More on Monoclonal Gammopathies

To the Editor:

We would like to add our support to the basic approach recommended by Kahn (Clin Chem 1989;35:508–8) for the detection and typing of monoclonal gammopathies in serum. We have the additional advantage of quantification of the proteins most likely to be confusing in the interpretation of high-resolution agarose gel electrophoresis: transferrin, C3, C-reactive protein, and apolipoprotein B (as an indicator of beta lipoprotein). Genetic variants of the first two of these can cause difficulties in interpretation, as can the unexpected appearance of fibrinogen in what was thought to be a serum sample. We therefore add immunofixation (IFE) for these proteins when questionable bands are present in their normal regions of migration.

We agree with Kahn that quantification of light chains is a relatively insensitive method of screening and typing, except in the presence of significant M-components, very low concentrations of polyclonal antibodies, or both. We would like to add two comments about “screening IFE.” First, we have found that antisera to the Fab portion of IgG is better than separate antisera to kappa and lambda chains; most of the latter are of relatively low titer and affinity, partly because of extensive ab/adsorption. Second, we believe that samples from patients with a clinical diagnosis of questionable multiple myeloma or macroglobulinemia should have IFE screening regardless of the electrophoretic pattern and the concentrations of immunoglobulins present.

Finally, when kappa or lambda light chains, but no heavy chains, are detected by IFE, it is important that IFE be repeated with antisera to IgD (\(\delta\) chains) and IgE (e chains).

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Influence of Blood Oxygen Tension on Dipstick Glucose Determinations

To the Editor:

The determination of glucose by enzymic oxidation with glucose oxidase (EC 1.1.3.4) consumes stoichiometric amounts of molecular oxygen:

\[
\beta-D\text{-Glucose} + H_2O + O_2 \rightarrow \text{D-gluconic acid} + H_2O_2
\]

Aqueous glucose reagents used in traditional “wet-chemistry” methods provide adequate amounts of dissolved oxygen. I examined three dry film (dipstick) glucose methods to see whether the oxygen tension of blood influenced the measured glucose concentration.

The three systems examined were BM1-44 Glycaemie/Reflolux II (Boehringer Corporation Ltd., Lewes, Sussex, U.K.), One Touch (LifeScan Inc., Mountain View, CA), and ExacTech (Baxter Health Care Ltd., Egham, Surrey, U.K.). BM1-44 and One Touch are colorimetric methods, the latter utilizing an automatic sample sensor. The ExacTech pen-sized meter is the first small meter to use electrochemical detection (1).

Heparinized whole blood containing glucose concentrations between 3.2 and 17.3 mmol/L was equilibrated with a nitrogen/oxygen mixture at different partial pressures. The oxygen tension of the equilibrated whole blood was determined in an ABL2 blood gas analyzer (VA Howe and Co., Ltd., London, U.K.), immediately followed by measurement of glucose on each glucose meter. A fluoride/oxalate-preserved sample was taken simultaneously for determination in a YSI glucose analyzer (Clandon Scientific Ltd., Aldershot, Hampshire, U.K.).

The glucose meter measurements, expressed as the percentage difference from the laboratory YSI measurement, demonstrated sensitivity to oxygen tension in the ExacTech but not in the two colorimetric systems (Table 1). Contrary to what would be predicted by the above equation, measured glucose concentration increased as oxygen tension decreased. The percentage difference was not influenced by glucose concentration at the levels studied, and it varied between 0% at an oxygen tension of 40 kPa and 80% at 0.5 kPa (Figure 1).

The apparently paradoxical response to oxygen tension may relate to competition between two oxidative pathways. The reduced form of glucose oxidase can either return to its former oxidized state by electron transfer to oxygen or through a suitable mediator (ferroene in the case of the ExacTech) to the measurement electrode. At very low oxygen tension with the ExacTech, only the latter pathway is available and therefore the result displayed is a falsely increased glucose concentration.

Because the interference is greatest at very low oxygen tension, the instrument is unsuitable for measurement of glucose in patients who are severely anoxic or whose oxygen tension is unstable during treatment of respiratory distress or failure. Venous blood has a lower oxygen tension than does capillary blood, the oxygen content of which depends greatly upon sampling technique (2). These samples are clearly not interchangeable, and higher measurements of glucose concentrations can be expected in venous blood.

Table 1. Effect of Blood Oxygen Tension on Dipstick Glucose Measurement

<table>
<thead>
<tr>
<th>(P_{O_2}) kPa</th>
<th>YSI</th>
<th>One Touch</th>
<th>ExacTech</th>
<th>Reflolux II</th>
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<tr>
<td>0.5</td>
<td>10.2</td>
<td>10.0 (-1)*</td>
<td>17.1 (69)</td>
<td>14.1 (39)</td>
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<td>0.9</td>
<td>13.1</td>
<td>12.6 (-4)</td>
<td>22.9 (74)</td>
<td>17.4 (33)</td>
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<tr>
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<td>9.8</td>
<td>9.4 (-4)</td>
<td>15.8 (62)</td>
<td>13.5 (38)</td>
</tr>
<tr>
<td>1.8</td>
<td>3.7</td>
<td>4.7 (27)</td>
<td>5.8 (57)</td>
<td>5.7 (54)</td>
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<tr>
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<td>13.5</td>
<td>12.7 (-6)</td>
<td>19.2 (43)</td>
<td>16.4 (22)</td>
</tr>
<tr>
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<td>12.9</td>
<td>12.9 (0)</td>
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<td>15.8 (22)</td>
</tr>
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<td>11.6</td>
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<tr>
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<td>12.7 (30)</td>
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<tr>
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<td>3.3 (-10)</td>
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<td>16.9 (41)</td>
<td>16.2 (35)</td>
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<tr>
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<td>14.2 (34)</td>
</tr>
<tr>
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<td>17.3</td>
<td>14.7 (-15)</td>
<td>17.9 (3)</td>
<td>18.5 (7)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the % difference from the YSI values.
blood. Oxygen tension is a further variable to consider when assessing the performance of blood glucose meters, and when selecting material for their quality control, particularly when the meter uses electrochemical detection.

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References

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Two spokespersons for the manufacturer of the ExacTech respond:

To the Editor:

The ExacTech instrument in Mr. Halloran’s study has been incorrectly used after calibration. Each pack of ExacTech blood glucose test strips contains a calibrator, which enables the system to correctly determine the glucose content of fresh capillary blood.

The method of detection in the ExacTech system is based on the dynamic examination of the catalytic process as it occurs; unlike the other devices in Mr. Halloran’s study, it is not an endpoint method. Mr. Halloran used old venous blood that had been treated with heparin and subjected to extensive gas manipulation/equilibration in a tube, which might introduce nonphysiological rheological properties.

In addition we are concerned about the physiological credence of his study; most standard clinical chemistry textbooks confirm that, at arterial blood PO2 values <2.7 kPa, death occurs. Surely testing values below this is purely academic. It is well known that fluctuations in PO2, capillary blood, are modest. In practice, values seldom decrease below 8.5 kPa or increase above 13.5 kPa. If we apply generous limits of 2 kPa and 20 kPa and use Mr. Halloran’s own data, then a completely different picture emerges (Figure 1). The ExacTech system, despite the nonphysiological samples, performs most consistently and is clearly reproducible, showing no trend over this range. The Lifespan One Touch and B.M. Refilux machines show scatter and inconsistency. It is likely that the overall bias observed in this study with ExacTech reflects inappropriate sample handling, likely to induce extreme rheological change.

The ExacTech system is fully capable of delivering excellent results across a wide range of (physiological) PO2 values likely to be encountered in capillary blood.

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Alcohol Detection By Microdiffusion

To the Editor:

A unique delivery system was developed to detect ethanol in urine and serum. In the presence of acid, chromium trioxide (CrO3) is reduced to chromic oxide, Cr2O3 (1). When a reducing volatile such as ethanol is absorbed in an acid dichromate solution, the reaction produces a blue color, the intensity of which is proportional to the concentration of ethanol and to time (2, 3):

\[
3C_2H_5OH + 2K_2CrO_7 + 8H_2SO_4 \rightarrow \quad \text{(yellow)}
\]

\[
3CH_3COOH + 2K_2SO_4 + 2Cr_2(SO_4)_3 + 11H_2O
\]

The procedure for this system calls for the dropwise application of chromic acid reagent onto a sheet of glass microfiber paper held in position with a two-piece acrylic spotting guide (Figure 1). This assembly is placed above disposable concentration cups containing 1 mL of a specimen of urine, blood, or saliva. Filled disposable concentration cups are located in a heating block (OMEGA-12 Extraction Solvent Concentrator) at a temperature of 80 to 120 °C. Under these conditions the chromic acid is reduced by ethanol-positive specimens within 5 min. This reduction is made visible by a distinct change from yellow to blue at the reaction sites on the glass fiber sheet (Figure 2). Ethanol concentrations as low as 100 mg/L can be detected with this method. Negative samples remain yellow. A 200 mg/L ethanol standard can be used as a comparative reference. Alternatively, a series of ethanol standards can be used for semiquantitative results.

The disposable concentration cups used with this method offer convenience and eliminate sources of contamination. Specimen handling capacity is flexible with this method, allowing for the design of test configurations such that 24 specimens can be processed in 10 min. This method is