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An evaluation of photoelectric haemoglobinometers

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SYNOPSIS A number of photoelectric haemoglobinometers have been evaluated as to their suitability for haemoglobinometry in the routine clinical laboratory. They have been tested for accuracy, precision, linearity of response, instrument comparability and, where relevant, for carry-over. The advantages and defects of each are described.

As a project of the British Committee for Standards in Haematology, and at the request of the Laboratory Equipment and Methods Advisory Group of the Department of Health and Social Security, a number of photoelectric haemoglobinometers that are currently available in the United Kingdom have been tested and evaluated as suitable for clinical haemoglobinometry in the routine laboratory. Manufacturers or their agents were invited to provide, on short-term loan, instruments as available from stock, ready for delivery to the customer. On receipt the instruments were assembled in accordance with the operating instructions provided. Then, over a period of three to four weeks, their performance was assessed by established methods for accuracy, precision, instrument comparability, and when relevant, for carry-over and sample cross-contamination. For evaluation of accuracy and precision, haemoglobin (as cyanmethaemoglobin) was determined repeatedly on five specimens over a wide range of haemoglobin concentrations. In some of the runs the samples were handled sequentially, in others they were interspersed with routine specimens in a batch. For instrument comparability a batch of routine specimens was measured in the instrument under test and in a conventional photoelectric colorimeter of known reliability. Accuracy was assessed by measuring the haemoglobin concentration of the test samples with a spectrophotometer which had been calibrated in accordance with the recommendations of the International Committee for Standardization in Haematology (1967). In order to determine linearity of response of the galvanometer, optical density was recorded with progressive dilution of cyanmethaemoglobin. The result given is the mean of measurements of four samples. Total carry-over was measured only in instruments with automatic sampling units by determining the influence of (a) a high-concentration specimen on (b) a subsequent low-concentration specimen. Three tests were carried out consecutively, first on specimen a (a_1, a_2, a_3) and then on specimen b (b_1, b_2, b_3), and the carry-over was expressed as $k = \frac{b_1 - b_3}{a_3 - b_3}$. Routinely, specimens were diluted in Van Kampen-Zijlstra's cyanide-ferricyanide reagent (*p*H 7·2) (International Committee for Standardization in Haematology, 1967) and measurement was delayed for at least five minutes to ensure complete conversion. This reagent contains a non-ionic detergent. In some instances parallel tests were carried out using the cyanide-ferricyanide reagent without detergent at a *p*H of 9·6 (Lewis, 1967). For these tests measurement was delayed for at least 15 minutes. In general, identical results were obtained with both reagents, but where differences did occur this will be noted in the relevant section below.

Some of the instruments are intended speci-

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fically for haemoglobinometry, whereas others are general-purpose photoelectric colorimeters. They have, however, been evaluated only with regard to their use as haemoglobinometers, and it should be emphasized that the standard of performance which was considered acceptable was that required for routine clinical haemoglobinometry. In this report the analytic data have been summarized. Detailed protocols of results are available.

EEL Haemoglobinometer

Evans Electroselenium Ltd, Halstead, Essex (£78).

DESIGN

This is a sturdy instrument, 33 × 18 cm in area, made of cast aluminium. Operation is manual. The controls are easily accessible. Cuvettes are optically matched test tubes which have a capacity of 4 to 6 ml and a light path of 12 mm; single beam with tungsten filament. Haemoglobin is read directly as oxyhaemoglobin or cyanmethaemoglobin on adjacent scales. The scales are cramped in the 13-18 g/100 ml region. Standardization is relatively complex by means of a 'high standard' (18 g/100 ml) and a 'low standard' (3 g/100 ml) which have to be used consecutively. For setting the instrument for oxyhaemoglobin measurement whole blood 'standards' are required; calibration for cyanmethaemoglobin measurement requires cyanmethaemoglobin solutions of the appropriate concentrations as indicated. There is a relatively long stabilization time of 30 minutes, after which the instrument remains stable with negligible drift during the course of the day. There is, however, risk of accidental movement of the standard control knobs which cannot be locked after they have been set. The instrument casing is designed to hold tubes of high and low standards, intended for use during the day. However, these become heated by the instrument lamp and are thus liable to deterioration.

COMMENTS

Only cyanmethaemoglobin reference preparations in a concentration of 55 to 85 mg/100 ml conform to the specifications established by ICSH (1967). When intended for cyanmethaemoglobin measurements this haemoglobinometer requires, for its calibration, the use of cyanmethaemoglobin preparations of high and low concentrations which are outside this range and which are not subject to the rigid control which can be applied to preparations which do conform to the ICSH recommendations. Furthermore, if used for oxyhaemoglobin measurement, calibration requires whole blood 'standards' of uncertain reliability (Dacie and Lewis, 1968), which may result in serious error if they have not maintained their reputed values. In the evaluation of the haemoglobinometer the tests indicated an unsatisfactory level of accuracy. To be certain that this was not due to discrepancies in the 'standard' preparations used for calibrating the instrument, these were checked against the ICSH cyanmethaemoglobin reference preparations and were shown to be true to their reputed concentrations. Hence the poor performance was apparently inherent in the instrument. The presence of the two scales for cyanmethaemoglobin and oxyhaemoglobin respectively adjacent to each other is a potential source of reading error and confusion, and the method of calibration is unnecessarily complicated.

SUMMARY OF ANALYTIC DATA (HAEMOGLOBIN MEASURED AS CYANMETHAEMOGLOBIN)

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·4	15·3	10·7	7·8	6·0
As read (mean of 20)	15·17	15·01	10·37	7·44	5·76
SD	0·2519	0·3090	0·3585	0·3841	0·2653
V%	1·64	2·02	3·35	4·92	4·42
Linearity of Response					
Regression line (*y*)	= 0·146*x* − 0·41				
Correlation coefficient (r)	= 0·9997				
Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)					
Regression line (*y*)	= 0·996 − 0·303*x*				
Correlation coefficient (r)	= 0·9978				

EEL Spectra Colorimeter

Evans Electroselenium Ltd, Halstead, Essex (£125).

DESIGN

An attractive, robust and well designed instrument, 46 × 23 cm, made of cast aluminium. Operation is manual. The controls are easily accessible. There is a continuous spectrum interference filter with wavelength indicator dial covering the range 400-700 nm at 35 nm band widths. There are matched cuvettes in a range of sizes (0·75 ml, 1·5 ml, 3 ml, 6 ml) providing light paths of 2·5 mm, 5 mm, 10 mm, and 20 mm respectively; single beam with tungsten filament. Standardization is by one point setting against an ICSH-approved reference preparation; galvanometer scale measures OD indicated by light spot and hairline; haemoglobin is obtained by calculation or from a linear graph. After a

stabilization time of 10 minutes there is negligible drift of zero and standard sample during the course of a day.

COMMENTS

An instrument of acceptable accuracy and precision for routine haemoglobinometry by manual method. The continuous spectrum and range of cuvette sizes makes it a more versatile instrument for use with a wide range of sample concentrations and analytic reactions.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·3	11·2	10·7	7·8	6·0
As read (mean of 20)	15·29	11·28	10·70	7·70	5·93
SD	0·1392	0·1931	0·0883	0·1166	0·0916
V%	0·91	1·71	0·82	1·50	1·54

Linearity of Response
Regression line (y) $= 0{\cdot}005x - 0{\cdot}006$
Correlation coefficient (r) = 0·9999

Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)
Regression line (y) $= 0{\cdot}992x + 0{\cdot}102$
Correlation coefficient (r) = 0·9997

EEL Flowthrough Spectra Colorimeter

Evans Electroselenium Ltd, Halstead, Essex (£125).

DESIGN

The instrument is basically similar to the EEL Spectra (q.v.) It is of the same size (46 × 23 cm) but in addition it requires the use of a sink or trap bottle for waste from its flow-through cell. The cell consists of a single cuvette of 5 ml capacity; light path 10 mm. An item for criticism is the fact that the flow-through control plunger is difficult to manipulate, making its operation cumbersome.

COMMENTS

The EEL Spectra colorimeter has been described in the previous section. The flow-through unit is an attachment for facilitating its use. Its design is open to some criticism (see above) but in general it can be recommended as a useful aid for the manual method of haemoglobinometry when there is a relatively large throughput of specimens.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·3	11·2	10·7	7·8	6·0
As read (mean of 20)	15·29	11·06	10·60	7·67	5·95
SD	0·1356	0·1723	0·943	0·1100	0·0943
V%	0·88	1·55	0·88	1·42	1·58

Linearity of Response
Regression line (y) $= 0{\cdot}003x - 0{\cdot}003$
Correlation coefficient (r) = 0·9999

Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)
Regression line (y) $= 1{\cdot}00x - 0{\cdot}039$
Correlation coefficient (r) = 0·9997

EEL 171/178 Automatic Colorimeter and Autosampler

Evans Electroselenium Ltd, Halstead, Essex (£1,520).

DESIGN

This is a large apparatus consisting of three separate units of the following dimensions: electronic unit 57 × 45 cm, photometer 48 × 22 cm, autosampler 48 × 41 cm. In practice this will require a bench area of about 120 × 50 cm. The apparatus is made of aluminium and steel and operation is automatic. There is a continuous spectrum interference filter with wavelength indicator dial covering the range 400-700 nm at 30 nm bandwidths. It has a single hour-glass-shaped cuvette, nominal capacity 2·5 ml, light path 5 mm; single beam with tungsten filament. Samples are taken up and subsequently discharged by a motor-driven syringe. Galvanometer scale measures OD; the automatic read-out is a three-digit counter and printer mechanically coupled to a potentiometer, and results are given either in OD units or in concentration of haemoglobin (g/100 ml) after presetting against an ICSH-approved reference preparation. The print-out has specimen identification.

The autosampler holds four racks, each of 12 tubes (100 × 15 cm), to contain prediluted samples. As the holding pins are very short the racks are easily dislodged from the autosampler; this may cause accidental spilling of the sample. Throughput time (rate of analysis) is 18 seconds per sample. After a stabilization time of 30 minutes, zero drift is approximately 1% per hour, but deviations will be corrected automatically when the auto-zero or auto-standard circuits are brought into operation.

COMMENTS

During a one-month testing period the parti-

cular instrument under trial developed several major faults requiring extensive technical service from the manufacturer on three occasions. There was yet another breakdown during the linearity of response measurements which prevented this test from being completed and the instrument was then returned to the factory.

During preliminary tests it was found that results obtained with Van Kampen-Zijlstra's cyanide-ferricyanide reagent as recommended by ICSH showed considerable discrepancies, in contrast to good performance when a conventional Drabkin's reagent without detergent was used. It seems likely that the discrepancies were caused by frothing and air bubbles. It is not clear whether criticism for this failure of performance should be levelled at the reagent or the instrument. For the present evaluation only conventional Drabkin's reagent was used, and with this an acceptable level of accuracy and precision was obtained.

This is an expensive instrument intended for automatic performance of haemoglobinometry. The faults in the instrument under test made it unacceptable.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·3	11·2	10·7	7·8	6·0
As read (mean of 20)	15·19	11·28	10·77	7·83	6·03
SD	0·1805	0·2762	0·1876	0·1587	0·1466
V%	1·18	2·40	1·70	2·00	2·40

Linearity of Response
This was not carried out owing to technical failure (see comment below)

Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)
Regression line (y) = 0·980x + 0·329
Correlation coefficient (r) = 0·9994

	Total Carry-over: High Concentration	Total Carry-over: Low Concentration	$K = \frac{b_1 - b_2}{a_2 - b_2}$
1	18·1	7·3	0·0092
2	19·0	7·7	0·0088
3	17·7	7·8	0·0100
4	18·4	6·4	0·0000
5	18·2	7·7	0·0000
6	16·9	7·6	0·0000
7	16·1	7·0	0·0000
8	17·5	8·2	0·0107
9	17·3	7·9	0·0106
10	16·3	7·9	0·0000
			Mean of k = 0·005

Gallenkamp Mark III Colorimeter

A. Gallenkamp & Co Ltd, London EC2 (£82; with three filters and three cells).

DESIGN
This is a robust, well designed instrument, 21 × 35 cm in area, made of sheet steel. Operation is manual. It has easily accessible controls. Eight filters are available in the range 450 nm-670 nm, including one at 540 nm. It has matched cuvettes, 4 ml-6 ml capacity, with light path of 10 mm; single beam with tungsten filament. Standardization is by one-point setting by an ICSH-approved reference preparation. The galvanometer scale measures OD on a mirror scale with pointer. Haemoglobin is obtained by calculation or from linear graph. After a stabilization time of 10 minutes there is negligible drift of zero and standard sample during the course of a day.

COMMENTS
An instrument of acceptable accuracy and precision suitable for routine haemoglobinometry by manual method.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·3	11·2	10·7	7·8	6·0
As read (mean of 20)	15·17	11·13	10·62	7·65	5·83
SD	0·1486	0·1345	0·1414	0·1191	0·1652
V%	0·97	1·20	1·33	1·55	2·84

Linearity of Response
Regression line (y) = 0·004x − 0·002
Correlation coefficient (r) = 1·000

Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)
Regression line (y) = 0·992x + 0·102
Correlation coefficient (r) = 0·9997

Linson Junior Haemoglobinometer

A.B. Lars Ljunberg & Co, Stockholm (distributed by Grant Instruments, Cambridge, £81)

DESIGN
A compact instrument of attractive design made of moulded plastic; it is 22 × 14 cm in size with accompanying transistorized stabilizer (17 × 11 cm). Operation is manual. Its controls are simple and easily accessible. It has a single cuvette but a flowthrough cuvette is also available. The cuvette is square, optically flat, with a capacity of 2·5 ml (least volume) to 12·5 ml and a light path of 12·7 mm; single beam with tungsten filament. Standardization is by one point setting against an ICSH-approved reference preparation. Haemoglobin is read directly on a galvanometer scale

indicated by a pointer. Cramped scales make reading difficult, and as the scale is placed horizontally, slight strain occurs when the operator attempts to avoid error of parallax in reading. After stabilization time of five minutes there is negligible drift of zero and a drift of up to 0·0075 OD in a standard sample during the course of a day.

COMMENTS

A compact, elegant instrument with a fair degree of precision and accuracy. It is one of the cheaper instruments of the type available and it can be recommended, especially for the smaller laboratory or clinic rather than for the laboratory with a heavy workload.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	17·4	14·4	10·0	7·3	3·8
As read (mean of 20)	17·93	14·42	10·15	7·32	3·83
SD	0·2993	0·3937	0·2114	0·1872	0·2149
V%	1·70	2·72	2·14	2·53	5·60

Linearity of Response
Regression line (y) = $0·027 + 0·007$
Correlation coefficient (r) = 0·9998

Comparison with Standardized Photoelectric Colorimeter (x) (*100 observations*)
Regression line (y) = $1·01x + 0·186$
Correlation coefficient (r) = 0·9948

Linson 3 Photometer

A.B. Lars Ljunberg & Co, Stockholm (distributed by Grant Instruments, Cambridge, £107)

DESIGN

This instrument occupies an area of 35 × 29 cm. It is made of painted metal, of attractive design. Operation is manual. The controls are easily accessible. It has a single cuvette but a flow-through cuvette is also available. The cuvette is square, optically flat, with a capacity of 2·5 ml (least volume) to 12·5 ml and a light path of 12·7 mm; single beam with tungsten filament. Standardization is by one-point setting with an ICSH-approved reference preparation. Results are read as extinction values on galvanometer, indicated by scale pointer. Haemoglobin is obtained by calculation or from a linear graph. Cramped scale makes reading difficult, and as the scale is set horizontally, reading may cause some strain when the operator tries to avoid error of parallax. After a stabilization time of five minutes there is negligible drift of zero and a drift of up to 0·0075 OD in a standard sample in the course of a day.

COMMENTS

An instrument of acceptable accuracy and precision, suitable for routine haemoglobinometry by manual method. The design of the scale is open to criticism.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	17·4	14·4	10·0	7·3	3·8
As read (mean of 20)	17·74	14·24	10·15	7·35	3·90
SD	0·2819	0·1875	0·2212	0·1731	0·1905
V%	1·60	1·35	1·12	2·30	4·90

Linearity of Response
Regression line (y) = $0·006x + 0·003$
Correlation coefficient (r) = 0·9999

Comparison with Standardized Photoelectric Colorimeter (x) (*100 observations*)
Regression line (y) = $0·989 + 0·337$
Correlation coefficient (r) = 0·9956

Vitatron HBF 200 Haemoglobinometer

Distributed by Fisons Ltd (£112; vacuum pump for flowthrough operation £13)

DESIGN

This instrument has a plastic moulded top with a metal base; it is 28 × 26 mm in area and requires additional space for a small vacuum pump and trap-bottle. Operation is manual. The working controls are easily accessible but the standard-setting control is very difficult to reach as it is positioned at the bottom of the instrument beneath the cuvette. The cuvette is a single flow-through type, requiring prediluted samples. It is glass in a plastic moulding with a capacity of 2·5 ml and a light path of 10 mm. Emptying is achieved by simply applying finger-tip pressure to a hole in the support stand. Standardization is by one-point setting with an ICSH-approved reference preparation. Haemoglobin is read directly (as g/100 ml cyanmethaemoglobin) on the galvanometer scale. Unusually, the scale is marked right to left but it is easy to read except in the 18-20 g/100 ml region where it is cramped. After a stabilization time of 10 minutes there is slight drift of zero and also in the standard sample during the course of the day, requiring some adjustment with each batch of specimens.

COMMENTS

A neat looking instrument but it requires some redesign of controls. It gives an acceptable performance; it is slightly more expensive than other instruments of this type, but it does not appear to have any significant advantages over the other instruments.

SUMMARY OF ANALYTIC DATA

	(a)	(b)	(c)	(d)	(e)
Precision and Accuracy					
True Hb content (g/100 ml)	15·4	15·3	10·7	7·8	6·0
As read (mean of 20)	15·43	15·31	10·77	7·81	6·15
SD	0·1090	0·1161	0·1360	0·1204	0·1688
V%	0·71	0·76	1·27	1·54	2·81

Linearity of Response
Regression line (y) = 0·153 + 0·003
Correlation coefficient (r) = 0·9999

Comparison with Standardized Photoelectric Colorimeter (x) (100 observations)
Regression line (y) = 0·986x + 0·148
Correlation coefficient (r) = 0·9980

Vickers Haemoglobinometer

This instrument is no longer marketed and was not available for testing.

I.L. Haemoglobinometer Model 231

Instrumentation Laboratories, Lexington Mass., USA.

This is advertised as an automatic instrument for direct measurement of haemoglobin concentration as the combined absorbencies of reduced haemoglobin, oxyhaemoglobin, and carboxyhaemoglobin at 548·5 nm against a standard red dye consisting of amaranth and 6% propylene glycol in water. By means of a proportioning pump the blood sample is diluted in a ratio of approximately 1:15 in a 'buffered haemolyzing agent' (as supplied by the manufacturer) and a reading is obtained in less than 15 seconds. There has been one favourable report (Gambino and Waraksa, 1969) but another colleague (G. Izak, personal communication) has commented on serious trouble with the instrument in practice, notably that the lumen of the tubing, which is compressed by the rotating cylinder to obtain the proportional dilution, is subjected to uncontrolled changes, and that this leads to inaccuracies and inconsistencies manifested by ± 15% variation in the same blood sample examined repeatedly within a three-hour period. Moreover, the principle of the instrument is also open to a criticism, at least on theoretical grounds, that its performance cannot be checked directly by means of a cyanmethaemoglobin reference preparation in conformity with current international practice.

Unfortunately, as the UK agents have not yet provided an instrument for evaluation, we have been unable to make personal observations, and its acceptability remains in doubt.

Conclusion

It is not the purpose of this report to provide a 'best buy' recommendation. There have been previous reports of comparative evaluation of a number of general purpose photoelectric colorimeters (Broughton and Riley, 1965; Broughton, Riley, Cook, Sanders, and Braunsberg, 1966). In the present study each instrument has been assessed, essentially in terms of its accuracy and precision, as to its suitability for haemoglobinometry in the setting of a clinical laboratory. Undoubtedly, the accuracy of measurement of haemoglobin depends largely upon accuracy in pipetting, sampling, and the correct use of the standard (Lewis and Burgess, 1969). Thus minor variation in the colorimeter is unlikely to be of serious consequence and should be put into perspective. It must, moreover, be remembered that the tests were carried out on one instrument of each make, and that these accuracy and precision evaluations do not necessarily apply to all other instruments of the same make. Faults may occur even when there has been a rigid factory inspection; it is important that a 'rogue' instrument should be recognized, and it is suggested that a testing procedure similar to that used in this study should be applied individually to all newly purchased instruments.

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