the hybridoma technique, combined with the low intensities of centrifugation.

Giemsa stained smears of a patient's CSF that had this artefact showed only lymphocytes without neutrophils, indicating that it was the lymphocyte from which this artefact arose.

Our results suggest that if sufficient numbers of cryptococci are present on a Gram stained smear, centrifugation should be avoided before microscopy with the India ink technique. If centrifugation is to be used, caution is warranted in interpreting such artefacts as cryptococcus, especially when the capsular antigen detection test and culture give negative results.

We thank Dr J S M Peiris for comments on centrifugation in the hybridoma and shell-vial culture techniques.

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# Measurement of haemoglobin using single drops of skin puncture blood: is precision acceptable?

A M Conway, R F Hinchliffe, J Earland, L M Anderson

## Abstract

**The study aimed to investigate local concerns about clinically important discrepancies between repeat HemoCue haemoglobin measurements from single drops of blood. Two biomedical scientists and two health visitors each obtained a series of paired haemoglobin values by fingerprick sampling from healthy volunteers. Seven of 20 paired values obtained by health visitors and three of 20 obtained by scientists from the first drop of blood forming at the puncture site differed by ≥ 10 g/l; 11 of 20 paired values obtained by health visitors and one of 20 by the scientists from the fourth drop of blood differed by ≥ 10 g/l. After collecting and mixing a number of drops in EDTA tubes before analysis, seven of 40 paired values differed by > 5 g/l, and none by > 10 g/l. Pooling drops of blood before analysis improves precision of HemoCue haemoglobin measurement and allows users to achieve results comparable to those obtained by experienced laboratory staff. Measurement of haemoglobin from single drops of skin puncture blood should be discontinued.**

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Keywords: haemoglobin measurement; skin puncture blood sampling

**Sub-department of Paediatric Haematology, The University of Sheffield Department of Paediatrics, The Sheffield Children's Hospital NHS Trust, Western Bank, Sheffield S10 2TH, UK**
A M Conway
R F Hinchliffe
L M Anderson

**Centre for Human Nutrition, The University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU, UK**
J Earland

Correspondence to:
Mrs Conway.

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The HemoCue portable haemoglobinometer (HemoCue Ltd, Sheffield, UK) has been available commercially for some years and has been found to be reliable and to give precise and accurate results.[1 2] Nevertheless, concerns exist among local general practitioners about clinically important differences sometimes found between repeat HemoCue measurements made on single drops of skin puncture (capillary) blood. This study was performed to investigate the problem.

## Subjects and methods

Two biomedical scientists and two health visitors took blood from four groups of 10 healthy adult volunteers. The health visitors had experience of using HemoCue in their daily work, and the biomedical scientists had considerable experience of skin puncture blood collection. All had received training by staff of HemoCue Ltd in the proper use of the instrument. Three samples were collected from one standard lancet puncture to the middle finger of each hand, producing three paired haemoglobin measurements from each volunteer. Blood from the first and fourth drops forming at the site was collected into HemoCue cuvettes, that from the second and third drops were wiped away with a sterile swab. About 20 drops were then taken into a miniaturised EDTA tube (Teklab Ltd, Co Durham, UK). After mixing, the haemoglobin of this sample was measured using both HemoCue and a Bayer H1 automated blood counter (Bayer plc, Newbury, Berks, UK). Venous blood from both arms of a further 10 volunteers was collected by one biomedical scientist and haemoglobin measured using both instruments. Coefficients of variation obtained by the health visitors and scientists on each sample type were determined, as was the mean and the 95% confidence intervals of the differences between duplicate measurements. Differences between paired values were assessed using the Wilcoxon signed rank test.

## Results

Figure 1 shows the differences between paired values obtained with HemoCue; the results are summarised in table 1. Seven of 20 paired values obtained by health visitors and three of 20 samples obtained by the biomedical scientists from the first drop of blood differed by ≥ 10 g/l. Eleven of 20 paired values obtained by health visitors and one of 20 obtained by the biomedical scientists from the fourth drop

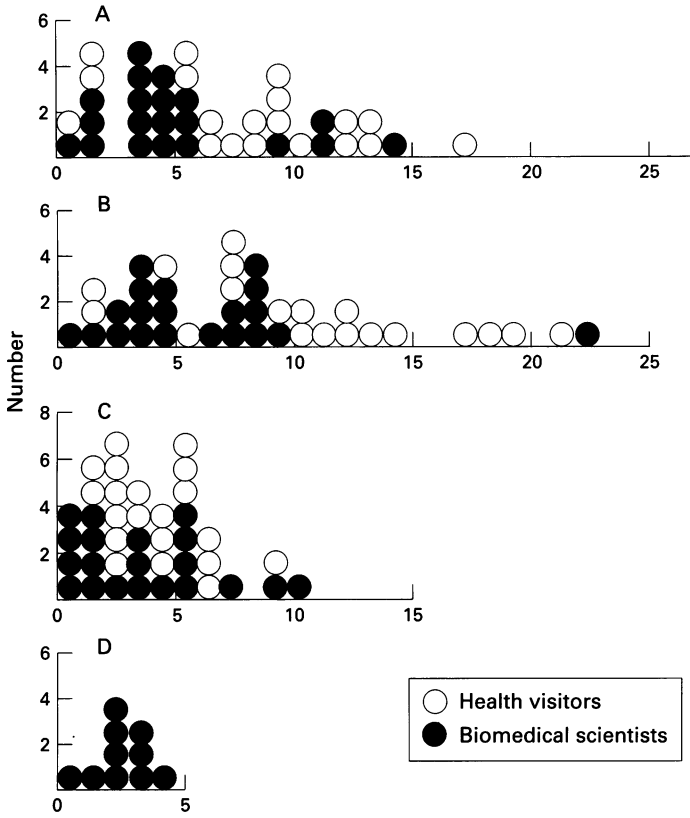


*Figure 1 Difference between paired HemoCue haemoglobin measurements (g/l). (A) First drop of skin puncture blood. (B) Fourth drop of skin puncture blood. (C) Pooled drops of skin puncture blood. (D) Venous blood.*

of blood differed by ≥ 10 g/l. No paired values from the pooled blood differed by > 10 g/l; four obtained by health visitors and three by biomedical scientists differed by > 5 g/l. Differences between paired values obtained by the biomedical scientists were significantly lower than those of the health visitors for both the first and fourth drops of blood ($p < 0.01$). Differences between paired values obtained by the health visitors on pooled drops using HemoCue were significantly lower than those they obtained on both the first and fourth drops ($p < 0.001$). The precision of HemoCue compared well with that of the automated analyser on both the larger volume skin puncture and venous samples. Haemoglobin values obtained ranged from 108–172 g/l: mean 137.5 g/l, HemoCue, first drop; 137.8 g/l, HemoCue, fourth drop; 141.7 g/l HemoCue, pooled drops; and 137.2 g/l, Bayer H1, pooled drops.

## Discussion

This brief study confirms a high incidence of disturbingly wide variation between duplicate HemoCue measurements made on single drops of blood collected directly from the skin puncture site. Most of the discrepant results were obtained by the health visitors, despite their being among the most experienced local users of HemoCue. These occurred despite our use of a homogeneous group of healthy and willing volunteers to rule out, as far as possible, the problems sometimes associated with skin puncture sampling in a patient population, such as poor peripheral circulation or lack of cooperation. The health visitors tended to work slowly, sometimes allowing a large drop of blood to form before filling the cuvette. On several occasions there was almost instantaneous sedimentation of red cells in such drops, and sampling from this non-homogeneous source probably caused some discrepancies.

A mean difference between duplicate skin puncture haemoglobin measurements of 5 g/l, with 10% of paired values differing by ≥ 10g/l, has previously been reported.[3] These results are almost identical to those obtained with single drops of blood by the biomedical scientists in the present study. Although details of sample collection were not given in the earlier report, the similarity leads us to suggest that these figures are the best precision obtainable with this type of sample. In a patient population this degree of precision may be unobtainable.

Mills and Meadows[4] showed improved precision of haemoglobin measurement using HemoCue is possible by pooling and mixing drops of skin puncture blood before analysis. Despite this report, the manufacturer continues to recommend the use of a single drop of blood for analysis and, based on its own studies, suggests the fourth drop forming at the puncture site provides the most representative sample. Our results do not support this contention, but confirm the previous report and extend it to show the precision of haemoglobin measurement in pooled drops of skin puncture blood approaches that obtainable with venous blood under ideal conditions—that is, where samples are collected by an experienced phlebotomist from healthy volunteers with good veins. Furthermore, this improved precision was obtained by both biomedical scientists and health visitors, despite the latter

*Table 1 Coefficients of variation, mean differences between duplicate haemoglobin measurements, and 95% confidence intervals of the differences obtained by both groups of workers on each type of blood sample*

| | | *Biomedical scientists* | | | | *Health visitors* | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Sample* | *Instrument* | *No of paired values* | *CV (%)* | *Mean difference between duplicate samples (g/l)* | *95% CI* | *No of paired values* | *CV (%)* | *Mean difference between duplicate samples (g/l)* | *95% CI* |
| Skin puncture blood | | | | | | | | | |
| First drop | HemoCue | 20 | 2.9 | 4.7 | 3.0 to 6.4 | 20 | 5.4 | 8.9 | 6.1 to 11.7 |
| Fourth drop | HemoCue | 20 | 3.6 | 5.7 | 3.6 to 7.8 | 20 | 6.3 | 10.5 | 7.8 to 13.2 |
| Pooled drops | HemoCue | 20 | 2.1 | 3.2 | 1.9 to 4.5 | 20 | 2.1 | 3.3 | 2.1 to 4.5 |
| Pooled drops | Bayer H1 | 20 | 1.2 | 1.9 | 1.3 to 2.5 | 20 | 1.8 | 2.7 | 1.7 to 3.7 |
| Venous blood | HemoCue | 10 | 1.2 | 2.2 | 1.5 to 2.9 | | | | |
| Venous blood | Bayer H1 | 10 | 1.5 | 2.5 | 1.5 to 3.5 | | | | |

never having used this method of collection before.

This study has confirmed the precision of haemoglobin measurement using HemoCue, and shown that skin puncture blood samples can be simply and reliably used with this instrument with little training, provided a number of drops are anticoagulated and mixed before analysis. In contrast, analysis of single drops of blood, even by experienced personnel, can give rise to misleading results. This approach to haemoglobin measurement should be discontinued.

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# Unacceptably high site variability in postmortem blood alcohol analysis

P A Sylvester, N A C S Wong, B F Warren, D L Ranson

**Abstract**

**Blood alcohol concentration is a frequently requested test in forensic pathology. The variability of this value was studied by measuring the blood alcohol concentration from six sites in nine subjects at necropsy in whom alcohol was the implicated cause of death. There were small consistent differences in the blood alcohol concentrations between the sites in the nine subjects ($p < 0.04$). Calculation of the mean blood:vitreous humour alcohol concentration ratio (B:V ratio) showed that vitreous humour alcohol concentration most closely reflected the concentration at the femoral vein (B:V ratio = 0.94, $r = 0.98$), which is considered the optimal site for blood alcohol measurement. The correlation of left heart blood with femoral blood was lower compared with the other sites. There is a potential for an unacceptably large variation in the postmortem measurement of blood alcohol within each subject.**

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Keywords: postmortem examination; blood alcohol concentration; vitreous humour

**University Department of Surgery, University of Bristol, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, UK**
P A Sylvester

**Department of Pathology and Microbiology, University of Bristol, Bristol Royal Infirmary**
N A C S Wong

**Department of Cellular Pathology, John Radcliffe Hospital, Headley Way, Headington, Oxford OX3 9DU, UK**
B F Warren

**Department of Forensic Medicine, Monash University (Victorian Institute of Forensic Medicine), 57–83 Kavanagh Street, Melbourne, Victoria, Australia 3006**
D L Ranson

Correspondence to:
Dr Sylvester.

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The measurement of postmortem blood alcohol concentration is one of the most commonly requested tests in forensic pathology. The importance of this measurement is clear as it is used as evidence in court to determine the part played by alcohol in both criminal and civil trials. Great care must be taken in the sampling and storage of postmortem blood used to measure alcohol concentration to avoid artefactual changes from—for example, putrefaction.[1] Despite such considerations, variability in blood alcohol concentration has been found between different sites of the same cadaveric specimen.[2 3] One explanation for this is postmortem diffusion of alcohol from the gastric lumen into neighbouring blood vessels and viscera.[3 4] The principal aim of this study was to determine the degree of this variability by measuring blood alcohol concentrations from five separate vascular sites in nine cadaveric subjects. We also studied whether this variability had any bearing on the use of vitreous humour alcohol measurement to estimate blood alcohol concentration.

## Methods

Blood samples were collected from nine consecutive subjects in whom alcohol was implicated in the cause of death. All subjects had been involved in road traffic accidents with minimal delay in refrigeration of the cadaver (within four hours of death) so that putrefaction should have been negligible. Samples from axillary and femoral sites were collected by percutaneous external puncture before the start of the necropsy; cardiac blood was collected by opening the pericardial sac and aspirating from the right and left ventricles; and the internal jugular vein was dissected and sampled directly. The samples were obtained before dissection of the abdominal contents or disturbance of the viscera. Blood (10 ml) was collected from each of these five sites from each subject. Collection of vitreous humour involved adducting the eye and inserting the needle through the lateral sclera; 2 ml of vitreous humour was collected from each subject. All specimens were collected by one person using separate 20 ml syringes and 18 gauge needles for each site. Vitreous humour was not sampled from one subject (subject 3) as the eyes had been damaged.

The samples were immediately transferred into 2% fluoridated tubes and frozen at −18°C until further analysis. All samples were analysed by headspace gas chromatography as a single batch by the same operator. All assays were performed in duplicate and mean values