

Proposal for Distribution of a Hemoglobin Standard

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There is probably no procedure more commonly used in clinical laboratories than hemoglobinometry and few that are less satisfactory in their results. The deficiencies may reside, in part, in inadequacies of the chosen method. In larger part, however, they result from manipulative errors in the measurement and processing of the samples of blood. The latter involve questions of technical proficiency that are entirely within the control of the analyst himself. When, however, he converts his observations—gasometric or photometric readings, measurements of specific gravity, and so forth—to grams of hemoglobin in 100 ml of blood, he must depend upon his own calibration or a calibration supplied by an instrument maker, both procedures involving a conversion factor taken from the literature.

To be satisfied that the calibration remains valid for the instrument, reagents, and technical routines in use in his laboratory, he should periodically undertake a precise and time-consuming series of standardizations. If a uniform hemoglobin standard were available nationally and if a single method of analysis were widely practiced, the individual laboratory would then have assurance not only that its results would be comparable from month to month but that they would also be comparable with those from other laboratories employing the same standard. Inconsistencies in results that persisted under these conditions could then be clearly attributed to technical and manipulative errors.

The purpose of this report (1) is to describe a plan for the distribution of a certified hemoglobin standard and to advocate the wide adoption of a recommended analytic procedure. The use of both the new procedure and the standard is recommended. At the same time it is pointed out that the standard may be employed for the calibration of other methods of hemoglobin analysis in laboratories that do not choose to adopt rou-

tinely the advocated photometric procedure.

In 1941 the British Medical Research Council instituted an extensive study of the problem of standardizing hemoglobinometry and emerged with (i) a recommended analytic procedure, (ii) a simple hemoglobinometer for general use, and (iii) a certified national hemoglobin standard for distribution to cooperating laboratories (2).

In the United States the initiative was taken by the Army Medical Service Graduate School with a limited field trial (3) of a cyanmethemoglobin solution proposed by David L. Drabkin. The success of the Army plan so impressed the Hematology Study Section of the National Institutes of Health that it requested the National Research Council to explore the possibility of establishing a national hemoglobin standard for general use throughout the country.

To this end the NAS-NRC established an *ad hoc* panel under the subcommittee on blood and related problems of the Division of Medical Sciences. This panel has sought the cooperation of the College of American Pathologists, the American Society of Clinical Pathologists, the American Association of Blood Banks, the Department of Defense, the Veterans Administration, the National Institutes of Health, and the National Bureau of Standards. It has also maintained close liaison with the committee on hemoglobinometry of the Medical Research Council of the United Kingdom and with the National Research Council of Canada.

The panel gave serious consideration to the British plan but decided that, in respect to simplicity and adaptability, the cyanmethemoglobin method (4-6) adopted by the U.S. Army would be more suitable for use in the United States and Canada. It was agreed that the choice of a solution of some form of hemoglobin as a standard for hemoglobinometry was both logical and direct. In contrast to a glass standard, it would have the advantage of adaptability to a variety of photometric instruments and cuvettes. Among the forms of hemoglobin well adapted to photometry, cyanmethemoglobin has outstanding advan-

tages. It has been shown that solutions of this pigment are stable for years when they are preserved at refrigerator temperatures (7). The absorption band of cyanmethemoglobin in the region of 540 m μ is broad rather than sharp, so that its solutions are suitable for use in filter-type photometers as well as in narrow-band spectrophotometers (5, 8). Finally, all forms of hemoglobin likely to be found in blood, with the exception of sulf-hemoglobin, are quantitatively converted to cyanmethemoglobin upon the addition of a single reagent (5).

Recommendations

On the basis of these considerations, the panel reached agreement on the following recommendations.

1) There shall be a standard of reference in the form of a preparation of crystalline human hemoglobin prepared by the method of Drabkin (9). The acceptable criteria for this preparation shall be that a solution containing 1 milliatom of hemoglobin iron per liter shall have a millimolar extinction coefficient of 11.5 at a wavelength of 540 m μ , when measured as cyanmethemoglobin (5, 8). Certification of this standard of reference shall rest upon the results of spectrophotometric measurements and of analyses for iron made independently by the National Institutes of Health, the Army Medical Service Graduate School, the National Bureau of Standards, and Drabkin's laboratory at the University of Pennsylvania.

E. J. King of the Postgraduate Medical School in London will also characterize the standard of reference both chemically and spectrophotometrically and compare the results of its use with determinations of hemoglobin employing the British standard.

2) The iron content of hemoglobin shall be accepted to be 0.335 percent (10). This value for iron is the traditional figure used in this country and is in substantial agreement with that adopted by the British. It corresponds with an equivalent weight for hemoglobin of 16,700 per atom of iron and with an oxygen capacity of 1.34 ml per gram of hemoglobin (10).

The adoption of an agreed figure for the iron content is necessary in order that the spectrophotometric measurements in terms of iron may be translated into grams of hemoglobin. Should any change be made in the future in the accepted values for the extinction coefficient of cyanmethemoglobin and the iron content of hemoglobin, results based on the use of the afore-mentioned standard may be readily recalculated.

3) There shall be a standard for distribution in the form of a certified solu-

This plan was drafted by the *ad hoc* Panel on the Establishment of a Hemoglobin Standard of the Division of Medical Sciences, National Academy of Sciences-National Research Council. Dr. Cannan is chairman of the Division of Medical Sciences.

tion of cyanmethemoglobin that shall be prepared directly from the standard of reference. The standard for distribution shall be packaged as three separate solutions containing certified concentrations of approximately 20, 40, and 60 mg of hemoglobin in the form of cyanmethemoglobin per 100 ml of solution.

These three solutions will correspond to 1 to 250 dilutions of blood containing approximately 5, 10, and 15 g, respectively, of hemoglobin per 100 ml of solution. After bottling, samples of these standard solutions will be spot-checked for correctness of optical density by the four analytic laboratories that have been designated in recommendation 1. The batch will then be certified and distributed to cooperating laboratories by designated national agencies.

4) In conjunction with the use of the proposed standards, it is recommended that clinical laboratories consider the adoption of the cyanmethemoglobin method of hemoglobin determination described by Drabkin.

The use of the cyanmethemoglobin method follows from the concept that it would be logical to adopt a method of analysis that converts hemoglobin into the same pigment as that used in the standard. It does not, however, preclude the use of the cyanmethemoglobin standard for the calibration of other methods of hemoglobin analysis that may be in routine use in some laboratories. However, it should be realized that such a procedure may lead to some loss of accuracy.

The cyanmethemoglobin method employs a single solution containing potassium ferricyanide and potassium cyanide, which converts the hemoglobin in blood quantitatively to cyanmethemoglobin. The ferricyanide converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with potassium cyanide to produce the stable pigment cyanmethemoglobin. These two reactions are rapid and stoichiometric.

There should be no reluctance to employ this standard and reagent because they contain cyanide. The concentration of cyanide in the reagent that is proposed for use is only 50 mg of potassium cyanide per liter. Its lethal dose for man approaches 4 lit (11). Most clinical laboratories use, for the determination of uric

acid, a reagent containing 50 g of this salt per liter. In view of this and of the fact that laboratories of clinical pathology are disciplined in the use of such dangerous materials as isotopes and virulent pathogens, it would seem that the handling of the proposed reagent constitutes a quite negligible hazard.

Field Trial

The panel also agreed to undertake a 1-yr field trial of the standard for distribution described in recommendation 3. It is now felt that the plans for this field trial have progressed to the point where the participation of laboratories desiring to cooperate may be invited. The standards, which are prepared by Drabkin, are being distributed without charge to clinical laboratories on application, provided that the applicants agree to meet the following minimum requirements for participation: (i) to conduct and report at 3-mo intervals, for 1 year, measurements of the actual photometric readings of the three standard solutions in the photometer routinely in use for hemoglobin measurements in that laboratory; (ii) to cooperate in answering a simple questionnaire designed to furnish information on the influence of various factors on the results of the hemoglobin determinations that will assist the panel in its long-range plans for making the standard available on a national scale; (iii) to cooperate in the analysis and reporting of an unknown solution of cyanmethemoglobin and an unknown sample of blood.

The standard for distribution, consisting of the three solutions described in recommendation 3, is packaged as a single unit. Details of the procedure for the determination of hemoglobin as cyanmethemoglobin, as well as details of the procedure for calibrating another method in terms of the cyanmethemoglobin standard, are furnished with the standard.

Distribution is made to civilian laboratories by the College of American Pathologists, 203 N. Wabash Ave., Chicago, Ill.; to military and government laboratories by the Army Medical Service Graduate School, the Navy Bureau of Medicine and Surgery, the Air Force Surgeon General's Office, and the Vet-

erans Administration; and to laboratories in Canada through the Division on Medical Research, National Research Council, Ottawa, Ont. Cooperating laboratories are requested to apply to the distributing agency with which they are most closely associated. Because of limitation in the number of sets of the standard available, distribution is determined by priority of application and willingness to comply with the conditions listed. It is assumed that application for standards indicates acceptance of these conditions. Distribution of the standard began 1 July.

References and Notes

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7. D. L. Drabkin, personal communication.
8. D. L. Drabkin and J. H. Austin, "I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood," *J. Biol. Chem.* 98, 719 (1932).
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10. G. Hüfner, "Neue versuche zur bestimmung der sauerstoffcapacitat des blutfarbstoffes," *Arch. Anat. Physiol. Physiol. Abt.* 130 (1894).
11. T. H. Sollmann, *Manual of Pharmacology* (Saunders, Philadelphia, ed. 7, 1948), p. 713.

Let no youth have any anxiety about the upshot of his education, whatever the line of it may be. If he keeps faithfully busy each hour of the working-day, he may safely leave the final result to itself. He can with perfect certainty count on waking up some fine morning to find himself one of the competent ones of his generation, in whatever pursuit he may have singled out.—WILLIAM JAMES, *The Principles of Psychology*.