained results do not suggest any impact of method on the variation observed - both with direct and indirect blood sampling, variation was high for the samples collected from the tail (Figure 2).

LIMITS OF DETECTION FOR ONE TOUCH ULTRA IN RATS
According to the manufacturer, One Touch Ultra can detect human blood glucose levels in the range of 1.1-33.3 mmol/L. The upper limit of detection in rats was outside the scope of the present study. Using the corresponding equation (Figure 1A, B-C), the lower limit of detection of glucometer (1.1 mmol/L) was found to correspond to rat plasma glucose levels of 2.6 mmol/L and to rat serum glucose levels of 2.5 mmol/L.

The suggested interpretation of the values obtained by the glucometer is given in Table 1 below. The low limit of detection of the glucometer is regarded as sufficient for identification of a hypoglycaemic state requiring intervention in rats.

DISCUSSION
The present study demonstrated that the glucometer can be used to monitor rat blood glucose levels using sublingual whole blood. Specific formulae have to be used for conversion of values obtained using the glucometer to the corresponding glucose values in plasma and serum. The precision of evaluation of the hypoglycaemic status in rats appears to be somewhat lower than in dogs (in dogs, the values were quantifiable down to 2.1 mmol/L), while in rats it was only down to 2.6 mmol/L), but was still regarded as sufficient for animal health status evaluation purposes.

The possible reason for the discrepancy in the results and poor correlation for the blood collected from the tail and plasma/serum (collected sublingually) may be physiological (difference in blood flow in the tail compared to the whole body), further enhanced by rapid changes in glucose levels induced by treatment with short-acting insulin.

CONCLUSION
(1) The One Touch Ultra glucometer can be used for monitoring of glucose status in rats resulting in less stress for the animals, smaller blood sampling volume, and faster initiation of corrective actions for counteraction of hypoglycaemia.
(2) Measurements of sublingual blood provide more reliable results than measurements of blood collected from the tail vein.