Papers and Originals

Quality Control in Haematology: Report of Interlaboratory Trials in Britain

S. M. LEWIS,* M.D., B.SC., M.C. PATH.; B. J. BURGESS,† F.I.M.L.T.

British Medical Journal, 1969, 4, 253–256

Summary: An interlaboratory quality control scheme has been established in Britain by the British Committee for Standards in Haematology. In the first instance this has been confined to haemoglobin, red blood cell count, and packed cell volume. The materials which have been circulated include whole blood, stabilized red cell preparations, lysates, cyanmethaemoglobin solutions, and cyanmethaemoglobin reference preparations. The first two trials have been completed, and there are implications for instrument calibration, dilution techniques, and the use of standards. Periodic interlaboratory trials, at a national level, in conjunction with regular individual interlaboratory quality control procedures, are necessary in order to achieve acceptable levels of accuracy and precision.

Introduction

There is no doubt that some form of quality control is essential in the clinical laboratory in order to ensure reliability of results of tests, with acceptable levels of accuracy and precision. In clinical chemistry a number of primary standards and reference preparations are available, and also secondary standards and stable control preparations of established composition for a wide range of investigations. These have made it possible for quality control procedures to be carried out within individual laboratories, and also interlaboratory, with relative ease (Whitby, Mitchell, and Moss, 1967).

In haematology the problem is a much more difficult one because of the lack of suitable standards and reference preparations. The use of natural blood as a control preparation is limited by its rapid deterioration on storage and by the difficulty in establishing accurate cell-count values. A number of individual laboratories do, in fact, carry out some forms of internal quality control; these include (a) a statistical method—for example, analysis of frequency distribution of the routine laboratory results by the “number plus” system (Hoffman and Waid, 1963); (b) maintenance of constancy of normal range, and checking of inconsistent results; (c) performance of duplicate and replicate tests on selected blood samples from one batch in a subsequent batch of routine tests. Methods based on statistical analysis of data have severe limitations (Frankel and Ahlvin, 1967) and cannot be depended on as the only method of quality control. Duplicate testing of blood samples within a single laboratory will be a check of precision but may fail to detect a consistent error. Furthermore, electronic cell counting systems depend, as a rule, on arbitrary calibration settings, so that lack of a reference standard becomes a serious limitation to an assurance of accuracy.

There is increasing awareness of the need for the exchange of accurate data between laboratories, whether for research and collaborative studies or for patient care in this era of mobility. Interlaboratory quality control is essential for this, and also for checking the adequacy of the day-to-day control within individual laboratories.

Interlaboratory haematology trials have been carried out at intervals during the past several years in the Netherlands (Holtz, 1964) and more recently at an international level by the International Committee for Standardization in Haematology (Coster, 1965; Holtz, 1969). In addition, haemoglobin assay has been included in some of the clinical chemistry surveys which have been carried out in some countries (Whitby et al., 1967). The conclusion from these trials has been that though they have led to some improvements the quality of performance remained less than might be expected, and the need for continuing trials, both nationally and internationally, was emphasized.

The British Committee for Standards in Haematology has launched a pilot scheme in collaboration with a large number of laboratories in Britain in order to determine how a national scheme might be run successfully. Ideally, interlaboratory trials should include relatively stable working samples which are indistinguishable from routine specimens and can be treated as such in routine analysis. There is need for considerable experimental work in order to develop suitable materials of this type as well as reference standards and stable reference preparations. In this regard the British Committee for Standards in Haematology is engaged in collaborative work with the International Committee for Standardization in Haematology, and the scope of future quality control trials will depend on the increasing availability of such materials. Our experience with the materials which are at present available is the subject of this report.

Trial 1

The material for the first trial consisted of three items.

1. Stabilized Red Cells—This was a glutaraldehyde-fixed suspension, prepared by the method of Lewis and Burgess (1966), slightly modified as recorded by Dacie and Lewis (1968). It was dispensed in sealed rubber-capped glass bottles; participants were instructed to shake the material vigorously by hand to ensure resuspension, then to mix for at least 10 minutes on a mechanical mixer, and then to aspirate the required amount into a syringe by means of a fine needle through the rubber stopper to avoid opening the bottle. This material was provided for red blood cell count (R.B.C.) and packed cell volume (P.C.V.). In order to establish its R.B.C. and P.C.V. as accurately as possible, five laboratories

* Senior Lecturer.
† Senior Technician.
Department of Haematology, Royal Postgraduate Medical School, London W.12.

‡ For the current series of trials these were London Hospital, London E.1 (Dr. G. Jenkins, Mr. P. Hall); Middlesex Hospital, London W.1 (Professor J. W. Stewart, Mr. D. Barnard); Northern General Hospital, Sheffield (Dr. S. Varadi, Mr. J. M. Bevington); Royal Postgraduate Medical School, London W.12 (Dr. S. M. Lewis, Mr. B. J. Burgess); St. Thomas’s Hospital, London S.E.1 (Professor G. Wetherley-Mein, Miss U. H. Glass).
were invited to act as reference centres, and they were asked to carry out the R.B.C. both by an electronic method and by visual counting on a haemocytometer, and to use calibrated pipettes and other equipment of known accuracy.

(2) Haemolysis.—This was prepared from human blood by the method described by Holtz (1965). Its true haemoglobin content was determined spectrophotometrically, and checked against the International Committee for Standardization in Haematology international reference preparation of cyanmethaemoglobin. This material was provided for haemoglobin estimation, and it was intended as a check on equipment, reagent, secondary standard, and dilution technique.

(3) Cyanmethaemoglobin Solution.—This was made available by courtesy of the International Committee for Standardization in Haematology; it was, in fact, a sample of the international reference preparation but was provided unmarked, and participants were asked to measure its haemoglobin content by reference to the commercial standards used in their laboratories. It was intended that this should provide a check on these.

**Results and Comments**

Results were returned by 83 participants. They are illustrated in Figs. 1–6.

R.B.C.—Because of technical limitations the “true” R.B.C. cannot be measured with absolute certainty by any one laboratory; nor can it be assumed on the basis of a statistical analysis that the mean from a large number of laboratories will necessarily provide this result. In the present exercise it has been assumed that the mean of results from the reference centres would most likely provide the correct result. This was 3.98 x 10⁶/cu. mm. A number of other participants did visual counting (Fig. 1), and though there was a spread in distribution of results this was surprisingly narrow, and the mean was close to that of the reference centres (3.96 x 10⁶/cu. mm; n=59; S.D.=0.39). By contrast, the results of counts by electronic counters (mainly various models of Coulter counters) were unexpectedly variable (Fig. 2). There appeared to be a Gaussian distribution at one end, and taking these results alone the mean was 3.89 x 10⁶/cu. mm. (n=51; S.D.=0.38). The wide range of results suggested that some counters were incorrectly calibrated for measuring the test suspension, which consisted of donkey red cells with mean corpuscular volume (M.C.V.) of 45 μ; this had been deliberately chosen, as counters should be so calibrated as to be capable of measuring cells of this size as well as normal human cells (Fig. 3).

P.C.V.—With correct condition of centrifugation the glutaraldehyde-fixed red cell suspension should pack to a constant volume, though not as completely as fresh cells. Accordingly, M.C.V. cannot be derived from the results. The purpose of this test was to indicate the effectiveness of centrifuging and the limits of accuracy of the procedure of reading the P.C.V. The results have shown (Fig. 4) that this, too, is an area where greater accuracy is desirable.

---

**Fig. 1.—Trial 1. Red cell count by haemocytometer on stabilized cell suspension.** The mean of all results (n=59) is shown. Fig. 2.—Trial 1. Red cell count by electronic counters on stabilized cell suspension. The mean of results from the five reference centres is shown.

**Fig. 3.—Effects on cell count of various settings of Coulter counter (model FN), with normal human blood, stabilized red cell suspension of donkey blood and blood from patient with thalassaemia.**

---

**Fig. 4.—Trial 1. P.C.V. measurements on stabilized red cell suspension.**

---

**Fig. 5.—Trial 1. Results of haemoglobin measurements on lysate.** Spectrophotometric measurement gave a result of 13.5 g./100 ml. 1% and 2% variations from this are indicated. Fig. 6.—Trial 1. Estimation of haemoglobin content of provided cyanmethaemoglobin solution by means of commercial standards. The true value (International Committee for Standardization in Haematology [I.C.S.H.] certified) was 59 mg./100 ml., equivalent to 11.8 g./100 ml. at dilution of 1:200. 1% and 2% variations from this result are shown. The diagram indicates which commercial standards had been controlled by British Committee for Standards in Haematology.
Haemoglobin.—The wide scatter of results was disappointing in view of the introduction of the International Committee for Standardization in Haematology reference preparation and publicity on haemoglobinometry in recent years—for example, Lewis (1967). Errors occurred when the full procedure was required for measuring haemoglobin on the lysate (Fig. 5), and also when only a limited technique was required for reading the cyanmethaemoglobin solution provided against a commercial standard (Fig. 6). It was thought that the errors on the latter test would be due to use of incorrect standards, but in fact this was found to be important in only a minority of cases as in general relatively small differences were found between results with British Committee for Standards in Haematology certified standard solutions and uncontrolled solutions. This study did, however, draw attention to the value of using certified standards in order to eliminate at least this potential source of error.

Trial 2

The second trial was carried out two to three months after the first trial. The material consisted of the following:

1. Sample of the stabilized red cell suspension, the value of which had been established in five reference laboratories, as described above. Participants were provided with this information and were asked to use it in order to ensure correct calibration settings of their instruments.

2. Sample of the International Committee for Standardization in Haematology cyanmethaemoglobin reference preparation of stated value.

3. Two test samples of blood in Alsever solution. These were of two different cell sizes, about 57 cu. μ and 106 cu. μ respectively. Preliminary studies had shown (Fig. 7) that the red cell values underwent changes during the first few days, but were then constant for at least two weeks. Accordingly the trial was organized so that tests (Hb, R.B.C., and P.C.V.) could be carried out by all participants between the 10th and the 15th day after collection of the blood.

Results and Comments

Results were returned by 91 participants (Figs. 8–13).

R.B.C.—Only measurements by Coulter counters have been used for analysis. The results were much more precise than in the previous trial, and it was found that by setting the instruments on the basis of the reference preparation calibrations suitable for counting the unknown samples could be obtained. It was noted, however, that some instruments were being used at settings widely different from those recommended in the instruction manual for the particular model of counter; the effect of this on accuracy and precision will be studied further in a subsequent trial.

It is of interest to note (Figs. 8 and 9) that for both samples the mean obtained from all participants was slightly higher than that from the reference centres. This raises the question of which figure should be used as the “accurate” count. On statistical grounds, especially in view of the Gaussian distribution obtained, it seems reasonable to suggest that the figure derived from the larger number of tests should be accepted. The preference between using a small number of specially selected laboratories and a large number of randomly chosen laboratories for obtaining the “true” count of reference and standard preparations requires further consideration.

Haemoglobin.—Accurate measurements were obtained by spectrophotometric assay. Colorimetric measurement by the participants showed, once again, a disturbingly wide scatter of results (Figs. 10–11). The distribution was the same with the
Observations

Techniques and results confirmed that on this occasion all commercial preparations used were satisfactory. In a few cases there were inconsistent errors between the two test samples, suggesting perhaps a variable instrument fault—for example, drift, air bubbles, dirty or unmatched cuvettes. In most cases the error was similar with both samples, and pipetting errors seemed to be a more likely explanation. These occurred both with manual techniques and with autodiluters and is in accordance with other observations that a major source of error in this type of laboratory assay is in the dilution procedure (Lewis, 1969).

P.C.V.—The results are shown in Figs 12 and 13. Though the mean and mode included over half the results, the spread of results confirmed the observation of the previous trial that this is not as reliable a measurement as might be hoped. There are a number of possible sources of error (de Boroviczény, 1966; Stewart, 1966), not least of which seems to be due to limitations in the scaled readers in general use. The outliers in Fig. 12 were undoubtedly due to errors of reading and recording.

![Fig. 12](image)

Fig. 12.—Trial 2. P.C.V. measurements on sample C. The mean of all results is indicated. Fig. 13.—Trial 2. P.C.V. measurements on sample D. The mean of all results is indicated.

Conclusion

The two trials reported here have shown the potential value of this procedure for quality control of blood counts. The use of fresh blood for this requires a speed of distribution that is impractical for a national service. On the other hand, in a country the size of Britain it is possible to use blood preserved in Alsever solution, as postal distribution of samples to all participants can be achieved within an acceptable time. It is also possible that the blood may be preserved even longer by the addition of nucleotides to A.C.D. (citric acid, trisodium citrate, dextrose solution) or to the Alsever solution—for example, Akerblom et al. (1967), Strumia et al. (1968), and Carville and Lee (1969). The extent to which the red cell values remain constant with these additives requires further study in order to determine whether this procedure can increase the scope of the scheme.

There is a place for both fresh blood samples and appropriate reference preparations, international and national standards and working, or secondary, standard solution. Cyanmethaemoglobin solutions conforming to international specifications as set out by the International Committee for Standardization in Haematology (1967) are now universally available for clinical haemoglobinometry, but their use does not ensure that all steps in haemoglobin estimation are controlled, and the present trial has shown that there may be significant errors despite the use of reliable cyanmethaemoglobin standard solutions. Thus adequate quality control of haemoglobinometry requires whole blood or lyase of known haemoglobin content as well as samples of whole blood for blind testing. Similarly, even with the event of standards for the other criteria, there will be continued need for interlaboratory trials with blind testing on samples of whole blood. It would be valuable to have such samples indistinguishable from routine specimens in order that they could be handled without special attention. This is, however, not practicable and each individual laboratory should have its own daily interlaboratory control with replicate tests on routine specimens, to be integrated with the facility provided at less frequent intervals for interlaboratory control at a national level. The former controls precision while the latter procedure is to ensure accuracy. This is becoming even more important in automated systems, as all values are measured in a single process relative to each other and the user is deprived of the opportunity for the correlation control which is available when some of the values are measured independently.

It is planned to have similar trials at intervals of two to three months, and also to extend their scope in order to include other haematological laboratory tests.

This project is being sponsored by a grant from Nuffield Provincial Hospitals Trust. We wish to thank Dr. R. Archer, Director of Equine Research Station, Newmarket, for invaluable cooperation, and especially for providing material for the stabilized red-cell preparations; the International Committee for Standardization in Haematology for providing the ample supply of the International Cyanmethaemoglobin Reference Preparation; Coulter Electronics Ltd. for helpful discussions; Mr. M. J. R. Healey, M.R.C. Clinical Research Centre, Division of Computing and Statistics; the reference laboratories named in the text; and our many colleagues who have participated in the trials. Their continuing interest, favourable comments, and helpful criticisms have ensured the success of the scheme, and encourage us to continue with it.

References