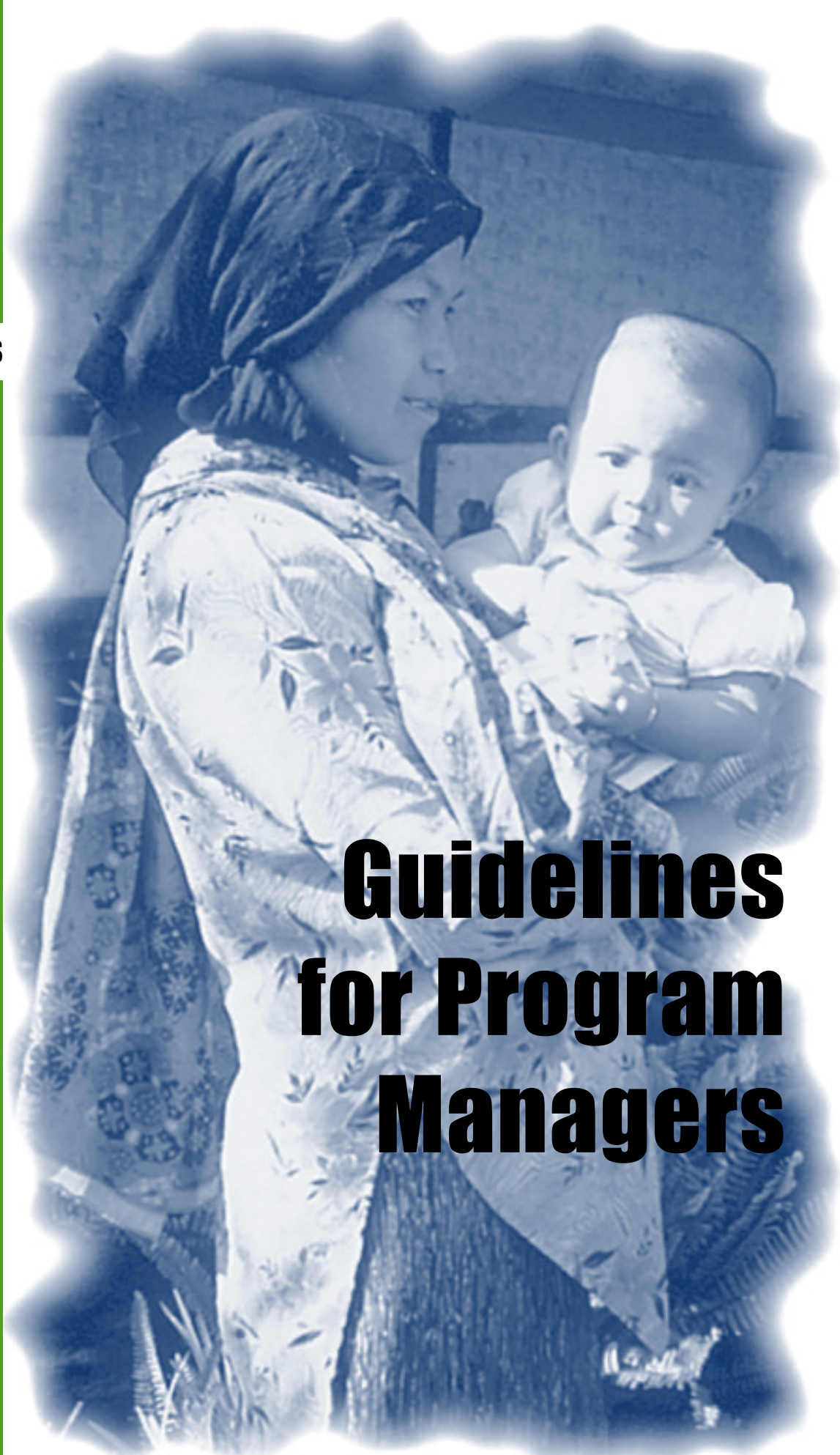


ANEMIA DETECTION

in Health Services



Guidelines for Program Managers

path



OMNI



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Anemia Detection in Health Services: Guidelines for Program Managers

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path

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Anemia Detection In Health Services

Guidelines For Program Managers

INTRODUCTION

Iron deficiency is the most common micronutrient deficiency in the world¹ and has far-reaching and serious adverse effects on health. Anemia detection is often used as a screening test for iron deficiency. Because the condition is so widespread, anemia control activities should be an integral part of health care services.

These guidelines are intended to help program managers establish anemia detection services or enhance existing services. They include a general overview of the programmatic issues of anemia screening to provide a context for method choice. Existing commonly available anemia detection methods are presented in a standardized format to help managers make appropriate decisions regarding technology selection. Although some technical information is provided about each of the methods, this is only to give the manager an idea about the complexity of tests in relation to personnel training and equipment maintenance needs. For more detailed information about individual technologies, the reader should consult a standard laboratory text. It is also beyond the scope of these guidelines to include the clinical management of anemia, which is well covered in other publications.

DEFINITION OF ANEMIA

Anemia occurs when the total volume of red blood cells (and/or the amount of hemoglobin in these cells) is reduced below normal values, as defined by healthy populations (see charts on pages 2 and 3). Anemia results from one or more of the following processes: defective red cell production, increased red cell destruction, or blood loss.²

There are often multiple causes for anemia. People suffer from both nutritional anemia (impaired red-cell production) and from parasitic diseases such as malaria (red blood cell destruction) and intestinal worms (blood loss).³ Although **iron deficiency** is the most common cause of anemia, especially among younger children and women of child-bearing age, other nutrient deficiencies, such as folate and vitamin B12, can also contribute to anemia.⁴

IRON DEFICIENCY

Iron is necessary for the synthesis of hemoglobin, which carries oxygen to the body's cells and transports carbon dioxide from the tissues to the lungs. Anemia is a late sign of deficient iron stores.⁵ Nearly twice as many people have deficient iron stores as have overt anemia.⁶ Iron-deficiency anemia results in impaired cognitive and motor development in children and decreased work capacity in adults.⁷ The effects are

particularly severe in infancy and early childhood and probably cannot be reversed by subsequent therapy. In pregnancy, iron-deficiency anemia can lead to perinatal loss, prematurity, and low birthweight.⁸ Iron-deficiency anemia also adversely affects the body's immune response.⁹

WHO IS AFFECTED?

- Nearly one fourth of the world's population is currently anemic.¹⁰
- All ages and both sexes are affected, but the prevalence of anemia varies by group.
- Vulnerable groups include women of reproductive age (because of menstruation), pregnant and breastfeeding women, and children from 6 months to 2 years of age (because of weaning from breastfeeding).
- Half the pregnant women in the world are anemic (in developing countries between 55% to 60% of pregnant women are affected vs. 18% in developed countries).¹¹
- The prevalence of anemia is the most severe in Southeast Asia where 75% of pregnant woman are affected.¹²
- Anemia is the sole or major contributory cause in 20% to 40% of the half million maternal deaths yearly.¹³
- Approximately 43% of young children are presently anemic.¹⁴

Anemia is most commonly detected by measuring **hemoglobin** (the iron-carrying part of red blood cells) or by determining the **hematocrit** (the volume of red blood cells in a specified amount of blood).

The World Health Organization (WHO) proposes the following cut-off hemoglobin values for **anemia**:¹⁵

Children under 5 years of age	Hb less than (<) 110 grams per liter (g/L)
Non-pregnant women	Hb < 120 g/L
Pregnant women	Hb < 110 g/L
Men	Hb < 130 g/L

Severe anemia has been defined as < 70 g/L and very severe anemia as < 40 g/L.

WHO lists the following ranges for **normal** hematocrit (Hct) values:

Children under 5 years of age	Hct 38-44%
Women	Hct 37-43%
Men	Hct 40-50%

Cut-off levels for hemoglobin and hematocrit must be shifted upwards for people living at higher altitudes and for those who smoke.

CURRENT PROGRAM APPROACHES TO ANEMIA DETECTION

Because anemia is so prevalent and because it has such wide-ranging effects on health, screening for this condition should be one of the most common primary health care activities.

Two basic approaches to anemia assessment exist:

- individual screening.

Individual screening is usually done in a clinic setting as part of routine services to groups at risk. These services usually include antenatal care and well-child programs, such as growth monitoring.

Some screening of pregnant women occurs outside of clinic settings, where services are often delivered by community health workers such as traditional birth attendants.

- population-based screening.

This type of screening usually takes place at a regional or a national level. Baseline and follow-up surveys are usually done in the community.

In both cases, two types of assessment activities are undertaken:

- making a diagnosis (establishing a baseline).
- monitoring the effects of interventions.

Current program approaches are based on judgments about which groups are vulnerable and on possibilities for measuring these groups.

The choice of diagnostic methods for anemia depends on the purpose of the assessment and the resources available. These choices vary by country and even by settings within countries. In **resource-limited settings**, the most commonly used

measures of anemia are levels of hemoglobin and hematocrit. Biochemical tests (such as serum ferritin and transferrin) that measure iron stores are not practical in these settings because of the complexity of the equipment needed.

The choice of methods also depends on the degree of anemia typically encountered. If a test only detects severe anemia and the level of anemia encountered is low to moderate, the number of those correctly identified as having anemia may be lower than if a test with broader detection ability is used. Hemoglobin values are more accurately measured when they fall in certain ranges for some anemia detection tests (see charts of individual technologies on pages 11 and 12). Individual case management requires anemia detection devices that are able to discriminate differences of 10g/L hemoglobin, so that monitoring of interventions can be assessed as recommended by WHO Safe Motherhood management guidelines.

MAKING DECISIONS ABOUT ASSESSMENT ACTIVITIES

Baseline surveys assess the prevalence of anemia in a country or region. Accurate and reliable anemia detection devices are needed to generate this data. Program managers should request this information from the national levels of health care, if it is available. Once prevalence levels of anemia are known, decisions can be made about the type, frequency, and locations of anemia screening activities.

Routine screening may not be appropriate in areas with either high prevalence (greater than $>20\%$) or low prevalence ($<5\%$) of iron-deficiency anemia.¹⁶ Instead, general iron supplementation of at-risk groups may be considered for the high-prevalence areas.¹⁷

Before making decisions about interventions, it is important to distinguish iron-deficiency anemia from anemia caused by additional factors (see page 1). One simple way to identify iron deficiency is to look at the distribution curves for anemia in a population by age and sex. This information may be available from national level surveys. When nutritional iron is the major factor, women and children will be disproportionately affected and men not usually affected. When multiple factors are responsible, all ages and both sexes will be equally affected.¹⁸ Alternatively, one can treat the suspected conditions with and without iron and monitor changes with a suitable diagnostic test.¹⁹

ASSESSMENT STRATEGIES BY LEVELS OF RESOURCE

Program managers must consider resources available, patterns of anemia in the population, and existing health care services. Needs will vary depending on the profile of the population served and on the purpose of anemia assessment. In one setting, it may be important to provide precise estimates of the degree and type of anemia, and, in other settings, general screening may be adequate. Considering that

resources will almost always be less than optimal, managers should be careful to match the assessment methods to the situation. Thus, appropriate anemia detection devices will not necessarily be the most sensitive, complex, or expensive.

Resource-Limited Settings

These are settings in which laboratory facilities are minimal, expendable goods are in short supply, and health care providers have only basic minimum training. This situation can apply equally to country situations or to areas within countries. Some countries will have adequate resources at the central level but have fewer resources in rural and/or remote areas.

Generally, resource-limited settings are characterized by high levels of anemia from multiple causes. At the community level, general screening is typically done using **clinical signs** or by use of the **filter paper method**. At the clinic level, iron-folate supplements are often given as a preventive to groups at risk of anemia.

In these settings, the low cost and ease of operation of a screening method are overriding features. Inexpensive anemia detection methods that can withstand field conditions, such as the **copper sulfate** method, should be used for further screening or to identify individuals at risk. Many of these methods give ranges for hemoglobin levels rather than specific numerical measurements. Most are not dependent on electricity. Hematocrits and precise hemoglobin determinations are not usually warranted in these settings. The issues that influence the types of anemia detection methods to use in these settings are described.

Key Issues for Resource-Limited Settings:

- Cost of equipment and resupply items.
- Proper care and maintenance of equipment.
- Diagnosis and treatment of co-existing parasitic infections.
- Skill levels of personnel.
- Reliability of information obtained.

Resource-Mixed Settings

These are settings in which health care providers have some specialized training beyond basic primary health care and where expendable supplies are available most of the time. Dependable electricity may or may not be available. Often there are personnel trained in multiple laboratory procedures. In these settings, causes of anemia may or may not be mixed. Program managers have more choices about types of devices to use. At peripheral or remote settings clinical signs may still be used.

Most assessments take place in clinic settings. There may be a mix of simple methods for general screening and more accurate methods for confirmatory diagnoses. Emphasis is still focussed more on groups than on individuals. Iron-folate tablets may still be given as a preventive to high risk groups. Biochemical tests for assessment of iron stores are not usually done.

In this setting, where quantitative hemoglobin or hematocrit testing can be implemented, the accuracy and precision of a method, coupled with relatively low cost, are the important features. The portable hemoglobinometers would fit well at this level. The hematocrit by centrifuge is valuable as a supplement to the measurement of hemoglobin for determining the cause of anemia.

Key Issues for Resource-Mixed Settings:

- Appropriate choice of diagnostic tool for the setting.
- Proper care and maintenance of equipment.
- Appropriate follow-up for individuals diagnosed as anemic.

Resource-Adequate Settings

This setting usually includes only central or reference laboratories, or regional or intermediate levels in larger countries. Biochemical tests may be used for assessment of iron stores. Training of personnel and maintenance of equipment is more complicated and expensive. A reliable supply of electricity is necessary.

Key features influencing selection of tests include accuracy, precision, sensitivity, and ease of operation. Photometric methods are appropriate at this level.

Key Issues for Resource-Adequate Settings:

- Feedback to sources of referral is essential.
- Refresher training for laboratory personnel is crucial.

OTHER RESOURCE CONSIDERATIONS FOR CHOOSING A TEST

When choosing anemia detection equipment, all costs for purchase, operation, and maintenance must be considered. This includes costs for reagents and for disposable equipment, such as capillary tubes. Expenses can be direct, such as capital costs of equipment, and indirect, such as programmatic support. These considerations are similar for national programs and for individual clinics.

The following **personnel** considerations affect choice of method:

- Level of worker able to use test.
- Amount of training and supervision required.
- Subjectivity of results.

The following **equipment** considerations affect the choice of anemia detection methods:

- Similarity of equipment in other health settings.
- Costs incurred by changing equipment.
- Comparability of results.
- Number of units needed.
- Proportion of health budget allocated to this equipment.
- Life span of the equipment.
- Availability of replacement parts and/or expendable supplies.
- Ease and cost of repair.
- Strength and durability of equipment.
- Portability of testing equipment.
- Ease of calibration and standardization.

The following **setting** considerations affect the choice of methods:

- Availability and/or necessity of electricity.
- Availability of good, reliable lighting.
- Availability of running water.
- Space requirements for the equipment.
- Disposal considerations.

The last considerations pertain to the **program** into which the equipment must fit:

- Equipment should be the same as, or complementary to, that of the national program to ensure standardization of results.
- Choice of equipment will depend in large part on whether it is used primarily for population or for individual screening.

- Necessary follow-up should be available.
- The type of method chosen should be appropriate for the setting (clinic based or outreach).

USE OF ANEMIA SCREENING RESULTS

Accurate baseline data give program managers a basis for comparison of clinic data. For example, changes in patterns of anemia can be noted and used to guide clinic interventions or program strategies. Nationwide trends can be monitored by region, gender, age, season, and levels of parasitemia.

Different options for recording test results include **master clinic records, individual clinic records (including child growth charts), and home-based record cards.** Master records should be designed to facilitate compilation of data for surveillance purposes. If referrals are necessary, external laboratory results should be incorporated into existing clinic and client records. On client cards, color-coding, or other classification systems, will help health care providers explain results to clients.

QUALITY ASSURANCE MEASURES

Personnel

Health care personnel should be trained using standard guidelines. Refresher training at regular intervals is important for maintaining skills.

Training issues include:

- collection of blood samples.
- performance of laboratory test procedures.
- recognition of abnormal results and follow-up action.
- operation, maintenance, and repair of instruments.
- use of calibration standards, controls, and preparation of standard curves.
- preparation and storage of reagents.
- maintenance of an inventory of supplies.
- proper disposal of sharp objects and medical waste.

It is helpful to develop standardized procedures for each anemia detection method that can be referenced from manuals. This information should be readily available to clinic personnel.

Anemia detection tests should be done with care and questionable results repeated. Periodic evaluations of test performance by clinic personnel will help ensure consistency. This can be done by using the reference manual as a guide and by comparing results with an accurate standard method.

Equipment

Routine maintenance of equipment used in anemia detection is crucial if one is to obtain accurate and consistent results. Cleaning equipment after use is essential to proper maintenance. Pipettes and cuvettes must be carefully cleaned of dried blood. If instruments require calibration, this must be done on a regular basis using standard calibrating devices. Records should be kept of maintenance activities.

System

To optimize the performance of the anemia detection system, it is important to establish quality assessment procedures. This can be done on a country or regional basis by checking performance at sentinel surveillance sites. Even if less accurate anemia detection devices are used to screen populations, results are still meaningful for programmatic purposes. Trends can be noted, and individual errors are less important in a larger system.

To assess the performance of a system that uses simple anemia detection devices, a portable instrument capable of providing precise and accurate measurements, such as any photo-electric hemoglobinometer, can be used. A regional or district hospital can provide blood samples of known values as a point of reference. Individual clinic results can be tested against this reference method to identify the direction of error. Managers must take corrective action when results do not meet acceptable standards.

MAJOR METHODS FOR ASSESSING ANEMIA

Major methods can be divided into qualitative and quantitative methods. Quantitative methods are more accurate and precise. Among the quantitative methods, technologies that require dilution of blood are more complex and, therefore, more subject to error.

Methods	General Category	Requires Electricity		Chemical Reaction or Chemicals		Level of Skill	Complexity of Operation	Accuracy & Precision*	Time to Obtain Result (1 estimation)	Initial Cost of Instrument	Relative Cost Per Test
		Yes	No	Yes	No						
Clinical Exam for Anemia	Qualitative		✓		✓	Low	Low	+	2 minutes	None	Low
Filter Paper Color Comparison	Qualitative		✓		✓	Low	Medium	+	1 minute	None	Low
Copper Sulfate	Qualitative		✓	✓		Medium	Low	+	1 minute	None	Low
HCT/Centrifuge	Quantitative	✓			✓	Medium	Medium	+++	4 minutes	Ranges from Low to High	Medium
Lovibond	Quantitative		✓	✓		Medium	Low	++	5 minutes	Low	Low
Sahli	Quantitative		✓	✓		Medium	High	++	8 minutes	Medium	Medium
BMS/Grey Wedge	Quantitative		✓	✓		Low	Medium	++	2 minutes	Medium	Medium
HemoCue	Quantitative		✓	✓		Medium	Low	+++	30 seconds	High	High
HbCN Photometer	Quantitative	✓		✓		High	High	+++	5 - 20 minutes	High	Medium
HbO Photometer	Quantitative	✓		✓		High	Medium	+++	5 - 20 minutes	High	Medium

*+++High

++ Acceptable

+ Low

TECHNOLOGIES FOR ANEMIA DETECTION BY CATEGORY

No Blood Sample Required

- Clinical signs (visual inspection of physical characteristics)

Non-Dilutional (No Pre-Mixing Of Blood With Chemicals)

The following methods use NON-LYSED (red blood cells are intact when used in test) whole blood:

- Filter paper method
- Copper Sulfate
- Hematocrit/centrifuge
- Lovibond (can also be used with dilution technique)

The following methods use LYSED blood (red blood cells are broken down with a soap-like product):

- Grey wedge/BMS Hemoglobinometer
- HemoCue (lysis is automatic in the method)

Dilutional (Blood Is Mixed With Chemicals)

Accurately measured amounts of whole blood are mixed with chemicals that produce a new compound. Color intensity of the new compound is proportional to hemoglobin concentration.

This color intensity of the compounds can be measured two ways:

Visual Color Match

Compound used for tests: hydrochloric acid \Rightarrow acid hematin

- Sahli
- Lovibond

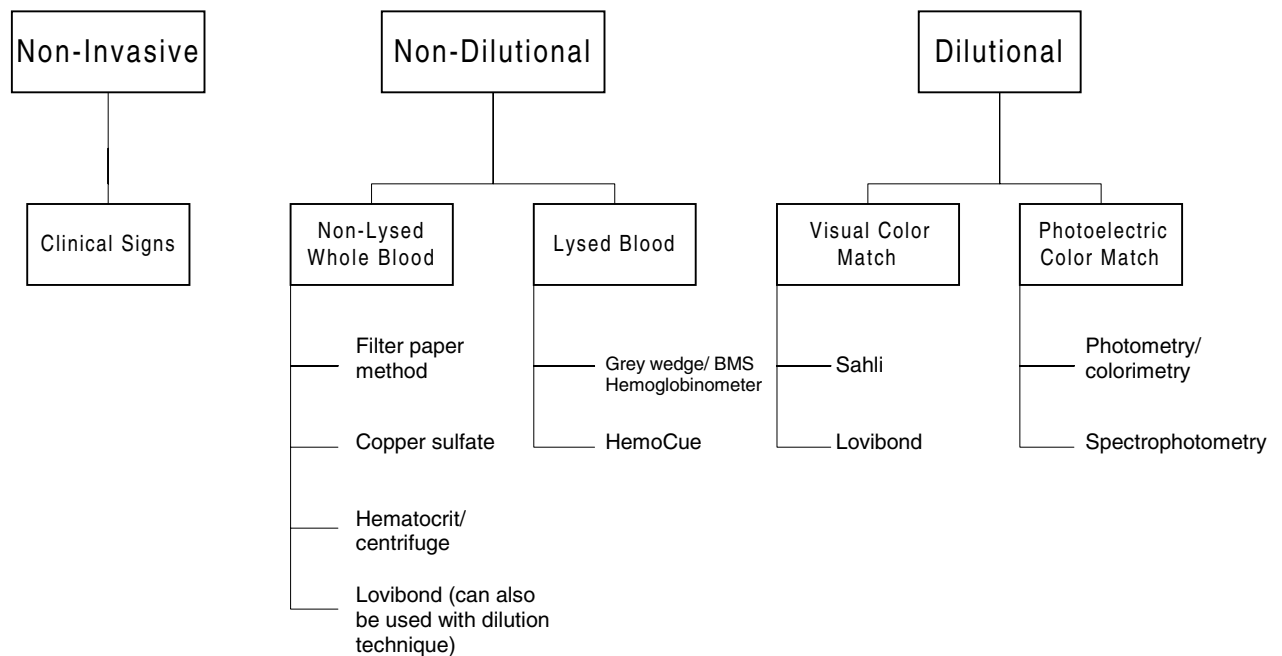
Photoelectric Color Match

Compounds: Drabkin's solution \Rightarrow cyanmethemoglobin
ammonia \Rightarrow oxyhemoglobin

- Photometry/colorimetry
- Spectrophotometry

TECHNOLOGIES BY CATEGORY

On the following pages are descriptions of individual technologies currently available. The basic mechanism for each technology is described briefly, along with its levels of use and advantages, its limitations, sensitivity and specificity, necessary equipment and supplies, and ways to lessen problems associated with its use. The technologies are presented in the order of the categories noted on the chart below.



NAME: Clinical Signs

CATEGORY: No Blood Sample,
Non-Invasive

DESCRIPTION

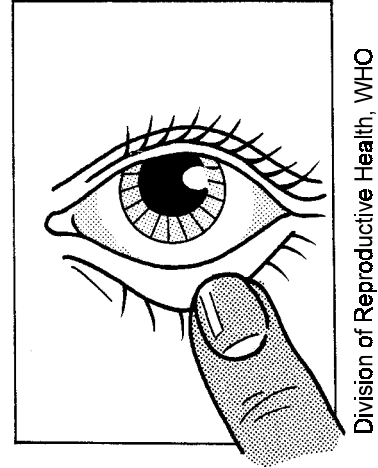
Inspection of conjunctivae, nailbeds, gums, and skin for pallor.

LEVELS OF USE/ADVANTAGES

- Used at the village level of health care.
- Minimal training and equipment required.

LIMITATIONS

- Method is highly subjective.
- Clinical inspection will not detect mild anemia.
- Adequate light source is required.



SENSITIVITY*/SPECIFICITY†

- Sensitivity with a color conjunctival chart ranges from 16% to 38%. It can be as high as 68% with experienced examiners.
- Sensitivity without a chart improves to 64% if hemoglobin is below 70g/L (severe anemia).
- Specificity ranges from 70% to 100%.

EQUIPMENT

- Color conjunctival charts may improve accuracy in some settings.

LESSENING PROBLEMS OF USE

- Health care providers may improve accuracy if actual hemoglobin values are used for feedback during training.

* The ability of a screening test to give a positive result when a condition is present (expressed as a percentage).

† The ability of a screening test to give a negative result when a condition is absent (expressed as a percentage).

NAME: Filter Paper Methods
Formerly: Talqvist
CATEGORY: Non-Dilutional,
Non-Lysed

DESCRIPTION

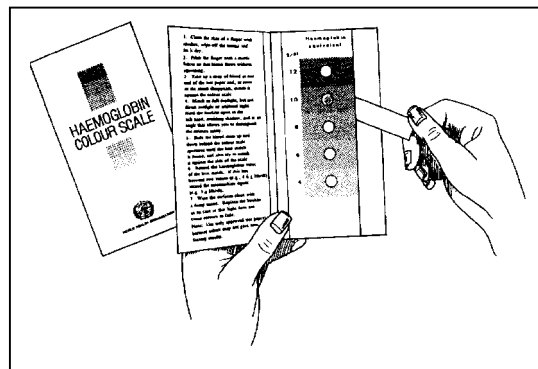
A capillary blood spot collected directly on filter paper is compared to a printed set of color standards.

LEVELS OF USE/ADVANTAGES

- Most useful for screening in rural settings.
- Inexpensive, simple, portable, and rapid.

LIMITATIONS

- Method is highly subjective.
- Inappropriate as a stand-alone test.
- Lighting conditions influence test result.
- Size and thickness of blood spot, temperature, and humidity all affect drying time, which, in turn, affects color.



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SENSITIVITY/SPECIFICITY

- Sensitivity and specificity of 60% at 100g/L.
- Accuracy increases at hemoglobin levels less than 90g/L.

EQUIPMENT

- Standard blotting/filter paper.
- Color comparison charts.

LESSENING PROBLEMS OF USE

- Adequate and consistent lighting conditions are important for consistent color matching.
- Lamination of color chart will provide better durability under field conditions.
- New formats are being developed that may improve performance and reliability.

NAME: Copper Sulfate
CATEGORY: Non-Dilutional,
Non-Lysed

DESCRIPTION

The test is based on the fall (or flotation) of a drop of whole blood in a copper sulfate solution of a known specific gravity. A drop of blood will sink or float for the first 10 to 15 seconds, indicating if the specific gravity of the blood is equal to, greater than, or less than that of the copper sulfate solution.

LEVELS OF USE/ADVANTAGES

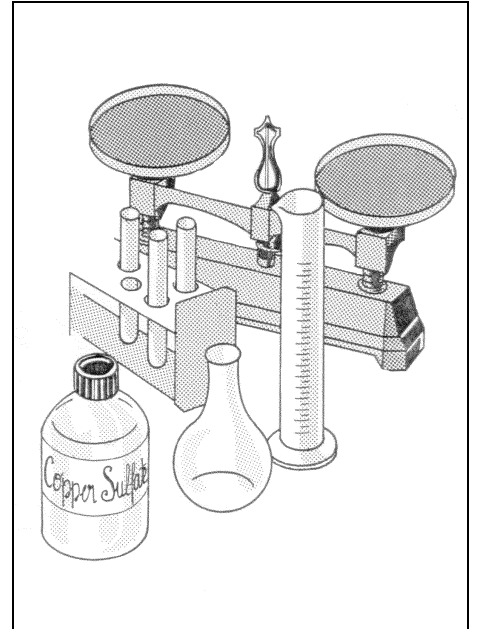
- Useful for screening programs.
- Simple, inexpensive, and rapid.
- Less subjective than visual color match methods such as filter paper, Lovibond, and Sahli.
- Solutions can be prepared to measure a range of hemoglobin levels.
- Solutions have a long shelf-life if tightly sealed to prevent evaporation.
- A 100ml solution can be used for as many as 50 specimens.
- Electricity not required.

LIMITATIONS

- Only gives ranges of hemoglobin levels.
- Stock solutions and dilutions must be made with precision.
- Must properly dispose of stock solutions containing blood.
- Error is introduced after 50 tests have been performed and increases progressively with continued use.

SENSITIVITY/SPECIFICITY

- Sensitivity of 87.5% and specificity of 99%.
- Test more sensitive at hemoglobin levels <90g/L.
- Interpretation is easier at low hemoglobin levels, as the drop of blood will remain suspended in solution for a longer time.



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EQUIPMENT

- Reagent-grade cupric sulfate.
- Glass containers for storage and dilutions.
- Hydrometer to measure specific gravities.
- Analytic balance/graduated cylinders/stir plate.

LESSENING PROBLEMS OF USE

- Can use either blood collected from a finger, heel, or earlobe stick in a capillary tube or from a venipuncture sample put into an EDTA blood collection tube.
- Blood sample must be large enough to form a free-falling drop from the optimum distance of 1cm above the solution.
- Blood sample should be dropped from a capillary pipette, a syringe, or a dropper, not directly from a finger.
- Solution should be carefully observed for first few seconds after releasing drop for accurate interpretation.
- Specific gravity of the solutions can be readjusted by adding 0.2ml or 0.4ml more of the standard solution.
- A piece of paper can be taped at the bottom of the tube and used to tally the number of drops put into the solution.
- A smaller drop size permits more tests per solution.

NAME: Hematocrit, Centrifuge
CATEGORY: Non-Dilutional,
Non-Lysed

DESCRIPTION

Whole blood is collected in a microhematocrit tube and centrifuged at sufficient speed (7,000-9,000 revolutions per minute) and for sufficient time to pack red blood cells into a mass measured on a reader as a percentage of total blood volume.

LEVELS OF USE/ADVANTAGES

- Useful at a health center level.
- Simple procedure.
- Several specimens can be measured at one time.

LIMITATIONS

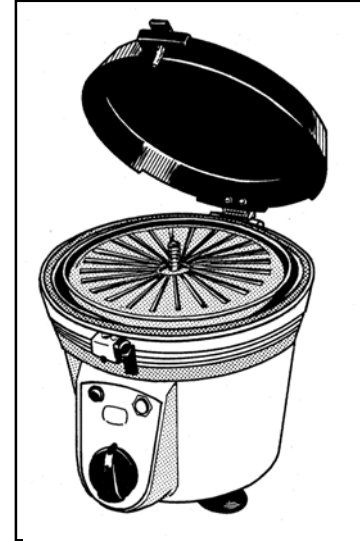
- Electricity- or battery-dependent (high consumption).
- Power supply must be consistent to get a true indication of packed cell volume.
- Sometimes difficult to differentiate between individual specimens once they have been placed in the centrifuge.
- Heating of the centrifuge may cause some lysis.
- Lysis of the blood sample will cause error in reading.

SENSITIVITY/SPECIFICITY

- Sensitivity >90%.
- Accuracy depends on consistent centrifugal speed.

EQUIPMENT

- Centrifuge with power supply.
- Plain capillary tubes, or tubes impregnated with heparin (an anti-coagulant), and clay sealant.
- Reference chart to calculate hematocrit value.
- Most microhematocrit centrifuges use standard size capillary tubes (70mm to 75mm in length and 1mm diameter).



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- Heparinized tubes are used for capillary blood samples obtained from finger, heel, or earlobe sticks.

LESSENING PROBLEMS OF USE

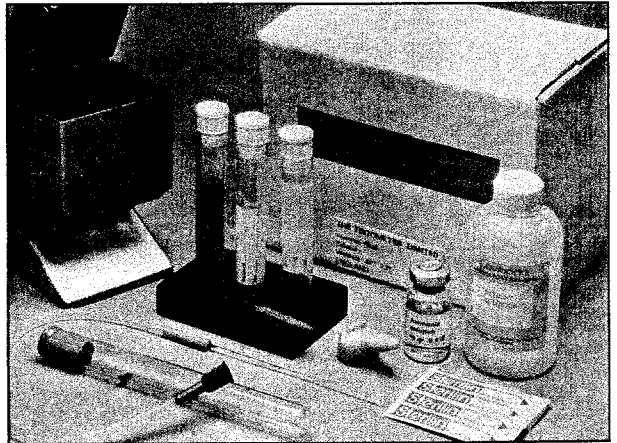
- Important to measure hematocrit within 6 to 8 hours after blood specimen taken.
- Revolutions-per-minute of centrifuge should be checked weekly with a tachometer or with a strobe light.
- Alternative method for checking the speed is to run the centrifuge at a range of speeds and times to ensure maximum cell packing and to check results against the cyanmethemoglobin method.
- Attach capillary tube to patient identification paper until test is performed.
- Plain capillary tubes should be used to determine the hematocrit when blood has been collected by venipuncture and put into tubes containing EDTA anticoagulant.

NAME: Lovibond Comparator

CATEGORY: Non-Dilutional Non-Lysed/
Dilutional Non-Lysed

DESCRIPTION

Either a whole blood sample or a blood sample diluted in ammonia or Drabkin's solution is put into a tube and compared to a series of colored glass standards (discs), using a visual comparator. The depth of the red color of each glass standard corresponds to a certain hemoglobin level.



LEVELS OF USE/ADVANTAGES

- The undiluted method is useful for routine screening.
- Does not require electricity.
- Simple and rapid method.
- Can measure a range of hemoglobin levels.
- Can be used with whole blood.
- Durable device.
- Comparator can also be used to measure blood glucose and urea with appropriate discs.

LIMITATIONS

- Requires subjective color matching.
- Initial cost of hemoglobin discs is high.
- Requires precise dilutions and calibrated pipettes for the dilutional method.
- Requires large drop of blood (50 μ l).
- Requires resupply of ammonia for the dilutional method.
- Needs to be read in direct sunlight.

SENSITIVITY/SPECIFICITY

- The non-dilutional method yields sensitivity and specificity values in the 90% range.
- Accuracy is high compared with the reference method.

EQUIPMENT

- The Lovibond disc (colored glass hemoglobin standards).
- The Lovibond comparator.
- Two Lovibond cells (tubes to hold the blood samples).
- Calibrated pipettes (50 μ l) for the dilution method.

LESSENING PROBLEMS FOR USE

- Take several readings on each specimen and average the results.
- Use the non-dilutional method where possible.

NAME: BMS Hemoglobinometer
Formerly: MRC Grey Wedge, A.O. Spencer
CATEGORY: Non-Dilutional, Lysed

DESCRIPTION

A glass chamber is filled with blood that has been lysed with saponin, then placed in a viewing instrument. A grey wedge in the viewer is moved until the two color fields match, and the results are read from a scale on the side.

LEVELS OF USE/ADVANTAGES

- Useful as a screening device in clinics.
- Accurate, portable, and inexpensive.
- A permanent glass standard is provided.
- Less subjective than filter paper or Sahli methods.

LIMITATIONS

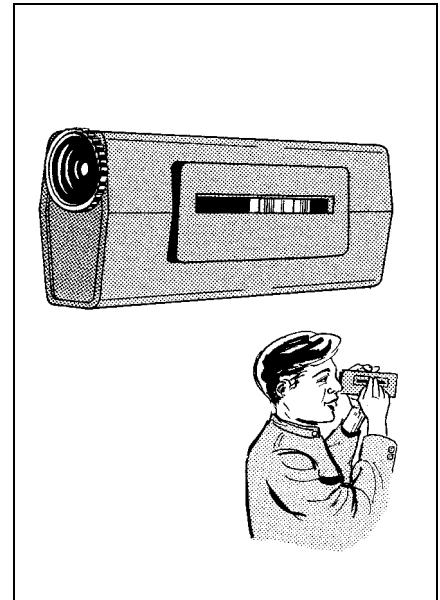
- Color matching is subjective.
- Glass chamber must be cleaned between uses.
- Blood specimen can disperse over chamber leading to difficulty in interpretation.
- Glass cuvette (blood collection receptacle) is very small.
- Can only process one specimen at a time.
- Each reading takes approximately 2 minutes, and it takes an additional 5 minutes between uses to clean and thoroughly dry the cuvette.
- Blood clots will result in an incorrect reading.

SENSITIVITY/SPECIFICITY

- Sensitivity of 77.5% and specificity of 96%.

EQUIPMENT

- BMS Hemoglobinometer.
- Size C batteries.
- Saponin sticks.
- Detergent.



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LESSENING PROBLEMS OF USE

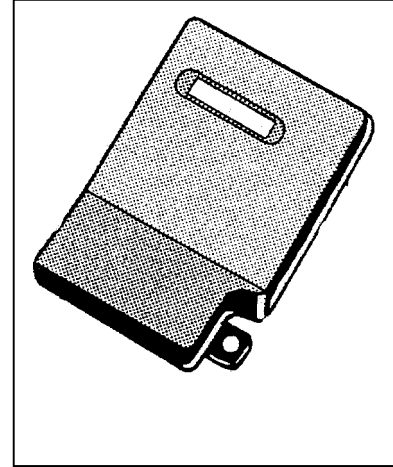
- Place small drop of blood from finger stick directly into chamber when cuvette is partially within clip.
- Cuvette is reusable, but it is useful to supply at least two.
- Cuvette should be thoroughly dry between uses or residual water will dilute the sample and falsely lower the results.
- A drop of blood from a finger stick can be placed directly onto the cuvette.

NAME: HemoCue

CATEGORY: Non-Dilutional, Lysed

DESCRIPTION

Whole blood is converted to azide methemoglobin in a disposable, chemically treated cuvette and then measured photometrically at a specified wavelength (565nm). The hemoglobin value is displayed digitally.



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LEVELS OF USE/ADVANTAGES

- Useful for surveys or where high accuracy is important.
- Method provides accurate, objective measurements comparable to cyanmethemoglobin method.
- Blood specimen needs no processing.
- Instrument is portable.
- Results are available in less than 45 seconds (entire procedure).
- Results are read directly without calculation.
- A permanent stable glass standard is available.
- Method can use either rechargeable nickel cadmium batteries or electricity.
- Little user training required.
- No reusable components to be cleaned between uses.
- Device lasts 5 to 7 years before components need replacing.

LIMITATIONS

- Cost of the instrument is very high.
- Uses only expensive, disposable cuvettes.
- Creates solid waste.
- High humidity can adversely affect performance.

SENSITIVITY/SPECIFICITY

- Sensitivity of 85% in field conditions, approaches 100% in controlled laboratory settings.
- Specificity of 94%.
- Sensitivity and specificity obtained from a range of hemoglobin values from 60g/L to 160g/L.

EQUIPMENT

- HemoCue instrument.
- Nickel cadmium batteries.
- Standard control cuvette.
- Disposable cuvettes.

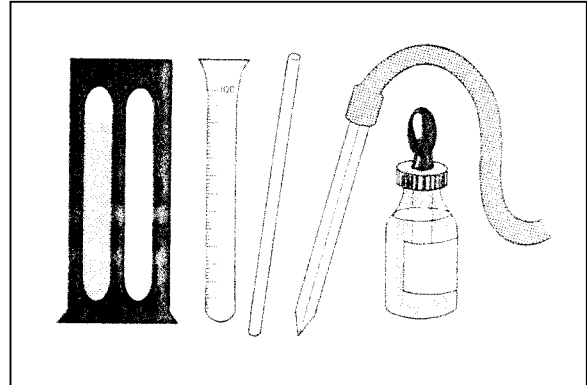
LESSENING PROBLEMS OF USE

- Careful cleaning and maintenance is crucial.
- An initial calibration should be done on a large number of blood samples to see if the machine has any built-in bias.
- Cuvettes fill directly from a finger stick.
- Nickel cadmium batteries will work better if allowed to run down completely before recharging.

NAME: Sahli
CATEGORY: Dilutional,
Visual Color Match

DESCRIPTION

Whole blood is pipetted into dilute hydrochloric acid (0.1mol), hemolyzed and converted to acid hematin. The solution is further diluted until the color matches that of two identical standards placed to the left and right of the dilution tube. The hemoglobin concentration is read from the graduated scale on the dilution tube as g/dl or as a percent of normal.



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LEVELS OF USE/ADVANTAGES

- Method is suitable for clinic use.
- Cost is low for procurement and re-supply.
- Test is relatively easy to perform.
- Electricity not required.

LIMITATIONS

- Results are subjective.
- Results are less accurate if readings are taken in fading daylight or in artificial lighting.
- Standard is not a true color match for the diluted blood.
- Graduated tubes must be cleaned between uses.
- Pipettes are impossible to clean once plugged with blood.
- Mouth pipetting should be discouraged.
- Glass pipettes are easily broken.
- Pipetting must be done carefully to avoid errors in measurement.
- Brown glass standards can fade with time.
- Easy to overshoot endpoint when adding diluent.
- Must properly dispose of acid hematin solution.
- Not suitable for photometry.
- A single measurement with the Sahli takes approximately 3 to 5 minutes; another 5 to 10 minutes is necessary for cleaning and drying the measuring tube between uses. Timing is crucial because it requires 3 to 5 minutes for the reagent to reach its peak. Readings must be taken immediately because levels fall quickly.

SENSITIVITY/SPECIFICITY

- Sensitivity is 85% to 90% and specificity is 85% to 100%.
- Accuracy is better at hemoglobin levels <100g/L.
- Accuracy may improve with operator practice.
- Precise sampling and dilution preparation are important for accurate results.

EQUIPMENT

- Sahli hemoglobinometer with brown standards.
- Sahli pipette (or 20µl microcapillary tube).
- Measuring tube.
- Stirring rod.
- Dropping pipette.
- 0.1mol/L (0.1N) hydrochloric acid.
- Small brush.

LESSENING PROBLEMS OF USE

- Wipe blood from exterior of Sahli pipette to accurately deliver the 20µl of blood.
- Allow sufficient time (3 to 5 minutes) for full reaction before taking measurement.
- Clean pipette immediately after use and rinse with cold water to prevent build-up of protein and plugging of pipette.
- Rinsing with hot water and/or alcohol will “fix” the blood and result in a plugged pipette.
- Advisable to have extra disposable 20µl capillary tubes available.
- Some brands of Sahli equipment use glass standards claimed to be non-fading.
- Visual matching should be done by taking a reading when facing a window, with the daylight behind the scale, as recommended by WHO Manual of Basic Laboratory Techniques.

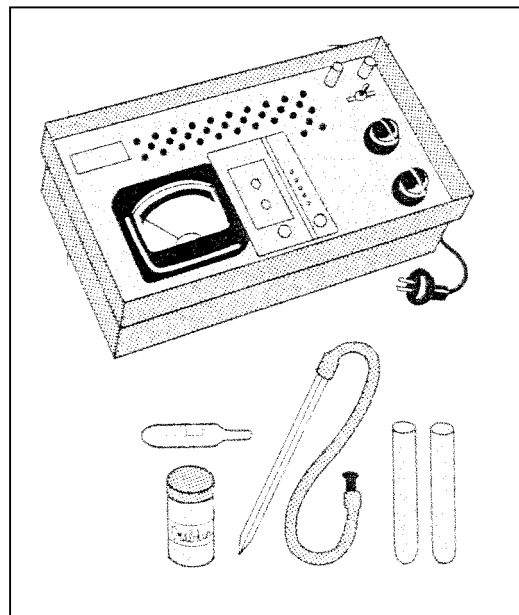
NAME: 1. Photometry Colorimetry
2. Spectrophotometry

CATEGORY: Dilutional Photoelectric
Color Match

DESCRIPTION

To accurately measure hemoglobin, photoelectric devices are used to assess the amount of light absorbed by a blood sample. When a colored solution is illuminated with visible light, certain wavelengths of light will be absorbed while others will be transmitted. By measuring the amount of light absorbed, one can measure the concentration of a substance in the colored solution.

The hemoglobin level is derived by comparing absorbance of the sample to known standards. Cyanmethemoglobin and oxyhemoglobin are the two compounds most commonly used for spectrophotometric and photometric/colorimetric measurements. Two types of instruments can be used to measure absorbance: filter photometers/colorimeters and spectrophotometers.



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LEVELS OF USE/ADVANTAGES

- Technique is suitable mostly for central reference laboratories.
- Method is highly accurate; results are objectively quantified.
- The cyanmethemoglobin method is the international standard for hemoglobin determination, as stable reference solutions are available for calibration.

LIMITATIONS

- Technique requires extremely accurate measurements.
- Sophisticated equipment is necessary.
- Reliable, stable supply of electricity required.
- Requires handling and disposing of toxic reagents, such as cyanide.
- Requires developing a calibration curve.
- Processing time when blood must sit in Drabkin's solution can be as long as 10 minutes prior to taking a reading.

- Cloudiness of the sample (caused by hyperlipidemia or hyperproteinemia) will alter the result.
- Oxyhemoglobin method does not have a permanent standard and requires preparation of a standard using a locally obtained fresh blood sample.

SENSITIVITY/SPECIFICITY

- Sensitivity approaches 100%.
- Specificity is greater than 90%.

EQUIPMENT

- Filter photometer, colorimeter, or spectrophotometer (range from simple to sophisticated).
- Drabkin's reagent.
- Calibrated pipette (to 20 μ l).
- Amber bottles.

LESSENING PROBLEMS OF USE

- Inspect specimens for cloudiness prior to taking measurement.
- Allow sufficient time for reaction to go to completion before taking measurement.
- Cloudiness can be minimized by the addition of surfactant (detergent-like additive) to the solution.

COLLECTION, HANDLING, AND DISPOSAL OF BLOOD SAMPLES

The amount of blood needed to measure the hemoglobin or hematocrit levels depends on the method used. Capillary blood samples (obtained by finger, heel or earlobe stick) are adequate for most anemia detection methods. Capillary blood is 1% to 3% lower in red cell volume than venous blood. When anemia is severe, results derived from capillary blood are less accurate than venous blood. If blood is collected by venipuncture for other tests and placed in a tube containing an anticoagulant, this sample also can be used for anemia detection.

When taking blood samples from children, especially those who are malnourished, health care workers may want to obtain the specimen from the earlobe. This area is not as sensitive as the finger tip. Both the lancet and the blood are not visible to the child if the worker stands behind while taking the specimen. Pricking the earlobe after rubbing it allows gravity to produce a good size drop.

If specimens are taken from patients who have been waiting in the hot sun for several hours, they are likely to show higher hemoglobin levels due to dehydration.

Proper training in collection of blood samples (capillary or venous) is crucial. For instance, excess squeezing of the finger tip is sometimes done to get an adequate drop of blood. This practice introduces extra plasma and falsely lowers the hemoglobin reading.

Supplies

Supplies needed to collect capillary samples from finger, heel or earlobe sticks include:

- alcohol and gauze (to clean the skin)
- lancets (to make the incision)
- capillary tubes to collect the blood sample

The supplies needed to collect venipuncture samples are:

- alcohol and gauze (to clean the puncture site)
- needle and syringe
- blood collection tubes containing EDTA anticoagulant
- plain capillary tube (used to collect a sample of blood from the EDTA tube).

National guidelines for the safe handling of blood products should be used. Health care workers must be trained in the safe collection, handling, and disposal of blood products and items with blood on them. Where available, gloves should be worn when performing a venipuncture or finger, heel and earlobe sticks. All blood samples should be considered

potentially infectious and handled with care. Mouth pipetting of blood samples or chemicals should be avoided. Proper hygiene such as frequently washing hands with soap and water will reduce the risk of exposure to infectious materials.

Waste disposal is an important consideration when choosing anemia detection devices. Proper disposal of sharp items protects the health care worker from possible infection acquired from an accidental cut or needle stick with used sharp blood collection materials. Sharp items, such as needles and lancets, must be collected in a puncture-proof container (plastic, glass, or metal) and then burned. Glass blood collection tubes, syringes, and used gauze should be placed in a separate cardboard container and burned.

GLOSSARY OF TERMS

Accuracy	The extent to which the measured value agrees with the true value.
Acid hematin	The compound that results when hemoglobin is added to hydrochloric acid. Used in the Sahli method.
Colorimetry	A procedure to measure specific wavelengths of light through a solution by means of a colored filter. Also called filter photometry.
Cyanmethemoglobin	The compound that results when Drabkin's solution is added to hemoglobin. When measured photometrically at 560nm, the method is considered the "gold standard" for anemia detection.
EDTA	Ethylenediamine tetra acetic acid. Compound present in blood collection tubes and serves as an anti-coagulant.
Drabkin's solution	See above. It consists of potassium ferricyanide, potassium cyanide and a surfactant.
Hemoglobin	The pigment that gives color to red blood cells consisting of heme and a protein. Hemoglobin carries oxygen from the lungs to the tissues and carbon dioxide from tissues to lungs.
Oxymethemoglobin	The oxygenated form of hemoglobin used in several methods of anemia detection, including the BMS Hemoglobinometer, the Lovibond Comparator, and photometric methods.
Precision	The ability of an instrument to reproduce a measured value.
Saponin	A compound that dissolves the red blood cell wall and releases hemoglobin.
Sensitivity	The number of true positives correctly identified among all samples tested. Expressed as a percentage.
Specificity	The number of true negatives correctly identified among all samples tested. Expressed as a percentage.
Specific gravity	A measure of the density of a material in grams per milliliter, compared to the density of water. This principle is used in the copper sulfate method.
Spectrophotometry	A procedure that uses a continuous spectrum of light wavelengths to measure the concentration of a substance in a solution.

Literature Review of Anemia Detection Devices

Method	Sensitivity	Specificity	Sample Size	Limitations of Each Study	Comments
Clinical signs only	52.0	38.9	219 Healthy subjects	Interobserver variability high for three field workers who performed assessments. Cutoff for classification of anemia was 99g/L. Does not state ages nor gender of subjects. Inspection of conjunctiva alone—did not include other signs. Binary designation of pink or red was used.	Field workers improved in identifying health subjects as the study progressed. Low prevalence of anemia. Perception of color varies in individuals. ¹
	93.0	60.0	64 male agricultural workers	Sensitivity and specificity given for one observer. Overall scores not given. Hematocrit values, used as reference method, were found unreliable when compared with cyanmethemoglobin values. Inter- and intraobserver variability statements are made on the basis of five subjects rather than total number of subjects in study.	Interobserver variability was less than intraobserver variability. Previous clinical experience did not improve accuracy. Training using actual hemoglobin values may improve the accuracy of the technique. ²
	38.0	90.0	180 inpatients (mean age = 69)	Interobserver variability is high. Observers vary in cutoff criteria. Hospital setting may not translate to field use.	Not useful for moderate anemia. Increase sensitivity at Hb <70g/L. ³
Clinical signs with Anemia Recognition Card	N/A	N/A	568 children 207 adults 151 pregnant women 926 Total	Overall sensitivity and specificity not given, although sensitivity and specificity are 100% for severely anemic (Hb <60g/L). Half of patients with Hb of 90-110g/L were correctly identified. Interobserver variability not described.	Village workers can be trained to detect severe anemia (Hb <90g/L) with the use of the Anemia Recognition Card. ⁴
	67.0	41.0	211 pregnant women	96% of women had Hb below 110g/L. Cutoff of 80g/L for classifying anemia may be too low.	The Anemia Recognition Card was not useful in distinguishing moderate from severe anemia in this study. ⁵
	63.0	72.0	200 randomly selected outpatients 200 randomly selected employees	Conjunctivitis and other eye diseases may confound result. Only three subjects had Hb below 80g/L.	Sensitivity improves with Hb <110g/L. Training improves performance Interobserver variability is minimized with chart. ⁶
Filter paper method	70.0	65.0	100 determinations	Study conducted in laboratory; may not translate to field conditions	Values improve in high anemia prevalence. ⁷
	60.5	59.1	100 determinations	Sensitivity and specificity values are given only for Hb at 100g/L for field assessment. Whole blood was diluted with saline to provide a range of Hb values for laboratory assessment.	The paper filter method was not sufficiently reliable when used in the field. A range of hemoglobin values would be more meaningful to assess ability to identify low values. Difficulty in color matching. ⁸

Method	Sensitivity	Specificity	Sample Size	Limitations of Each Study	Comments
Copper Sulfate	87.5	98.9	100 determinations	Sensitivity and specificity values are given only for Hb at 100g/L	Specificity improves with Hb <100g/L. Health workers perceive this method as less scientific. Less certainty in how to treat based on results obtained with this method. ⁸
	75.8	N/A	218 determinations	Use of blood collected in EDTA may not be appropriate for the copper sulfate method.	The copper sulfate method had good agreement with the reference method proving it to be an appropriate method for screening. Accuracy improved at higher hemoglobin levels in this study. ⁹
Spencer Hemoglobinometer (BMS)	77.5	95.9	100 determinations	Sensitivity and specificity values are given only for Hb at 100g/L.	The Spencer was well liked, easy to use, and the preferred instrument when compared with Tallqvist, copper sulfate, and Lovibond. ⁸
	90.0	93.7	120 measurements	Number of operators and amount of training is not described.	High anemia prevalence data is given. When instrument is used to test a low prevalence population: Sensitivity=98.9/Specificity=99.9. ¹⁰
	N/A	N/A	100 determinations	Study assesses interobserver variability in estimating Hb level—not of actual sample preparation.	BMS performed accurately across a broad range of hemoglobin values. ¹¹
Hematocrit (PCV) automated	97.0	98.2	120 measurements	Number of operators and amount of training is not described.	High anemia prevalence data is given. When instrument is used to test a low prevalence population: Sensitivity=99.9/Specificity=99.9. ¹⁰
Hematocrit (PCV) manual	90.1	93.7	120 measurements	Requires simple calculations to determine hematocrit. Cannot be sure of adequate rotational speed or time.	High anemia prevalence data is given. When instrument is used to test a low prevalence population: Sensitivity=98.9/Specificity=99.9. ¹⁰
Lovibond (undiluted technique)	87.4	91.9	100 determinations	Study performed under laboratory conditions.	High anemia prevalence data is given. When instrument is used to test a low prevalence population: Sensitivity=98.1/Specificity=99.9. Easy and quick method. Very low interobserver variability. ⁷
	100	58	48 determinations	Sensitivity and specificity values improve with low Hb ranges <100g/L.	Specificity was unacceptably low. Matching colors was difficult. Error is introduced in making dilutions. ⁸
Sahli Technique	85.0	85.0	100 determinations	Study performed under laboratory conditions.	Cumbersome to use. Use of manual pipettes may introduce error when making dilutions. ⁷
HemoCue Hemoglobinometer	85.0	94.0	102 determinations	Method of blood collection influences results.	Operator training did not influence result—no difference in first half of study compared to second half. ¹²
	88.5	77.6	103 samples	Blood samples were collected in EDTA tubes; cuvettes were not filled directly from the finger stick method.	Accuracy was decreased considerably in actual usage as compared to laboratory evaluations; important to evaluate equipment intended for primary care by primary care staff. ¹³
	75.0	86.3	59 volunteers	Cutoff levels to determine sensitivity and specificity were adjusted according to sample site, e.g., finger stick versus earlobe. Study does not describe who performed testing or amount of training.	Standardization for blood donors may be achieved by specifying both required hemoglobin levels and minimum performance for screening methods. ¹⁴

REFERENCES FOR LITERATURE REVIEW OF ANEMIA DETECTION DEVICES

- ¹Sanchez-Carrillo CI. Bias due to conjunctiva hue and the clinical assessment of anemia. *Journal of Clinical Epidemiology* 42 (8): 751-754 (1989).
- ²Glass R, Batres C, Selle C, Garcia-Ibanez R, Solomons N, Viteri F. The value of simple conjunctival examination in field screening for anemia. *Nutrition Reports International* 21 (3):405-412 (1980).
- ³Gjorup T, Bugge PM, Hendriksen C, Jensen AM. A critical evaluation of the clinical diagnosis of anemia. *American Journal of Epidemiology* 124 (4):657-665 (1986).
- ⁴Ghosh S, Mohan M. Screening for anemia (letter). *The Lancet* 1 p823 (April 15, 1978).
- ⁵Gujral S, Abbi R, Anderson MA, Christian P, Gopaldas T. Agreement between haemoglobin estimation and anaemia recognition card in assessment of anaemia in pregnant women. *European Journal of Clinical Nutrition* 43:473-475 (1989).
- ⁶Sanchez-Carrillo CI, Ramirez-Sanchez TJ, Zambrana-Castaneda M, Selwyn BJ. Test of noninvasive instrument for measuring hemoglobin concentration. *International Journal of Technology Assessment in Health Care* 5:659-667 (1989).
- ⁷van Lerberghe W, Keegels G, Cornelis G, Ancona C, Mangelschots E, van Balen H. Haemoglobin measurement: the reliability of some simple techniques for use in a primary health care setting. *Bulletin of the World Health Organization* 61(6):957-965 (1983).
- ⁸Stone JE, Simmons WK, Jutsum PJ, Gurney JM. An evaluation of methods of screening for anaemia. *Bulletin of the World Health Organization* 62(1):115-120 (1984).
- ⁹Politzer WM, Myburgh WM, van der Merwe JF. Haemoglobin estimation—reliability of the copper sulphate specific gravity v. the cyanmethemoglobin colorimetric method. *South African Medical Journal* 73(2):111-112 (January 23, 1988).
- ¹⁰Kegels G, Cornelis G, Mangelschots E, Van Brabant R, van Lerberghe W. Haemoglobin and packed cell volume measurement: the reliability of some simple techniques for use in surveys or rural hospitals. *Annales de la Société Belge de Médecine Tropicale* 64(4):413-419 (1984).
- ¹¹Linegar AG, Knottenbelt JD, Wormald PJ. Accuracy of a portable hemoglobinometer in clinical practice. *South African Medical Journal* 79:547-548 (May 1991).

¹²Mills AF, Meadows N. Screening for anaemia: evaluation of a hemoglobinometer. *Archives of Disease in Childhood* 64, 1468-1471 (1989).

¹³Neville RG. Evaluation of portable hemoglobinometer in general practice. *British Medical Journal* (Clin Res Ed) 294:1263-1265 (May 16, 1987).

¹⁴Chambers LA, McGuff JN. Evaluation of methods and protocols for hemoglobin screening of prospective whole blood donors. *American Journal of Clinical Pathology* 91(3):309-311 (March 1989).

ENDNOTES

- ¹ DeMaeyer EM. *Preventing and Controlling Iron Deficiency Anaemia Through Primary Health Care: A Guide for Health Administrators and Programme Managers*. Geneva: World Health Organization; 1989.
- ² World Health Organization. *The Prevalence of Anaemia in Women: A Tabulation of Available Information* (2nd Edition). Geneva: World Health Organization; 1992. (WHO/MCH/MSM/92.2).
- ³ World Health Organization. *The Prevalence of Anaemia in Women: A Tabulation of Available Information* (2nd Edition). Geneva: World Health Organization; 1992. (WHO/MCH/MSM/92.2).
- ⁴ DeMaeyer EM. *Preventing and Controlling Iron Deficiency Anaemia Through Primary Health Care: A Guide for Health Administrators and Programme Managers*. Geneva: World Health Organization; 1989.
- ⁵ United Nations. *Controlling Iron Deficiency*. Geneva: World Health Organization; 1991. (ACCS/SCN State-of-the-Art Series Nutrition Policy Discussion paper No. 9).
- ⁶ Yip R. Iron Deficiency: Contemporary Scientific Issues and International Programmatic Approaches. *Journal of Nutrition* 124:1479S-1490S (1994).
- ⁷ Dallman PR. Iron Deficiency: Does it Matter? *Journal of Internal Medicine* 226:367-72 (1989).
- ⁸ World Health Organization. *Prevention and Management of Severe Anaemia in Pregnancy* (Report of a Technical Working Group). Geneva: World Health Organization; 1991. (WHO/FHE/MSM/93.5).
- ⁹ World Health Organization and Food and Agriculture Organization of the United Nations. *Requirements of Vitamin A, Iron, Folate and Vitamin B₁₂*. Rome: Food and Agriculture Organization of the United Nations; 1988. (FAO Food and Nutrition Series No. 23).
- ¹⁰ United Nations. *Controlling Iron Deficiency*. Geneva: World Health Organization; 1991. (ACCS/SCN State-of-the-Art Series Nutrition Policy Discussion paper No. 9).
- ¹¹ World Health Organization. *Prevention and Management of Severe Anaemia in Pregnancy* (Report of a Technical Working Group). Geneva: World Health Organization; 1991. (WHO/FHE/MSM/93.5).

¹² World Health Organization. *Prevention and Management of Severe Anaemia in Pregnancy* (Report of a Technical Working Group). Geneva: World Health Organization; 1991. (WHO/FHE/MSM/93.5).

¹³ United Nations. *Controlling Iron Deficiency*. Geneva: World Health Organization; 1991. (ACCS/SCN State-of-the-Art Series Nutrition Policy Discussion paper No. 9).

¹⁴ DeMaeyer EM. *Preventing and Controlling Iron Deficiency Anaemia Through Primary Health Care: A Guide for Health Administrators and Programme Managers*. Geneva: World Health Organization; 1989.

¹⁵ World Health Organization

¹⁶ Yip R. Personal Communication.

¹⁷ World Health Organization. *Prevention and Management of Severe Anaemia in Pregnancy* (Report of a Technical Working Group). Geneva: World Health Organization; 1991. (WHO/FHE/MSM/93.5).

¹⁸ Yip R, Gove S, Farah BH, and Mursal HM. Rapid Assessment of Hematological Status of Refugees in Somalia: The Potential Value of Hemoglobin Distribution Curves in Assessing Iron Nutrition Status. *Colloque INSERM* 197:193-196 (1990).

¹⁹ Freire WB. Hemoglobin as a Predictor of Response to Iron Therapy and its Use in Screening and Prevalence Estimates. *American Journal of Clinical Nutrition* 50:1442-9 (1989).