GLUCOSE MEASUREMENT

Why sample type matters

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The sample
Glucose concentration may be measured using a whole blood sample, a plasma sample obtained after centrifugation of anticoagulated whole blood, or a serum sample obtained after centrifugation of non-anticoagulated whole blood. The necessity of centrifugation determines that in general, plasma and serum samples are only used in the traditional laboratory environment.

Outside the traditional laboratory environment whole blood is used. In the intensive care unit and emergency room this may be arterial or venous blood collected via an indwelling catheter or puncture of a vein or an artery, whereas in all other point of care settings it is more often capillary whole blood collected by finger, earlobe or heel prick.

When, as is often the case, the term blood glucose concentration is used without qualification of sample type, an erroneous assumption is implied that sample type does not affect glucose concentration. In fact, for accurate interpretation of glucose results, it is important that the sample type be known.

The most significant systematic difference is between whole blood glucose concentration and plasma or serum glucose concentration.

Difference between whole blood and plasma/serum glucose concentration
To understand this difference, which can be up to 10 – 15%, it is necessary to look in a little more detail at the way glucose in blood is distributed between plasma and the cytoplasm of blood cells. Because they are quantitatively by far the most significant, it is only the red blood cells (erythrocytes) that are important for this discussion.

The red cell membrane is effectively freely permeable to glucose, so that glucose passes from plasma to red cell cytoplasm, as well as in the reverse direction, maintaining equal concentrations on either side of the membrane. But the measured glucose is present only in the aqueous (water) phase of both plasma and red cell cytoplasm, and crucially for this discussion, the water content of plasma is significantly greater than the water content of red cells. Although glucose concentration in plasma water is the same as that in red cell water, the different water contents (presence of solid matter) determine that glucose concentration of plasma and red cells are unequal. The magnitude of this inequality can be defined by the percentage difference in water content. The solid phase of plasma (principally lipids and proteins) occupies around 7% of plasma volume, so that the water content of plasma is approximately 93%. In contrast, the solid phase of red cells, due almost entirely to haemoglobin, occupies around 29% of red cell cytoplasm volume and thus the water content of red cells is approximately 71%.

Since glucose concentrations in plasma water and red cell water are the same, but water cell contents differ by (93 - 71/93) x 100 i.e. 23.6%, it follows that the glucose concentration of plasma and red cell also differs by 23.6% (plasma greater than red cell).

Whole blood glucose concentration is determined by three parameters:
- Plasma glucose concentration
- Red cell glucose concentration (approximately 23.6% less than plasma concentration)
- Percentage of whole blood volume that is occupied by red cells (haematocrit)

Thus, if plasma glucose concentration is 10.0 mmol/L, then red cell glucose concentration is 23.6% less (10.0 - 2.36) i.e. 7.6 mmol/L and whole blood glucose is (0.45 x 7.6) + (0.55 x 10.0) i.e. 8.9 mmol/L.

Table 1 describes how haematocrit affects whole blood glucose concentration and therefore the percentage difference in glucose concentration of plasma and whole blood. If haematocrit is at the midpoint of the reference range (i.e. 45%), the theoretical difference is 11%, but less if haematocrit is reduced and more if haematocrit is raised. Importantly, it should be noted that unlike whole blood glucose concentration, plasma glucose concentration is unaffected by haematocrit.

Table 1: Effect of haematocrit (Hct) on whole blood glucose concentration

<table>
<thead>
<tr>
<th>PLAASMA GLUCOSE (mmol/L)</th>
<th>RED CELL GLUCOSE (mmol/L)</th>
<th>WHOLE BLOOD GLUCOSE (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>7.6</td>
<td>9.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.92</td>
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<td></td>
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<td>8.50</td>
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</table>

<table>
<thead>
<tr>
<th>% difference between plasma and whole blood glucose concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
</tr>
<tr>
<td>10.8</td>
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<tr>
<td>14.4</td>
</tr>
</tbody>
</table>
Difference between venous plasma and venous serum glucose concentration

The glucose concentration of venous plasma and venous serum should theoretically be the same. Any difference is an artifact due to sample handling; specifically delay in separating serum or plasma from cells. Delay causes lowering of glucose concentration at an estimated rate of 5 – 7% per hour, due to continued metabolism of glucose (glycolysis) by blood cells.

This in vitro glycolysis is prevented or at least minimised in the case of plasma by the addition of sodium fluoride to the anticoagulant present in blood collection tubes. If serum glucose is to be measured, the blood sample must be centrifuged and serum analysed within an hour of collection, in which case the result should theoretically be the same as that from an equivalent plasma sample derived from anticoagulated blood containing a glucose preservative such as sodium fluoride.

However, a recent study demonstrated a slight (4.7%) negative bias in plasma glucose from blood collected into tubes containing sodium fluoride compared with serum glucose when serum was separated from cells very soon (within 15 minutes) after collection. This confirms that sodium fluoride cannot be relied upon to completely eliminate in vitro glycolysis, particularly during the first hour after blood collection.

Figure 1 describes the theoretical relationships of glucose concentration between the various sample types. Note that the difference between capillary blood glucose and venous sample types depends on the time since last meal. Arterial blood glucose approximates closely to capillary blood glucose.

Venous plasma – the reference sample

Of all the clinical contexts in which glucose is measured, diagnosis of diabetes is the one that demands greatest accuracy and precision. Because plasma and serum glucose concentration are unaffected by the confounding effect of haematocrit, they are a more accurate measure of circulating glucose than whole blood glucose concentration. Compared with serum glucose, plasma glucose is considered less likely, in the context of routine clinical practice, to be affected by the potential inaccuracy associated with in vitro glycolysis. Venous blood is more convenient and safer to collect than arterial blood, and is less prone to sampling error than capillary blood sampling.

Thus plasma, specifically venous plasma, is the sample that affords greatest accuracy and convenience, and the one recommended for diagnosis of diabetes.

The consensus that plasma be regarded as the reference sample against which all other sample types should be compared has led to the recommendation that if whole blood glucose concentration is measured, results should be converted to "equivalent plasma values" using a constant factor of 1.11.

Plasma equivalent glucose (mmol/L) = whole blood glucose (mmol/L) x 1.11

This factor is the theoretical ratio of plasma glucose concentration to whole blood glucose concentration derived from blood with normal haematocrit (43%) and assumes, as in the discussion above, that water occupies 93% of plasma volume and 71% of red cell volume.

Adoption of this policy, which in essence means reporting all glucose results as plasma glucose concentration, allows greater harmonisation between central laboratory results and those obtained at the point of care. The potential for errors in interpretation of glucose results, due to confusion or ignorance about the difference between whole blood and plasma glucose concentration, is much reduced. However, it must be remembered that plasma “equivalent” values are as dependent on haematocrit as whole blood glucose concentration. If haematocrit does not deviate from normal then plasma equivalent glucose concentration is theoretically the same as measured plasma glucose concentration.

A decrease in haematocrit causes an increase in plasma equivalent glucose concentration (relative to measured plasma glucose concentration) and vice versa. The magnitude of the increase and decrease is theoretically the same as that for the effect of haematocrit on whole blood glucose (see Table 1).

References