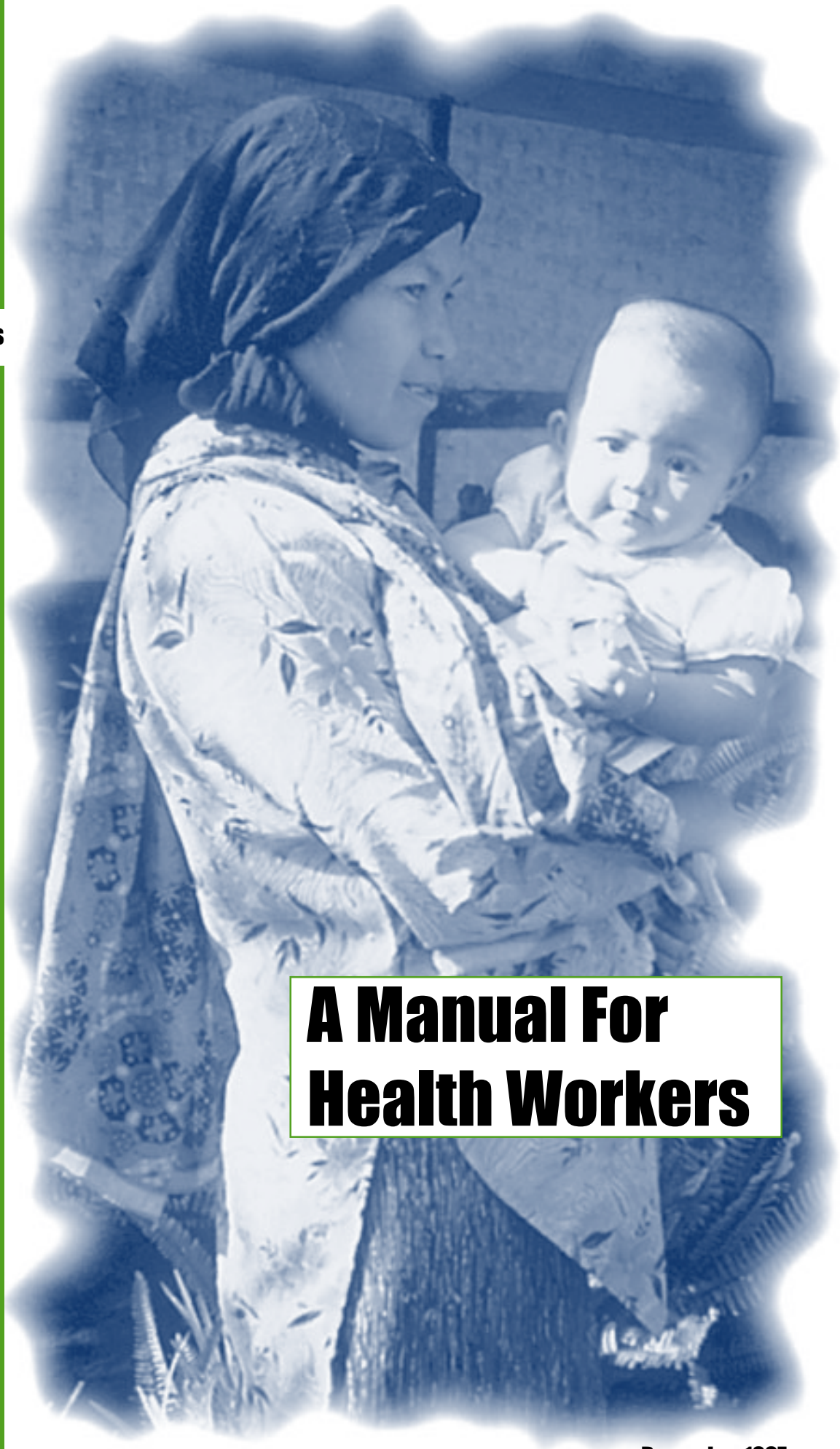
ANEMIA DETECTION METHODS

in Low-Resource Settings



A Manual For Health Workers

path

OMNI

USAID

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Anemia Detection Methods in Low-Resource Settings: A Manual For Health Workers

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Cover Photo: MotherCare Project

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As anemia persists in being a health concern throughout the world, we have attempted to write a manual that can be used by laboratory and health workers in many settings. The manual was sent to two locations in Indonesia and to Kenya and Tanzania for review by people familiar with various methods of detecting anemia under difficult working conditions. We would like to thank the following people for contributing to the development of the manual by thoroughly and thoughtfully reviewing its contents, sharing their insights regarding testing for anemia, and offering suggestions so that the manual addresses the realities faced by laboratory and health workers in low-resource settings:

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Table of Contents

Introduction

This manual describes common anemia detection tests for health workers in settings with limited resources such as rural clinics and hospitals. The manual provides instructions for performing the tests, suggestions for improving test performance, descriptions of test characteristics, advantages and disadvantages of each test, and the equipment and supplies needed for each test.

A manual aimed at helping administrators select tests appropriate to their situations, *Anemia Detection in Health Services: Guidelines for Program Managers*, was published in 1996. English, Spanish, and French editions can be obtained from OMNI (OMNI Project/JSI, 1616 N. Fort Myer Drive, 11th Floor, Arlington, Virginia 22209, USA).

Although many laboratory manuals provide instructions on how to perform anemia detection tests, they generally cover only a few of the methods available. Perhaps more importantly, few existing manuals provide information on how to optimize test performance under difficult field conditions, which are common in many developing countries. Laboratory technicians, service providers, and program supervisors often find existing reference materials cumbersome and at times incomplete with regard to anemia detection tests.

In this manual, step-by-step, illustrated instructions are provided for nine anemia detection methods:

- Clinical Signs
- Filter Paper Method
- Copper Sulfate Method
- Hematocrit by Centrifuge
- Lovibond Type Comparator
- Grey Wedge (BMS) Photometer
- Sahli Method of Hemoglobin Estimation
- Portable Hemoglobinometer (HemoCue)
- Colorimetry-Hemiglobincyanide

This manual has been extensively reviewed by international anemia experts and field tested by laboratory personnel in Africa and Asia. As part of this process, information was solicited about the key barriers facing laboratory workers conducting anemia tests, as well as the approaches they incorporated into their procedures to overcome or prevent difficulties that typically arise, particularly in lower-resource settings. Their suggestions for optimizing test performance have been included.

Chapter 1 provides basic but essential information on waste disposal, quality assurance, and record keeping as relevant to anemia detection. Chapter 2 focuses on safe blood collection and handling, and Chapter 3 presents instructions for using the nine tests. A glossary of commonly used terms and an appendix describing key factors to consider when evaluating anemia detection tests are included at the end of the manual.

Chapter 1—General Considerations in Anemia Testing

Definition of Anemia

Anemia is defined as having below normal values for the total volume of red blood cells, the number of normal red blood cells, or the amount of hemoglobin in these cells. Normal values are defined as those found in healthy populations. Anemia results from one or more of the following processes:

- Defective red cell production due to lack of essential nutrients in the diet, poor bioavailability of iron, or increased utilization of nutrients such as during pregnancy, lactation, or rapid growth periods.
- Increased red cell destruction (hemolysis) due to parasitic diseases such as malaria or genetic conditions such as sickle cell anemia or thalassemia.
- Blood loss, resulting from intestinal worm infestation, notably hookworm, or heavy menstrual flow.

Anemia is most commonly detected by measuring **hemoglobin** (the oxygen-carrying component in red blood cells) or by determining the **hematocrit** (the volume of red blood cells expressed as a percentage of the total blood volume).

The normal range for hemoglobin and hematocrit varies by age and sex as shown in the table below:

Normal Hemoglobin and Hematocrit Ranges

Population	Hemoglobin	Hematocrit
Infants (full term)	10.5 - 19.5 g/dl	32 - 60%
Children (1-9 years)	11.0 - 14.0 g/dl	33 - 40%
Children (10-12 years)	11.5 - 15.0 g/dl	35 - 45%
Men (adults)	13.0 - 18.0 g/dl	40 - 50%
Women (adults)	12.0 - 16.0 g/dl	36 - 44%
Pregnant women	11.0 - 14.0 g/dl	33 - 42%

Hemoglobin Ranges for Anemia **Children under five and pregnant women**	
Mild anemia	9.0 - 11.0 g/dl
Moderate anemia	7.0 - 9.0 g/dl
Severe anemia	<7.0 g/dl
Very severe anemia	<4.0 g/dl

Hemoglobin Ranges for Anemia **Children over five and nonpregnant women**	
Mild anemia	9.0 - 12.0 g/dl
Moderate anemia	7.0 - 9.0 g/dl
Severe anemia	<7.0 g/dl
Very severe anemia	<4.0 g/dl

Note: Hemoglobin levels are influenced by altitude. The formula developed at the U.S. Centers for Disease Control for calculating hemoglobin based on altitude, where altitude is known, is included in the Appendix.

Scope of the Problem

Nearly one quarter of the world's population is anemic. The groups most at risk of developing anemia include women of reproductive age (because of menstruation), pregnant and lactating women, and children from 6 months to 2 years of age (because of rapid growth). One half of the pregnant women in the world are anemic: in developing countries between 55% and 60% of pregnant women are affected while only 18% are affected in developed countries.

Maintenance of Equipment

Routine maintenance of the equipment used to detect anemia is crucial to obtain accurate and consistent results. Cleaning equipment after use is essential to proper maintenance. This includes cleaning pipettes and cuvettes.

If instruments require calibration, this must be done on a regular basis using standard calibrating devices. A preventive maintenance plan should be implemented to ensure that proper cleaning and calibration of the equipment are done on a regular basis and an inventory of parts that are likely to need replacement is available. It is important that a record book be kept of maintenance activities that include the date, type of maintenance, and any follow-up required.

Waste Disposal

Waste disposal is an important consideration when choosing anemia detection methods. The amount of waste generated varies depending on the method. Waste should always be rendered noninfectious by autoclaving or incineration, or buried in a specified and safe site when no other options are available. Waste disposal should be done on a daily basis, either immediately after work or before work the next day.

- *Needles and sharp items*

Proper disposal of sharp items protects the health care worker from possible infection from an accidental cut or needle prick. Sharp items, such as needles and lancets, must be collected in a puncture-proof container (plastic, glass, or metal such as a used coffee can), then autoclaved, incinerated, or buried. The container should be easily recognized (orange or red colored containers are good) and clearly marked as containing biologically hazardous waste (*Figure 1*).

Figure 1

- *Blood collection tubes, syringes, and used cotton wool*

Where blood collection tubes and reusable syringes are reused, they should first be rinsed thoroughly with water, soaked in detergent for two to three hours, and dried. Disposable syringes and cotton wool should never be reused. These items should be placed in a cardboard container before being incinerated or buried.

- *Disposal of blood and infected materials*

Blood and processed blood specimens can be disposed of in a plastic bucket with a lid containing a 0.5% hypochlorite solution (bleach) *(Figure 2)*. If local health codes permit, the bucket can be poured into a sink and flushed with running water. If not, dispose of the materials as instructed by the local health code.

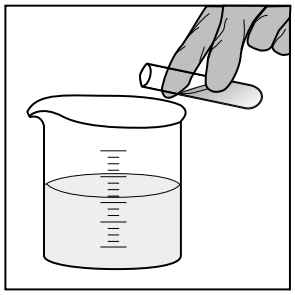


Figure 2

Immediately after completing the screening tests, the work area must be disinfected with 0.5% hypochlorite solution. All wrappings and soiled cotton wool should be put in the appropriate receptacle. Pipettes and tubes should be rinsed in water, then soaked in a 0.5% hypochlorite solution for at

least 30 minutes. Blood spills or centrifuge tube breakage should be cleaned using a 0.5% hypochlorite solution. Hypochlorite solutions should be prepared freshly each day and kept in containers with secure lids.

Good Laboratory Practices

Good laboratory practices include using established guidelines to ensure that standard laboratory and safety procedures are followed. It is important that there is adequate work space to avoid crowding, which, in turn, will reduce the risk of accidents. The laboratory room should be adequately ventilated. Laboratory surfaces and floors should be made of materials that can be easily cleaned and disinfected. Eating, drinking, and smoking should not be permitted in the laboratory at any time. Adequate and conveniently located puncture proof containers for the disposal of biological contaminated materials must be available.

Quality Assurance

Quality assurance is a concept that covers all aspects that can influence the quality of a product or a process. Quality assurance, as related to anemia detection, includes correct blood collection and handling, adherence to proper testing procedures, the use of reliable instruments and reagents, proper equipment maintenance, adequate record keeping and reporting, correct interpretation of laboratory test results, and systems to check the reliability of results.

Accuracy and precision are important in all laboratory work. *Accuracy* is a measure of how close an estimated value is to the true value. *Precision* is the degree of agreement between values obtained for repeat measurement of the same sample. Two important elements of overall quality assurance include *internal quality control,* which evaluates and controls precision, and *external quality control* which evaluates and controls accuracy.

<u>Internal Quality Control to Ensure Test Standardization</u>

Internal quality control measures are performed within the laboratory to ensure test-to-test consistency. Standardization of test procedures is essential.

The anemia detection methods described in this manual are standardized using a calibrator or reference standard. A calibrator is a device that conforms to a reference standard and is used to calibrate, or adjust, an instrument so that its readings are accurate. Reference standards, which are blood samples with a known hemoglobin concentration, are used to ensure that equipment and reagents are functioning correctly and that personnel are performing the procedures correctly and maintaining accuracy. If a reference standard is not available, blood samples can be obtained from a central laboratory where the hemoglobin values have been previously determined by an accurate method. A good method of checking day-to-day reproducibility, i.e. how well the test is performing, is to make three separate assessments on three different blood samples.

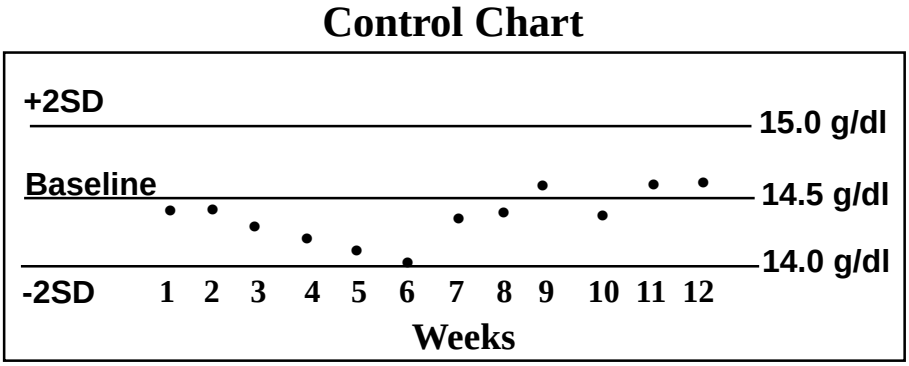
It is helpful if laboratory personnel are familiar with basic statistical concepts, such as calculating means, standard deviations, and coefficients of variation. The Appendix describes these concepts more thoroughly.

A control chart is a useful tool for monitoring trends with the use of reference standards. The mean and standard deviation for the reference standard as determined by the supplier are plotted against time in days. The line above the mean represents two positive standard deviations (+2SD) and one below the mean represents two negative standard deviations (-2SD). Each time the reference standard is used, its value should be plotted on the chart. Trends can be easily seen to determine

whether errors are random or whether readings have a tendency to rise or fall. The precision of the test should be questioned when reference standards deviate from the mean in the following ways:

- A value lies entirely outside the +/-2 standard deviation (SD) limits.
- Values are consistently in one direction and fall outside +/-2SD.
- Several consecutive values lie on one side of the mean.
- Two or more results per 20 tests lie on the +2 or -2 SD lines.

Anemia detection tests must be done with care, and tests with questionable results should be repeated. It is important to provide training so that laboratory personnel are able to recognize when results are not precise.



External Quality Control

External quality control determines the uniformity of test results between laboratories. To determine the accuracy of simple anemia detection devices, a portable instrument capable of providing precise and accurate measurements, such as a hemoglobinometer, can be used as the standard method. A regional or district hospital can provide blood samples with known hemoglobin values as a reference. Individual laboratories assess the hemoglobin concentration of the blood samples using their own test method to determine whether their method is accurate. If the results differ from the reference values by more than 0.5 g/dl for any of the photometric methods or by 1.0 g/dl for the visual comparator methods, then the sources of error must be determined as well as the correctional procedures.

Sources of Error

Errors can be either systematic or random. Systematic errors are introduced when things such as pipettes are improperly calibrated or reagents whose shelf life has expired are used. The error is systematic because it will occur every time the test is conducted until the source of error is discovered and corrected. Random errors are mistakes that occur by chance. An example of a random error is a sudden variation in ambient temperature that influences the test result.

The control of errors can be divided into three categories and include:

Pre-analytical Error Control Procedures
- Proper sample labeling
- Proper collection of the blood sample (wiping away the first drop, avoiding excessive squeezing)
- Collecting accurate blood volumes, avoiding bubbles where relevant
- Not using chipped or broken pipettes
- Wiping the outside of the capillary tube
- Using properly prepared reagents that are not outdated
- Using clean, distilled water
- Proper mixing of the sample

Analytical Error Control Procedures
- Correct use of the instrument; allowing sufficient time for warming up, calibrating, and taking the baseline measurements
- Checking that solutions are not turbid
- Avoiding the use of dirty cuvettes; using dry cuvettes so that blood samples are not diluted

Post-analytical Error Control Procedures
- Proper recording of results
- Proper dispatch of results to clinicians and health personnel

Where there are signs that error may have been introduced during the test procedure, the test must be repeated after the source of error has been eliminated.

Record Keeping

Proper record keeping is essential in any laboratory as it helps to ensure accuracy in diagnosing anemia and determining if treatment is effective. Blood specimens must be labeled with the date of collection and with either an identification number or the patient's name, depending on the system used.

It is important to keep a daily record of test results in a bound laboratory notebook in a central place in the laboratory, as loose reports are easily misplaced. The notebook should contain the following details: date; laboratory record number; patient's name, age, sex and residence; tests performed; the person who performed the tests; and test results. It is important to note any unusual findings on the specimen, such as the presence of clotted blood or lipid, as this may influence the result. In addition, records of all quality assurance and quality control exercises should be kept in a separate notebook.

Chapter 2—Safe Blood Collection and Handling

Because the infectious potential of any patient's blood or body fluids cannot be known without testing, all blood specimens and body fluids should be treated as though they are infectious. Health care workers must observe universal precautions when collecting and handling blood specimens. That is, the health care worker should avoid direct contact with any body fluid including blood specimens.

Guidelines for safe handling of blood products should be taught to all personnel who come in contact with blood. These guidelines should be posted visibly in the laboratory or clinic as a reference for staff. Health care workers must be trained in the safe collection, handling, and disposal of blood products and items with blood on them, as described below.

Avoiding Infection During Sample Collection

1. Health care workers should always wear a fresh, disposable pair of gloves when obtaining blood samples from patients, regardless of the method used to obtain the blood sample. If disposable gloves are not available, disinfect reusable gloves by swabbing them with 70% alcohol after each use with a patient. This will protect both the health care worker and the patient from accidental contamination with blood from a previous patient.

2. Pipetting of blood, blood products, or chemicals by mouth is dangerous and must never be done.

3. Always wash hands and other skin surfaces immediately after contact with blood or body fluids.

4. If blood is spilled on a surface, wear gloves to clean it up and wash the surface with a 0.5% hypochlorite solution.

5. In the case of accidental puncture with a used sharp object, immediately wash the affected area gently in running water while squeezing to ensure a free flow of blood. Cover the affected site with a bandage. Record all accidents, however minor, in the accident book and report them immediately to your supervisor.

6. Clean all working surfaces with a 0.5% hypochlorite solution after each use. Dispose of all used materials promptly and appropriately.

Considerations for Collecting Blood Samples

The amount of blood needed to measure hemoglobin or hematocrit levels will vary depending on the method used.

Capillary blood samples, obtained by finger, heel, or earlobe prick, are adequate for most anemia detection methods, although they are 1% to 3% lower in red cell volume than venous blood. When anemia is severe, results derived from capillary blood are less accurate than those from venous blood.

Venous blood samples are collected by venipuncture for other tests and placed in tubes containing anticoagulant. These samples can also be used for anemia detection.

Instructions for Collecting Blood

Prepare all supplies in advance and determine how much blood will be required for the tests to be performed. Having an adequate quantity of blood is important for getting an accurate result.

Reassure the patient that the blood draw may be slightly painful but will not take long. Tell the patient how much blood will be drawn.

Capillary Blood Collection

Capillary blood is obtained in adults and children from the fingertip or earlobe. In infants, the side of the heel is the preferred site. To ensure an adequate amount of blood from a fingerprick, run warm water over the fingertip, vigorously rub the fingertip, or shake the wrist to improve blood flow prior to capillary blood collection. With children, especially those who are malnourished, health care workers may obtain the specimen from the earlobe because this area is not as sensitive as the finger tip. Neither the lancet nor the blood are visible to the child if the worker stands behind the child while taking the specimen. To ensure an adequate amount of blood from the earlobe, rub the earlobe before puncturing.

Required Supplies

- 70% alcohol and cotton wool (to clean the skin)
- sterile, dry lancets (to make the incision)
- capillary tubes or Sahli blood pipette (to collect the blood sample)
- rubber tubing if using the Sahli blood pipette (to draw up the blood)
- beaker of saline solution (for flushing Sahli blood pipette after use)

Procedure

1. Swab the site using cotton wool soaked with 70% alcohol. Allow the site to dry.

2. Using a sterile, dry lancet, puncture the skin deeply enough so that large drops of blood emerge slowly but spontaneously (*Figure 3).*

   Do not squeeze the fingertip as this will dilute the blood sample with tissue fluid and result in an inaccurate low reading.

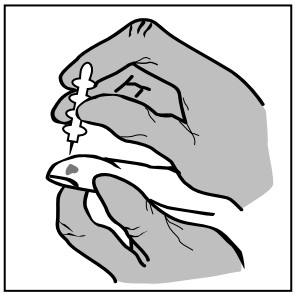


Figure 3

3. Wipe away the first drop of blood, as it is contaminated with skin particles, oil, and sweat.

4. If using a capillary tube, hold the capillary tube horizontally and allow the blood to flow into the tube until it is 75% filled.

   If using the Sahli blood pipette, fill the tube to the level required for the test. *Do not pipette by mouth.* Attach a piece of rubber tubing with a knot at one end of the pipette and draw up the blood by gently squeezing the rubber tubing. Keep a beaker of saline for flushing the Sahli pipette after use.

5. After collecting the sample, place cotton wool on the puncture site and apply light pressure to stop the bleeding.

6. Attach the capillary tube to a piece of paper marked with the patient's identification until the test is performed.

Venipuncture Blood Collection

Required Supplies

- 70% alcohol and cotton wool (to clean the puncture site)
- sterile needle (19- to 23-gauge; 25 to 40 mm long) and syringe or sterile vacutainer needle
- blood collection tubes containing EDTA anticoagulant
- plain capillary tube (used to collect a sample of blood from the EDTA tube)
- tourniquet

Procedure

1. Have the patient sit comfortably at a table with the arm extended to form a straight line.

2. Ask the patient to form a fist. Select a vein in the bend of the elbow.

3. Place the needle on the syringe.

4. Wrap the tourniquet firmly around the arm three or four inches above the elbow. Form a loop with one end, so it can be easily removed.

5. Feel the arm until the site where the blood will be drawn from is located. Cleanse the site with cotton wool soaked with 70% alcohol. Allow the alcohol to dry.

6. Introduce the needle bevel side up at a 15-degree angle into the skin and through the top wall of the vein. Pull the piston of the syringe slowly to prevent hemolysis while obtaining the amount of blood needed (*Figure 4*).

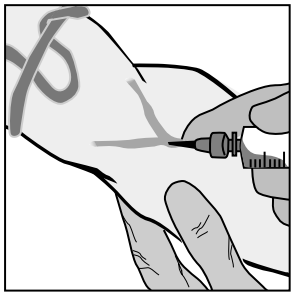


Figure 4

7. Once the blood starts to flow, release the tourniquet by pulling on the looped end.

8. Once the required amount of blood has been drawn, apply dry cotton wool over the hidden point of the needle, and rapidly remove the needle.

9. Have the patient apply pressure on the cotton wool for three minutes to stop the bleeding.

10. Mark the blood sample with patient identification information.

Chapter 3—Common Anemia Detection Tests

This chapter describes nine of the most commonly used tests for detecting anemia. The tests are presented in order of complexity of the test procedure or equipment required.

1. CLINICAL SIGNS

Noninvasive test based on clinical signs. No blood sample is required.

Principle

A clinical evaluation of the patient may be used to detect anemia. This method is more sensitive in detecting signs of severe anemia, and is less reliable in detecting moderate or mild anemia. The inner eyelid, lips, tongue, gums, and the area under the fingernails are examined and the degree of pallor indicates the severity of anemia. Symptoms of anemia may include weakness, tiredness, headaches, and shortness of breath.

Test Characteristics	
Appropriate settings:	Routine screening during physical examination Village level settings where drawing blood is not possible, and/or where cultural beliefs against blood drawing exist
Space requirements:	Well-lighted environment
Accuracy:	Sensitivity 64% in severe anemia (hemoglobin level below 7.0 g/dl) Specificity 70% to 100%

Equipment and Supplies

Required: Small flashlight (torch) or other light source

Test Procedures

Note: Ideally, the health care worker should wear gloves when performing the evaluation.

1. Assess the overall condition of the patient; note whether the patient reports tiredness or weakness, or appears pale.

2. To evaluate conjunctival pallor (degree of paleness and color), gently pull down the lower lid and determine if the membranes of the inner eyelid appear pale (white - pink) instead of red (*Figure 5*).

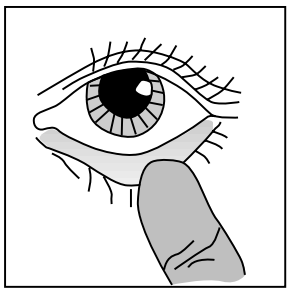


Figure 5

3. To assess tongue pallor, use a small flashlight (torch) to examine the top surface of the tongue. The lips, tongue, and gums will appear pale when anemia is present (*Figure 6*).

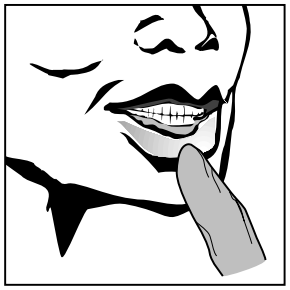


Figure 6

4. To assess pallor of the area under the fingernails, gently rotate the front surface of the hand towards you and observe the area under the fingernails directly. Do not apply any pressure to the hand (*Figure 7*).

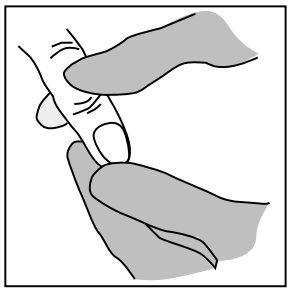


Figure 7

5. To assess the pallor of the palm of the hand, open the patient's hands and partially extend the fingers (*Figure 8*).

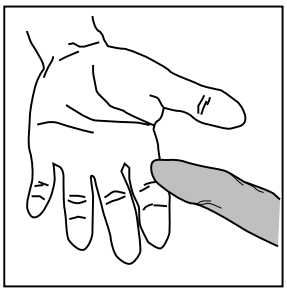


Figure 8

Interpretation

- The pallor of each site examined can be graded as *definite*, *probable*, or *absent*.
- The presence of *definite* pallor at any site that has been examined means the patient is anemic.
- If the pallor of several sites is *definite* or *probable*, the presence of anemia is likely.
- If pallor is absent, this does not rule out moderate or mild anemia.
- If anemia is suspected, particularly in patients who are high risk for anemia, confirm with a blood test whenever possible.

Suggestions for Improving Test Performance

- The conjunctivae, lips, tongue, gums, palms, and area under the fingernails should all be examined.
- Clinical examination of all sites is improved by good lighting and, where possible, by using a small flashlight to examine the top surface of the tongue.
- Training in distinguishing color shades and minimizing variation in color perception may improve the accuracy of this method.

- Accuracy also may be improved if actual hemoglobin values can be determined for comparison and used during training.
- The conjunctivae of crying children or persons with irritated or infected eyes may not appear to be pale, although anemia may still be present.

Advantages

- Minimal equipment required.
- Very low cost.

Disadvantages

- Method is highly subjective and will vary according to the viewing angle of the observer.
- Mild to moderate anemia may not be detected.
- Not suitable for follow up of patients receiving treatment for anemia.
- An adequate light source is required.
- The presence of eye infection, allergies, or other conditions may influence the shade of the conjunctivae, which may make this method less accurate.

2. FILTER PAPER METHOD
Formerly: Tallqvist

This method does not require lysis or dilution of blood.

Principle

The red color of blood corresponds to the amount of hemoglobin present. The degree of anemia can be visually assessed by matching the color of a drop of blood on filter paper against a standardized color chart. The color chart has been developed to represent the color range of normal to anemic blood on filter paper.

An improved method, the World Health Organization (WHO) Haemoglobin Colour Scale, is expected to be available in 1998. A brief description of the WHO Haemoglobin Colour Scale is described at the end of this section.

Test Characteristics

Appropriate settings:	Most useful for screening in rural settings
Space requirements:	Well-lighted environment
Amount of blood sample:	One drop of capillary blood
Preparation/processing time:	5 minutes
Sample/test stability:	10 minutes
Accuracy:	Sensitivity 60% at 10.0 g/dl
	Specificity 60% at 10.0 g/dl
	Accuracy increases with hemoglobin levels below 9.0 g/dl

Equipment and Supplies

Required: Filter/blotting paper that allows absorption and rapid drying of blood drop
A standardized color comparison chart that represents ranges of hemoglobin levels
Sterile lancets
70% alcohol and cotton wool

Maintenance and Storage

- Do not leave the color charts in the sun for extended periods of time as they may fade.
- Do not leave filter paper in the sun or in high humidity because the heat and humidity will damage them.
- Store color charts and filter paper at room temperature.

Test Procedures

1. Clean the earlobe or fingertip with cotton wool soaked with 70% alcohol. Allow the alcohol to dry. Obtain a drop of blood by puncturing either the earlobe or fingertip with a sterile lancet *(Figure 9)*. Wipe away the first drop of blood.

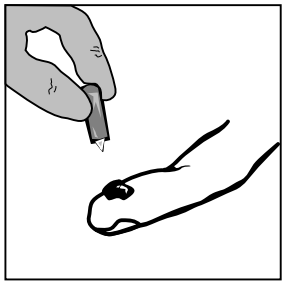


Figure 9

2. Collect the next drop onto the filter paper and wait a few seconds until the blood spot has almost dried (*Figure 10*).

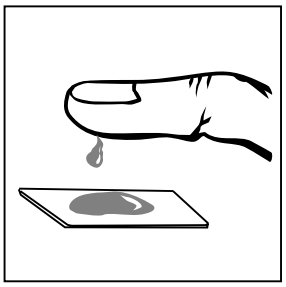


Figure 10

3. When the blood spot is no longer shiny, hold the filter paper behind the standardized chart so that the blood spot is visible through the hole in the chart (*Figures 11 and 12*).

   After the blood is dropped onto the filter paper, the reading must be performed within ten to fifteen minutes because the color of the blood spot on the filter paper will continue to darken as it dries.

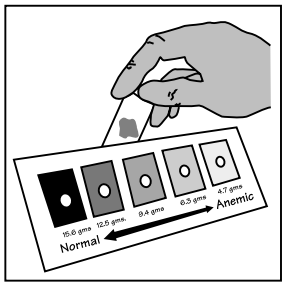


Figure 11

4. Find the color on the chart that most closely matches the color of the blood on the filter paper and record the result. If the color of the blood spot falls in between the colors on the chart, use the lower reading as the result.

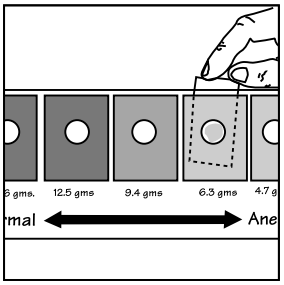


Figure 12

Interpretation

Some charts provide readings in grams per liter, while others provide readings in ranges of hemoglobin that indicate normal (>12.0 g/dl), mild anemia (<11.0 g/dl to 9.0 g/dl), moderate anemia (<9.0 g/dl to 7.0 g/dl), or severe anemia (<7.0 g/dl). If a result indicates moderate to severe anemia, follow up with a more accurate test where possible.

Suggestions for Improving Test Performance

- Use only the filter paper provided with the chart. Other papers, such as newsprint paper, will give inaccurate readings because they are not calibrated with the chart.
- Filter paper that has become discolored or has developed brown spots should not be used.
- Natural, direct lighting conditions are important for consistent color matching.
- The color chart will be more durable if it is laminated (sealed in a plastic covering).
- Observation of the patient's condition (clinical signs) will provide additional information to help determine whether or not anemia is present.

Advantages

- Inexpensive
- Rapid
- Simple
- No reagents required

- Portable
- Electricity not required
- Filter paper and color chart are durable if properly maintained and stored

Disadvantages

- The chart is supplied with a limited quantity of filter paper. Other types of paper can not be substituted because they are not calibrated with the chart.
- The scale may become contaminated with blood over time.
- Lighting conditions influence interpretation of the result.
- Size and thickness of blood spot, temperature, and humidity all affect drying time, which, in turn, affects color.

Special Update: WHO Haemoglobin Colour Scale

As this manual is being printed, a new color scale method is under development by the World Health Organization. In the new method, a capillary blood spot collected directly on a specific absorbent paper is compared with a set of printed color standards. Many of the sources of error identified with other filter paper methods are eliminated in the WHO Haemoglobin Colour Scale by the use of carefully chosen absorbent paper, an improved durable color scale, and detailed test procedures. The sensitivity and specificity from initial evaluations are both 90%.

Technical inquiries should be sent to:

Dr. S.M. Lewis
WHO Collaborating Centre for
Haematology Technology
Royal Postgraduate Medical School
London W12 0NN
UNITED KINGDOM

Fax: 44 181 946 9146

Questions about availability should be sent to:

World Health Organization
Programme on Health Technology
1211 Geneva 27
SWITZERLAND

Fax: 41 22 791 4836

3. COPPER SULFATE METHOD

This method does not require lysis or dilution of blood.

Principle

The specific gravity of blood is influenced by red blood cell volume. The copper sulfate test is based on the fall (or flotation) of whole blood when dropped into a copper sulfate solution of a known specific gravity. The drop of blood will either float or sink depending on whether it is lighter or heavier than the copper sulfate solution. Standard copper sulfate solutions are used to determine a particular hemoglobin level.

Test Characteristics	
Appropriate settings:	Screening programs, such as for blood donors or antenatal clinics
Space requirements:	Well-lighted environment; flat surface
Amount of blood sample:	One drop of capillary blood
Preparation/processing time:	20 minutes if stock solution must be prepared; 10 minutes if only the standard solutions are prepared from the stock
Sample/test stability:	10-15 seconds
Accuracy:	Sensitivity 87.5% Specificity 99% Test is more sensitive at hemoglobin levels below 9.0 g/dl

Equipment and Supplies

Required: Analytical-grade copper sulfate ($CuSO_4 \cdot 5H_2O$)
Weighing scale
Amber-tinted cylindrical bottles, 100 ml
Graduated transfer pipette
Volumetric flask, 1 liter
Volumetric flask, 100 ml
Clean distilled water
Heparinized or EDTA capillary collection tube
Sterile lancet, or needle and syringe

Optional: Stir plate
Hydrometer to measure specific gravities for the copper sulfate solutions
EDTA blood collection tube and needle

Maintenance and Storage

- Tightly seal stock and standard solutions and store where they will not be exposed to heat or direct sunlight in order to prevent evaporation.

Test Procedures

Preparation of stock solution with specific gravity of 1.100

1. Weigh 170.0 grams of copper sulfate and add this quantity to a large flask.

2. Add 1 liter of distilled water to the copper sulfate in the large flask. Rotate the flask to dissolve the copper sulfate.

3. If a hydrometer is available, confirm that the specific gravity of the solution is 1.100.

4. Store the stock solution in an amber bottle and label it as “Stock Copper Sulfate Solution” with the date of its preparation.

Preparation of standard solutions for qualitative assessment

Note: To detect the presence or absence of anemia (but not to discriminate between moderate and severe anemia), three standard solutions can be used; one for pregnant females, one for nonpregnant females, and a third for males.

The specific gravity of the standard solution for pregnant females is 1.050, which is equivalent to 11.0 g/dl hemoglobin. The specific gravity of the standard solution for nonpregnant females is 1.052, which is equivalent to 12.0 g/dl hemoglobin. The specific gravity of the solution for males is 1.053, which is equivalent to 12.5 g/dl hemoglobin. Instructions for preparing these solutions follows.

To determine the hemoglobin range with more precision, a set of standard solutions can be prepared representing additional hemoglobin values (see chart on next page).

To prepare the standard solution to assess pregnant female patients

1. Using a graduated transfer pipette, release 49.2 ml of the stock solution into a 100 ml volumetric flask.

2. Add distilled water in a rapid, steady stream to the 100 ml mark of the flask. Gently rotate the flask as the water is being added.

3. After the final volume is reached, place the standard solution in an amber bottle, cap the bottle, and invert it once to mix the solution.

4. Label the bottle with its gram equivalent of hemoglobin (11.0 g/dl).

To prepare the standard solution to assess nonpregnant female patients

1. Using a graduated transfer pipette, release 51.25 ml of the stock solution into a 100 ml volumetric flask.

2. Add distilled water in a rapid, steady stream to the 100 ml mark of the flask. Gently rotate the flask as the water is being added.

3. After the final volume is reached, place the standard solution in an amber bottle, cap the bottle, and invert it once to mix the solution.

4. Label the bottle with its gram equivalent of hemoglobin (12.0 g/dl).

To prepare the standard solution to assess male patients

1. Using a graduated transfer pipette, release 52.25 ml of the stock solution into a flask.

2. Add distilled water in a rapid, steady stream to the 100 ml mark of the flask. Gently rotate the flask as the water is being added.

3. After the final volume is reached, place the standard solution in an amber bottle, cap the bottle, and invert it once to mix the solution.

4. Label the bottle with its gram equivalent of hemoglobin (12.5 g/dl).

Preparation of additional standard solutions for semi-quantitative assessment of hemoglobin

Hemoglobin Level (g/dl)	Volume of Stock Solution (ml)	Volume of Water (ml)	Specific Gravity
6.0	39.2	60.8	1.040
8.0	43.2	56.8	1.044
10.0	47.2	52.8	1.048
12.0	51.25	48.75	1.052
14.0	55.3	44.7	1.056

Test Procedures

1. Clean the earlobe or fingertip with cotton wool soaked with 70% alcohol. Allow the alcohol to dry. Obtain a drop of blood by puncturing either the earlobe or fingertip with a sterile lancet, wipe away the first drop, and collect the next drop of blood in a capillary tube or EDTA treated capillary tube. If a plain, untreated capillary tube is used, the test must be performed rapidly before the blood starts to clot.

2. Select the standard solution that will give the cutoff value needed to assess the level of hemoglobin of the patient. Release a small drop of whole blood from 1 cm above the standard solution (*Figure 13*).

3. As soon as the blood enters the copper sulfate solution, observe the drop for 10 to 15 seconds.

4. If the blood drop sinks immediately, its **hemoglobin value is greater than** that of the hemoglobin gram equivalent of the copper sulfate solution. If the **blood drop floats** for 10 to 15 seconds, the **hemoglobin value is equal to or less than** the hemoglobin gram equivalent of the copper sulfate solution.

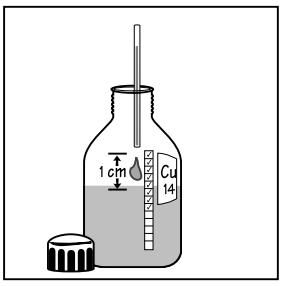


Figure 13

5. After each blood-drop test, mark the label on the bottle to record the number of uses (*Figure 14*). Discard the solution after 50 blood drops have been introduced to the solution.

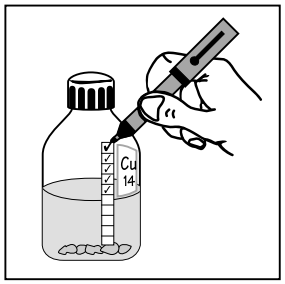


Figure 14

Interpretation

Select the copper sulfate solution representing the normal or expected level of hemoglobin for the patient. If the first assessment indicates the hemoglobin is less than the cutoff value of the copper sulfate solution (the blood drop floats in the copper sulfate solution), this indicates the patient's

hemoglobin value is less than normal. Using a range of copper sulfate solutions representing additional hemoglobin levels will allow a more accurate assessment of the hemoglobin value.

Suggestions for Improving Test Performance

- A piece of paper can be taped to the bottle to record the number of blood drops put into the solution. After 50 blood drops have been introduced, the level of accuracy of the reagent decreases and fresh solution must be prepared.
- The standard solutions should be mixed thoroughly.
- The blood should be used immediately after being drawn so it does not clot, unless it is drawn with anticoagulant.
- Both the water and the stock solutions should be at room temperature before use.
- The bottles containing standard copper sulfate solutions should be clearly marked with hemoglobin gram equivalent amounts.
- The blood sample should be dropped from a capillary tube, a syringe, or a dropper. It must not be dropped directly from the finger.
- Blood collected in a capillary tube from a finger, heel, or earlobe prick, or from a venipuncture sample put into an EDTA blood collection tube, can be used.
- The blood sample must be large enough to form a free-falling drop and dropped from an optimum distance of 1 cm above the solution.
- Check the specific gravity of the stock and standard solutions with a hydrometer periodically to be sure they have not changed.

Advantages

- Inexpensive.
- Performing the test is rapid and simple, though initial preparation of the solutions can take 10 to 30 minutes.
- Interpretation is more objective than visual color matching methods such as the filter paper method, Lovibond, and Sahli.
- Several solutions can be prepared to measure a range of hemoglobin levels.
- Solutions have a long shelf life if their containers are tightly sealed to prevent evaporation.
- A 100 ml volume of solution can be used as many as 50 times.
- Electricity is not required.

Disadvantages

- It is often difficult to obtain analytical-grade copper sulfate chemicals in rural areas.
- Provides only ranges of hemoglobin levels.
- Several solutions need to be prepared to measure a range of hemoglobin levels.
- Stock solutions and standard solutions must be prepared with precision and stored correctly to prevent evaporation (which may reduce their accuracy).
- Stock solutions should be obtained from facilities able to make accurate solutions.
- Laboratory workers must properly dispose of standard solutions containing blood.
- Unless fresh solution is used, error is introduced after 50 tests have been performed as the specific gravity of the standard solution will be altered. Error increases progressively with continued use of old solution.

4. HEMATOCRIT BY CENTRIFUGE

This method does not require lysis or dilution of blood.

Principle

The hematocrit level, or packed cell volume, is a measure of the ratio of the volume of red cells to the total volume of whole blood (plasma, white blood cells, and red blood cells) and is expressed as a percentage. The ratio is determined after centrifugation. The hematocrit level is approximately 3 times the hemoglobin level.

Test Characteristics

Appropriate settings:	Health clinics
Space requirements:	Flat surface; access to electricity or batteries
Amount of blood sample:	Capillary tube (length: 70-75 mm; diameter: 1 mm)
Preparation/processing time:	Average centrifugation time is 5 minutes at 10,000 x G
Sample/test stability:	The sample is stable for up to 6 hours
Accuracy:	Sensitivity is greater than 90% if centrifugal speed is consistent

Equipment and Supplies

Required: Disposable, standard-size, heparinized, capillary tubes (length: 70-75 mm; diameter: about 1 mm) or blood collection tubes treated with EDTA
Clay sealant
Microhematocrit centrifuge with following specifications:
- Radius greater than 8 cm
- Able to achieve maximum speed within 30 seconds
- Able to maintain a centrifugal force of 10,000 x G for 5 minutes without exceeding a temperature of 45°C

Arithmetic (graph) paper or reference chart to calculate hematocrit value based on packed cell volume
Electricity or battery power
Sterile lancets

Optional: Capillary tubes impregnated with heparin (anticoagulant)
Tachometer or strobe light

Maintenance and Storage

Check the centrifuge for accuracy by running several blood samples with known hemoglobin values from several hemoglobin ranges (5.0-10.0 g/dl and 10.0-15.0 g/dl). Operate the centrifuge at a range of speeds and times (5, 10, and 15 minutes) to determine the best conditions for maximum red blood cell packing.

Test Procedures

1. Obtain a blood sample from the finger, earlobe, or heel. The blood should be flowing freely. It is important to centrifuge the tubes within 6 hours after the blood sample is taken.

2. Holding the capillary tube horizontally, fill it with blood until it is 75% full. The tube fills more easily if it is kept horizontal so it is not working against gravity (*Figure 15*).

   If you are filling the capillary tube from anticoagulated blood collected by venipuncture, be sure to mix the blood in the anticoagulated tube well first.

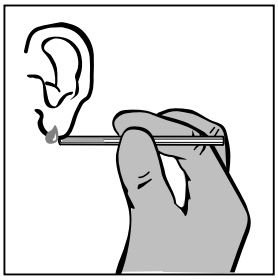


Figure 15

3. After blood is collected, place one finger over the dry end of the capillary tube, keeping the tube in a horizontal position.

4. Still holding the tube horizontally, remove your finger from the dry end and plug it with 2 mm of clay sealant (*Figure 16*). If clay sealant is not available, the tube may be sealed by heating the dry end of the tube rapidly in a flame.

5. Record the patient identification with the number of the slot where each tube is placed in the centrifuge.

Figure 16

6. Place the capillary tube into the slot in the centrifuge head. The plugged end of the tube should be placed away from the center of the centrifuge (*Figure 17*).

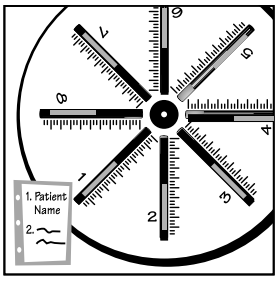


Figure 17

7. Centrifuge at high speed (10,000 x G) for five minutes (*Figure 18*).

8. Remove the capillary tubes in order, one at a time, and read the results.

Figure 18

Interpretation

If using the reference chart:

- Line up the bottom of the red cells at the zero mark (*Point A, Figure 19*).
- Slide the capillary tube along the scale until the top level of plasma reaches the 1.0 mark *(Point B, Figure 19).*
- The line passing through the top of the red cell column will indicate the packed cell volume *(Point C, Figure 19).*
- Do not include the buffy coat as part of the red cell level in the reading.

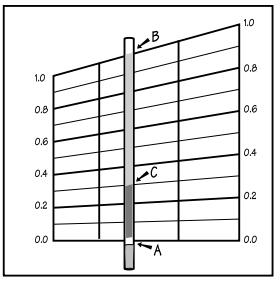


Figure 19

If using arithmetic paper:

- Measure the length of both the red-cell level and the total level of red cells and plasma in the tube.
- Use the following formula to calculate the packed cell volume (PCV):

$$PCV = \frac{\text{length of red cell column (mm)}}{\text{length of red cell column (mm)+ plasma column (mm)}}$$

Suggestions for Improving Test Performance

- Attach the capillary tube to a small piece of paper on which the patient's name or identification code is written to avoid sample mix up until the test is performed.
- Individual specimens must be clearly labeled with patient identification and with numbers corresponding to their placement in the centrifuge.
- Perform weekly maintenance of the centrifuge by checking the accuracy as described in the Maintenance and Storage section.
- Plain capillary tubes can be used to determine the hematocrit level when blood has been collected by venipuncture using tubes containing EDTA anticoagulant.
- It is important to have a constant source of power when running the centrifuge.

Advantages

- Rapid.
- Simple.
- Several specimens can be measured at one time.
- Accurate compared with the visual comparator methods.
- Useful for validating the accuracy of other methods.

Disadvantages

- Either anticoagulated blood or capillary tubes containing anticoagulant must be used.
- Electricity or battery power are required.
- Power supply must be consistent to get a true indication of packed cell volume.
- Heating of the centrifuge may cause some red blood cell lysis, which may result in an incorrect reading.
- Glass capillary tubes are fragile and may break when centrifuged.
- Glass capillary tubes containing anticoagulant must be discarded after each use and must be disposed of safely.
- The use of slower centrifuges may result in inaccurate readings due to trapped plasma, and is not recommended.

5. LOVIBOND TYPE COMPARATOR

This method requires dilution and lysis of blood.

Principle

The Lovibond visual color comparison method is based on comparing the depth of color that results when an accurate measurement of blood is added to a diluting fluid with a set of colored glass standards. The hemoglobin in the blood is converted to oxyhemoglobin or hemiglobincyanide depending on which diluting fluid is used. The color of the test solution is visually compared with a set of glass standards set in a disc that match the diluted hemoglobin fluid. The intensity of color in the test solution corresponds to a specific hemoglobin level.

Test Characteristics

Appropriate setting:	Health clinics
Space requirements:	Flat surface; adequate light
Amount of blood sample:	0.05 ml
Preparation/processing time:	5 minutes
Accuracy:	Sensitivity 100% dilutional method
	Specificity 60% dilutional method

Equipment and Supplies

Required: Comparator
Colored glass standards
Glass tubes or Lovibond type cells to make dilutions
A calibrated blood pipette able to accurately measure 0.05 ml of whole blood
Hemoglobin diluting fluid: ammonia or Modified Drabkin's diluting fluid

Optional: Parafilm or aluminum foil

Maintenance and Storage

- Keep the glass discs clean; wipe with a cloth if they become dirty.
- Store the discs in a box when not in use.
- Keep the discs out of a humid environment by storing in a desiccator and away from heat and direct sunlight.
- The ammonia solution can be stored in a polyethylene wash bottle for up to one week but should be filtered before use if it appears cloudy.

Test Procedures

Prepare either dilute ammonia or Modified Drabkin's diluting fluid

<u>Preparing the dilute ammonia</u>

1. Fill a one-liter cylinder with 1,000 ml of water (*Figure 20*).

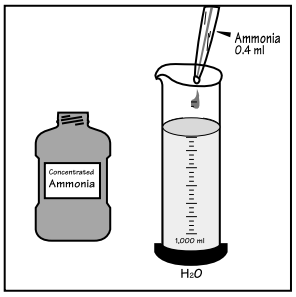


Figure 20

2. Add 0.4 ml concentrated ammonia using a pipette. This will provide enough diluting fluid for 100 tests.

Note: A smaller batch can be made by adding 0.1 ml of concentrated ammonia to 250 ml of water in a wash bottle.

Warning! Concentrated ammonia is hazardous and should be handled in a well-ventilated area. Ideally, it should be handled under a fume hood or with use of a respirator mask where available.

Preparing the Modified Drabkin's diluting fluid

In laboratories equipped with an accurate balance, Modified Drabkin's diluting fluid can be prepared as follows:

Ingredients:	Potassium ferricyanide	0.4 g
	Potassium cyanide	0.1 g
	Potassium dihydrogen phosphate	0.28 g
	Surfactant (such as Nonidet P40 or Brij 35)	2 ml
	Distilled water	2,000 ml

1. Dissolve the first three chemicals in the water and mix.
2. Add the surfactant (such as Nonidet, Storex, or Brij) and mix gently.
3. The reagent should be clear and pale yellow in color.
4. Store in a brown bottle. If the reagent appears cloudy, it may have microbial growth and should be discarded.

Warning! Potassium cyanide is a highly poisonous chemical and should be used only by experienced chemists. When not in use, it should be kept in a locked cupboard. After using the chemical, wash your hands thoroughly.

Measuring hemoglobin

1. Fill the Lovibond-type tube with exactly 10 ml of the diluting fluid you have prepared. Use only Lovibond-type tubes with this method. If too much fluid is added, use a pipette to remove the excess fluid.

2. Clean the earlobe or fingertip with cotton wool soaked with 70% alcohol. Allow the alcohol to dry. Obtain a drop of blood by puncturing either the earlobe or fingertip with a sterile lancet. Wipe away the first drop of blood.

3. Collect the blood into the blood pipette and fill to the 0.05 ml (or 50 µl) mark. Keep the pipette in a horizontal position to avoid spilling the blood. Wipe any excess blood from the exterior of the pipette. Do not pipette by mouth.

4. Expel the blood into the hemoglobin diluting fluid and flush the pipette in the fluid several times to make sure all the blood is released (*Figure 21).*

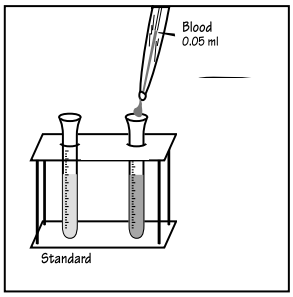


Figure 21

5. Place parafilm or foil wrapper over the Lovibond-type tube and invert the tube gently a few times to mix the blood and the diluting fluid (*Figure 22*).

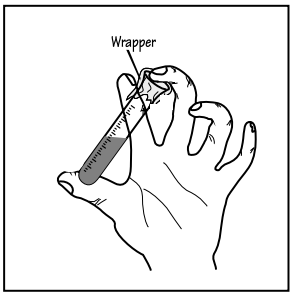


Figure 22

6. Place the hemoglobin disc (Lovibond 5.37X) into the comparator.

7. Place a clean tube with clear hemoglobin diluting fluid in the Standard slot. Place the tube containing the blood and test solution in the Test slot (*Figure 23*).

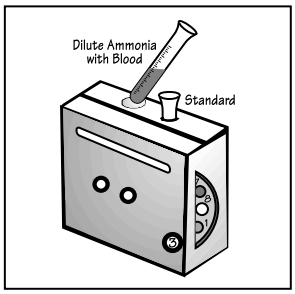


Figure 23

8. Hold the comparator up to the light and turn the disc until the colors from the two views match each other. It is helpful to take the color match to slightly below, then slightly above, in order to find the best color match (*Figure 24*).

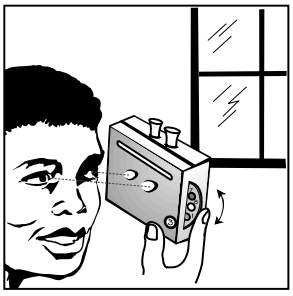


Figure 24

Interpretation

Record the corresponding level of grams of hemoglobin per 100 ml of blood from the answer slot. Grams per 100 ml is converted to grams per liter by multiplying by 10.

If you do not have an exact match, identify the color on the hemoglobin disc that is slightly lighter than the blood sample and the color that is slightly darker than the blood sample. Estimate the value based on the midpoint between the two disc colors.

Suggestions for Improving Test Performance

- Prepare and measure the reagents carefully for best results.
- When sampling the blood, hold the blood pipette horizontally. Use a tissue to wipe any excess blood from the exterior of the pipette.
- Take duplicate readings on each specimen to verify the results.
- Small clots in the blood may cause irregular distribution across the chamber.
- The results should be read within 10 minutes after the blood is introduced into the ammonia diluting fluid. The fluid will start to fade and give falsely low results after 10 minutes.
- If the sample is paler than the lowest value on the disc, repeat the test by adding twice the volume of blood and divide the results by 2.

Advantages

- Uses a durable device.
- Useful for routine screening.
- Does not require electricity.
- Simple and rapid.
- Measures a range of hemoglobin values.
- Comparator can also be used to measure blood glucose and urea with appropriate discs.

Disadvantages

- Requires subjective color matching.
- Cost of hemoglobin discs are high.
- Requires precise dilutions and calibrated pipettes.
- Requires a large drop of blood (50 µl).
- Requires ammonia or Modified Drabkin’s diluting fluid.
- Needs to be read in natural daylight.

6. THE GREY WEDGE (BMS) PHOTOMETER

This method requires lysis of blood.

Principle

The grey wedge is an optical wedge that ranges from very dark to very light and is encased in a viewing instrument. Light is absorbed simultaneously by both a defined layer of blood (sample) in a glass cell chamber and an empty glass cell standard. The amount of light absorbed is proportional to the amount of hemoglobin in the blood sample. The wedge is adjusted by moving a pointer on the scale until the colors of the two cells within the instrument match. The hemoglobin level is obtained by reading the position of the pointer on the scale when the colors of the two cells match.

Test Characteristics	
Appropriate setting:	Screening in health clinics and small laboratories
Space requirements:	Flat surface for sample preparation
Amount of blood sample:	0.005-0.01 ml
Preparation/processing time:	5 minutes (includes cleaning chamber)
Sample/test stability:	10 minutes
Accuracy:	Sensitivity of 77.5% Specificity of 96%

Equipment and Supplies

Required: A BMS grey wedge photometer
Saponin-treated sticks
Glass chamber for confining the blood sample during reading
Calibrating glass standard
"C" batteries (1.5 volt)
Detergent for cleaning the glass chamber after use

Maintenance and Storage

- Blood should never be allowed to dry in the glass chamber. Clean the glass chamber immediately after use with cold, soapy water then rinse thoroughly with clean water.
- The outer housing of the photometer can be easily cleaned with a soft cloth.
- The grey wedge inside the housing can be cleaned by opening the housing of the photometer and wiping the wedge with a soft cloth.

<u>Making Saponin-treated sticks</u>

Equipment: Bamboo skewers or toothpicks cut into 5 cm pieces
Saponin powder
EDTA powder
Distilled or deionized water

1. Bundle together 100 of the 5 cm sticks and tie with string or rubber bands.
2. Weigh 600 mg of Saponin and 200 mg of EDTA and mix these amounts together.
3. Add 1 ml of distilled or deionized water.
4. Stir gently until the powder is dissolved and the solution is slightly pasty. Try not to agitate the mixture as this increases air bubbles. Cover the solution so that it is airtight. It will dry out if not covered.
5. Allow the solution to sit for several hours or overnight.
6. Place the bundled sticks in the Saponin solution so that 1 cm of the stick is immersed in the liquid.
7. Soak the sticks in the solution for 3 hours. Dry the sticks by incubating overnight (for 12 hours) between 30 and 45 degrees Celsius.

Finished Saponin sticks should lyse blood within 60 seconds.

Test Procedures

Checking the calibration of the Grey Wedge Photometer

1. Insert the calibrating glass standard into the clip so that the label is closest to the clip handle (*Figure 25*).

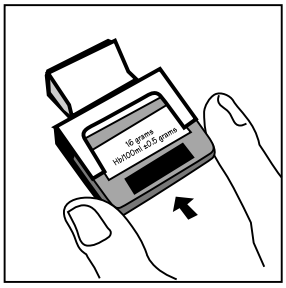


Figure 25

2. Insert the assembled clip and glass standard into the blood chamber compartment on the side of the photometer (*Figure 26*).

   If you have a glass block standard, insert the glass standard into the test cell.

3. Operate the photometer by turning on the light source.

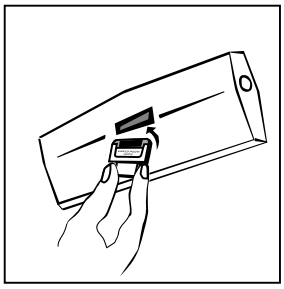


Figure 26

4. Visually evaluate the calibrating standard through the viewer and adjust the grey wedge until the two visual fields match *(Figure 27).*
5. Read the hemoglobin level from the side of the photometer. It should be within +/-0.5 g of the stated value on the glass calibrating standard.
6. If the photometer does not give a reading within +/- 0.5 g of the calibration standard, first change the batteries and recheck the reading. If

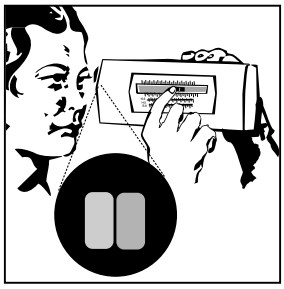


Figure 27

the reading still remains outside 0.5 g of the calibration standard, recalibrate the photometer before taking any readings (see next section).

7. Remove the assembled clip and glass standard when calibration is completed.

Calibrating the Grey Wedge Photometer

It is a good practice to check the calibration of the grey wedge when the lamp or batteries are replaced or when results do not match the glass calibrating standard.

1. Note the difference in grams from the reading of the instrument and that of the glass standard value.
2. Pull off the clip with the white indicator line.
3. Open the photometer and work with the upper housing only. Hold the housing in your left hand with the scale facing you and your thumb pressing up on the slide button to immobilize it.
4. While the base is immobilized, loosen the two exposed screws and move the adjustable indicator the correct distance (plus or minus) as noted in step one.
5. Tighten the two screws and push the indicator clip back into position.
6. Snap the instrument back together.
7. Insert the calibration glass standard into the photometer and take the reading. The result should correspond with the value marked on the glass standard.

Preparation of the blood sample

The blood chamber consists of an H-shaped glass chamber, a thick glass cover slip, and a metal clip to hold the two pieces of glass together. The chamber must be completely dry before adding the blood, as residual water will dilute the sample and influence the reading.

1. Partially assemble the two pieces of glass. Draw out the lower piece of glass so that the chamber is still accessible.
2. Clean the fingertip with cotton wool soaked with 70% alcohol. Allow the alcohol to dry. Obtain a drop of blood by puncturing the fingertip with a sterile lancet. Wipe away the first drop of blood. Allow the blood to freely flow onto the lower piece of glass of the chamber (*Figure 28).*
3. Hemolyze the blood sample on the glass surface by agitating slightly with a Saponin-treated stick. This takes 30 to 40 seconds to fully release the hemoglobin into the chamber. Be sure the blood is fully lysed so that the hemoglobin is fully released from the red blood cells and that the glass chamber has an even, well-lysed sample of blood.
4. Carefully push both glass chambers fully into the metal clip.

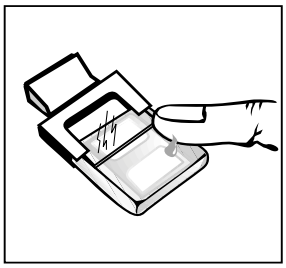


Figure 28

Hemoglobin estimation

1. Insert the assembled chambers and clip fully into the blood chamber compartment on the side of the instrument or in the test cell (*Figure 29*).

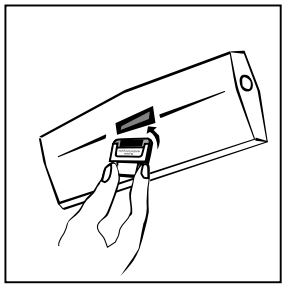


Figure 29

2. Adjust the wedge until the color of the two visual fields match (*Figure 30*).

3. Read the result of the corresponding hemoglobin level (or percent of normal), depending on the scale on your instrument.

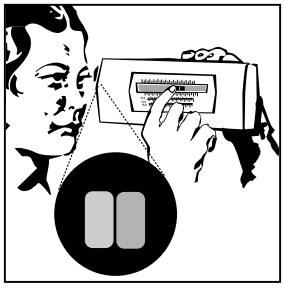


Figure 30

Interpretation

Some instruments may give the reading as a percent of normal, rather than as grams of hemoglobin per deciliter. The percent of normal can be converted to grams of hemoglobin using a conversion chart.

Suggestions for Improving Test Performance

- It is useful to have an additional glass chamber available if many readings will be taken in one session.
- Avoid allowing the drop of blood to dry in the glass chamber. Immediately after reading the result, remove and clean the glass chamber using cold, soapy water, then rinse with clean water.
- To determine if the blood is fully lysed, put the chamber on top of newsprint. Newsprint can be easily read through the blood sample when the blood is fully lysed.
- Ensure there are no air bubbles in the chamber before reading. If air bubbles are present, repeat the procedure with a fresh sample of blood.
- The batteries should be changed regularly to avoid unreliable test results.

Advantages

- Portable.
- Inexpensive.
- Rapid result.

Disadvantages

- Color matching is subjective.
- Glass chamber must be cleaned and dried thoroughly between uses.
- The blood specimen can disperse unequally across the chamber, leading to difficulty in interpretation, especially with anemic blood as the red cells settle during viewing.
- Glass chamber is tedious to work with and clean completely because of its small size.
- Can only process one specimen at a time.
- Saponin powder may not be readily available.
- Results become less reliable when the batteries are low.

7. SAHLI METHOD OF HEMOGLOBIN ESTIMATION

This test requires dilution of blood and visual color match.

Principle

The Sahli method is based on converting hemoglobin to acid hematin and then visually matching its color against a solid glass standard. Dilute hydrochloric acid is added to a graduated cylinder containing a blood sample until the color of the diluted blood sample matches that of the glass standard. The quantity of dilute acid added will be determined by the hemoglobin level of the blood sample.

Test Characteristics	
Appropriate settings:	Health clinics; mobile laboratories
Space requirements:	Flat surface; direct, natural, lighting
Blood sample amount:	0.02 ml
Preparation/processing time:	10 minutes
Sample/test stability:	10 minutes
Accuracy:	Sensitivity 85%
	Specificity 85%

Equipment and Supplies

Required: Sahli hemoglobinometer (solid glass standard and a calibrated graduated cylinder)
Sahli blood pipette (calibrated to 0.02 ml)
Small glass rod for stirring or wooden applicator sticks if glass rods are not available
Dropper for adding the hydrochloric acid
Dilute 0.1 M hydrochloric acid (0.1 N)
Detergent

Optional: Timing device

Maintenance and Storage

- The Sahli calibrated graduated cylinder must be cleaned after each use by washing with soapy water and rinsing with clean water.
- Storing the brown glass standard away from sunlight prevents the color from fading.

Test Procedures

1. Fill the Sahli graduated cylinder to the 2 g mark with dilute 0.1 M hydrochloric acid (approximately 0.15 ml) (*Figure 31*).

2. Clean the fingertip with cotton wool soaked with 70% alcohol. Allow alcohol to dry. Obtain a drop of blood by puncturing the fingertip with a sterile lancet. Wipe away the first drop of blood.

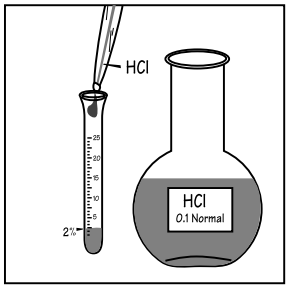


Figure 31

3. Draw the blood to the 0.02 ml mark using the Sahli blood pipette. Do not pipette by mouth (*Figure 32*).

4. Wipe any residual blood from the exterior of the pipette. Recheck that the blood still reaches the 0.02 ml mark.

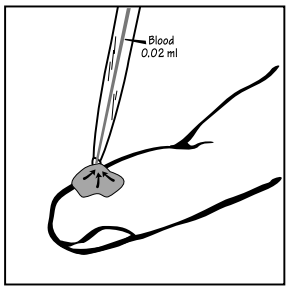


Figure 32

5. Add the blood to the dilute acid. Mix the blood and acid thoroughly by flushing the pipette several times (*Figure 33*).

6. Allow the acid/blood mixture to stand for five minutes.

7. Place the tube into the tube holder of the colored scale.

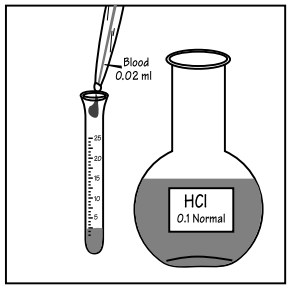


Figure 33

8. Hold the scale up to the light (*Figure 34*).

9. If the color of the solution is the same or lighter than that of the colored standard, the hemoglobin level is 4 g/dl or less.

   If the color of the solution is darker than the colored standard, continue to add dilute acid drop by drop. Stir the solution with the glass rod after each drop is added, and compare the solution to the colored glass standard.

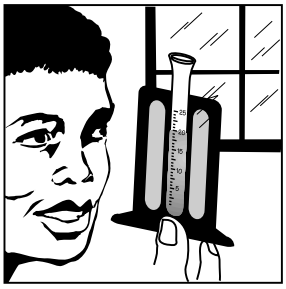


Figure 34

10. Keep adding the acid until the color of the solution matches the color of the glass standard (*Figure 35*). Hold the scale up to a window when assessing the color match.

11. Once the colors match, hold the instrument at eye level and record the value of percent hemoglobin indicated on the side of the tube by the level of fluid.

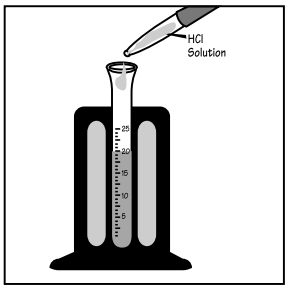


Figure 35

Suggestions for Improving Test Performance

- Precise measurements of blood will improve accuracy.
- Wipe any excess blood from the exterior of the Sahli pipette.
- Adding the dilute acid slowly and stirring the solution after each new drop will increase accuracy.
- Rinse the pipette with cold water after use and allow to dry thoroughly before reusing.
- It is useful to have several blood pipettes and calibrated cylinders available if many readings are to be taken during one session.
- Inspect the solution for cloudiness before taking measurements. If the solution appears cloudy, add a little surfactant (detergent).
- Allow a full five minutes after the blood is first added to the acid for the reaction to be completed.
- Practice doing the test with samples of known hemoglobin levels to improve accuracy.

- Visual matching should be taken facing a window with daylight behind the scale.
- At the end of each day, fill the graduated cylinder with 3% sodium hydroxide and leave overnight to remove proteinous deposits, then rinse thoroughly with water. Do not soak entire graduated cylinder in the sodium hydroxide as this will cause the numbers on the tube to fade.

Advantages

- Instrument and reagents are inexpensive.
- Test is easy to perform.
- Electricity is not required.
- Good method to have on standby in case more accurate methods fail.

Disadvantages

- Color matching is subjective.
- The color of the glass standard is not a true match for the color of diluted blood.
- Color of the standard will fade with time and needs periodic calibration to determine the correction factor. As described in the introduction, quality control should be done by comparing values obtained with the Sahli hemoglobinometer to known hemoglobin values determined by a reference standard. The shift of the colored standard can be determined by using a control chart.
- Graduated tubes must be cleaned before use.
- It is easy to add too much dilute hydrochloric acid during the color comparison process.
- Acid hematin is not a stable compound and readings must be taken within the recommended time interval.
- Samples can only be measured one at a time.
- After prolonged use, the numbers on the graduated cylinder fade and are difficult to read.

8. PORTABLE HEMOGLOBINOMETER (HemoCue)

This method requires lysis of the blood.

Principle

The HemoCue is an example of a rugged, portable, and accurate hemoglobinometer readily available for use. Other portable hemoglobinometers are available, but their test procedures may differ from those presented here. Consult the package insert that accompanies your hemoglobinometer.

The HemoCue uses a disposable cuvette that is treated with chemicals that rupture the red blood cell wall and combine with the hemoglobin to form a compound that can be measured photometrically. The result is displayed in digital form on the face of the instrument.

Test Characteristics	
Appropriate Setting:	Useful in rural settings, for surveys, and where high accuracy is important
Space Requirements:	Minimal
Amount of blood sample:	0.01 ml
Preparation/Processing Time:	1 minute
Sample/Test stability:	10 minutes
Accuracy:	Sensitivity: 85% in field settings 100% in laboratory
	Specificity: 94%

Equipment and Supplies

Required: HemoCue instrument
Disposable cuvettes
Nickel cadmium batteries
Standard control cuvette

Maintenance and Storage

- Store disposable cuvettes in their container with desiccants (to keep them dry). They are stable for three months after being opened.
- Store cuvettes away from humidity and heat.
- Store the HemoCue instrument away from heat and high humidity.
- The instrument should be carefully and regularly cleaned.
- Careful cleaning and maintenance of the photometer is crucial.
- Take a reference standard reading before each day's testing and keep a record of readings in a notebook to determine if the readout values vary over time.

Test Procedures

1. Move the switch at the back of the HemoCue instrument to the "Power On**"** position.

2. Pull out the cuvette holder to insertion position. This is noted by a distinct stop which should not be exceeded (*Figure 36*).

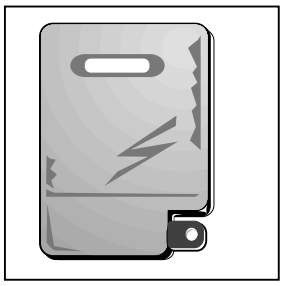


Figure 36

3. Calibrate the hemoglobinometer using the standard cuvette provided.

4. Clean the earlobe or fingertip with cotton wool soaked with 70% alcohol. Allow alcohol to dry. Obtain a drop of blood by puncturing either the ear lobe or fingertip with a sterile lance. Wipe away the first drop of blood.

5. Fill the disposable cuvette with the blood drop by placing the capillary tip of the cuvette on the blood drop (*Figure 37*).

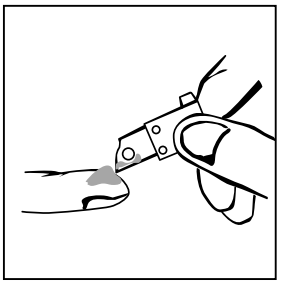


Figure 37

6. Be sure that the cuvette is entirely filled with blood, but do not overfill it. If air bubbles are present, discard the cuvette and fill a new disposable cuvette.

7. Place the filled cuvette in the cuvette holder and push it into the device until it stops (*Figure 38*).

Figure 38

8. After 45 seconds, the hemoglobin value is shown on the display (*Figure 39*).

   The disposable cuvettes cannot be reused and must be disposed of properly, as described in the waste disposal section in Chapter 1.

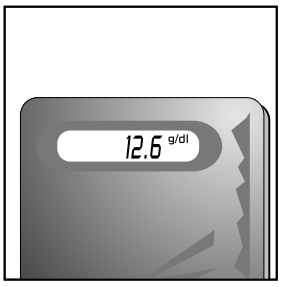


Figure 39

Suggestions for Improving Test Performance

- Take the reading within ten minutes of sampling.
- Replace the cap immediately after cuvettes are removed from the container.

Advantages

- Portable, lightweight, and sturdy.
- Accurate.
- Rapid result that requires no calculations.
- Instrument is sturdy and durable.
- Complete with standard control cuvette.
- Simple to use.
- Minimizes exposure to blood.

Disadvantages

- High initial cost of the instrument.
- High cost of disposable cuvettes.
- Disposable cuvettes cannot be reused and create solid waste.
- High humidity can adversely affect performance.
- Disposable cuvettes have a shelf life of three months once the container is opened.

9. COLORIMETRY-HEMIGLOBINCYANIDE METHOD

This method requires dilution of the blood.

Principle

The hemiglobincyanide (or cyanmethemoglobin) method is the most accurate method of measuring hemoglobin and is considered the "reference standard." The equipment is also the most complex among the nine tests described.

A measured sample of whole blood is added to Drabkin's diluting fluid. The red cells are hemolyzed and the hemoglobin is converted into a stable compound, hemiglobincyanide. The sample is placed in a colorimeter and the amount of light that is absorbed by the blood sample at a certain wavelength is proportional to the amount of hemoglobin in the blood. A reference solution of a known hemoglobin level is used to standardize the method.

Test Characteristics	
Appropriate settings:	District- and central-level laboratories
Space requirements:	A stable, flat surface; constant electricity source
Amount of blood sample:	0.02 ml
Preparation/processing time:	20 minutes
Sample/test stability:	Several hours
Accuracy:	Sensitivity 100% Specificity 100%

Equipment and Supplies

Required: Cuvettes
Test tubes
Pipettes calibrated to deliver 0.02 ml (20 µl) accurately, such as the Sahli blood pipette
Pipettes calibrated to deliver 5 ml accurately
Photoelectric colorimeter that transmits light at 540 nanometers
Drabkin's diluting fluid
Standard solution of hemoglobin at 15.0 g/dl
Graph paper
Watch or timer

Maintenance and Storage

- Keep the colorimeter clean and do not spill fluids inside the test slot.
- Power surges may harm the instrument.
- Store the colorimeter on a flat, stable surface.
- Calibrate the instrument before use.
- Drabkin's diluting fluid must always be stored in a brown bottle, away from light.

Test Procedures

Preparing a standard calibration curve
This procedure should be done frequently (every week or two) and when fresh reagents are prepared.

1. Ensure that the colorimeter is used with a yellow/green filter. If a spectrophotometer is used, adjust the wavelength to 540 nm. Switch on the colorimeter or spectrophotometer and allow it to warm up for ten minutes before use.

2. Prepare the Drabkin's diluting fluid or Modified Drabkin's diluting fluid (see page 25).

3. Label five tubes 1 through 5. Be sure the matched colorimeter test tubes or cuvettes are cleaned before using. Prepare the dilutions by adding the standard solution of hemoglobin at 15.0 g/dl to the Drabkin's diluting fluid in the quantities indicated below:

Tube No.	Standard Solution (ml)	Drabkin's Diluting Fluid	Hemoglobin (g/dl)
1	4.0	0	15.00
2	2.0	2.0	7.50
3	1.3	2.7	4.87
4	1.0	3.0	3.75
5	0.0	4.0	0.00

4. Mix solutions and allow them to stand for five minutes *(Figure 40).*

5. Fill a clean cuvette with the solution from Tube No. 5 (Modified Drabkin's or Drabkin's diluting fluid). Wipe the outside of the cuvette and remove any bubbles in the solution by tapping the cuvette. Place the cuvette in the colorimeter ensuring the transparent sides are facing the light path. Adjust the colorimeter to zero absorbance.

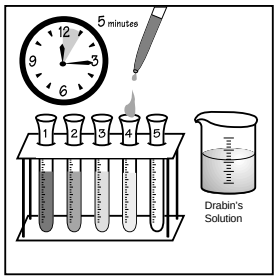


Figure 40

6. Fill four additional cuvettes with solutions from tubes 1 through 4. Place each cuvette in the test slot and read its absorbance from the colorimeter (*Figure 41*). Record the results.

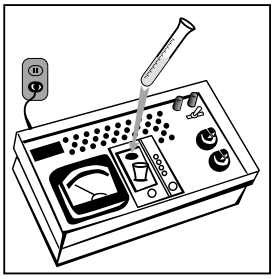


Figure 41

Preparing a calibration graph

1. Plot the absorbance readings of the diluted standards against their known concentrations of hemoglobin (*Figure 42*).

2. From the graph values, prepare a table of hemoglobin values from 2.0 to 18.0 g/dl, with their corresponding absorbance values.

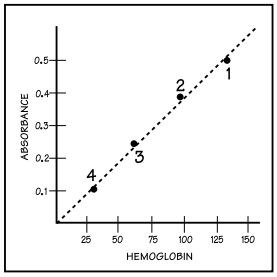


Figure 42

Hemoglobin estimation

1. Label tubes with patient identification numbers.

2. Use the pipette to add 5.0 ml of Drabkin's or Modified Drabkin's diluting fluid into each tube.

3. Draw venous or capillary blood to the 0.02 ml mark of a Sahli pipette. Do not allow air bubbles to enter.

4. Wipe any excess blood from the exterior of the pipette so as to deliver exactly 0.02 ml. Be careful not to absorb any blood from the interior of the pipette.

5. Place 0.02 ml of blood in 5.0 ml of the Drabkin's or Modified Drabkin's diluting fluid. Flush the pipette several times in the diluting fluid to ensure delivery of the entire quantity of blood. Inspect blood and diluting fluid mixture for cloudiness prior to taking measurements; cloudiness can be minimized by the addition of a small amount of detergent to the solution.

6. Mix the contents of the tube thoroughly and let it rest for five minutes at room temperature so that the reaction will be complete. Fill each cuvette with the amount specified.

7. Use Drabkin's or Modified Drabkin's diluting fluid to zero the spectrophotometer at 540 nm or for a control match in a colorimeter.

8. Read and record the absorbance of each specimen *(Figure 43).*

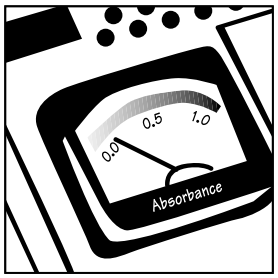


Figure 43

Interpretation

Use the table prepared from the calibration graph (see *Figure 42*) to determine the hemoglobin levels from their corresponding absorbance. The results are reported in grams per deciliter (g/dl) of hemoglobin.

Suggestions for Improving Test Performance

- Before performing a hemoglobin estimation, determine the hemoglobin concentration in triplicate of a known control sample. The three results should not differ by more than 0.5 g/dl. If the difference is greater than 0.5 g/dl, carefully repeat the procedure, paying careful attention to accurate volume measurement and thorough mixing of the control sample.
- All solutions should be at room temperature prior to taking readings.
- The blood and Modified Drabkin's diluting fluid must be thoroughly mixed and allowed to sit for at least five minutes before taking measurements so that results will be accurate. If using "ordinary" Drabkin's diluting fluid without surfactant, the blood and Drabkin's diluting fluid must sit for 10 minutes before taking measurements.
- Drabkin's or Modified Drabkin's diluting fluid is toxic and must be handled with care and disposed of properly as described in the waste disposal section in Chapter 1.

Advantages

- Provides the most accurate hemoglobin estimations.
- Many samples can be evaluated at one time.
- Samples can be prepared several hours in advance of evaluation.
- Allows a stable, reliable standard to be widely available.

Disadvantages

- Method requires extremely accurate measurements.
- A stable electricity source is required.
- Requires handling and disposing of toxic chemicals.
- Requires time to prepare the calibration curve using reference standards.

Appendix—Factors Influencing Anemia Detection Tests

In evaluating the appropriateness of an anemia detection test, there are several factors to consider. These include the test's sensitivity, specificity, accuracy, reproducibility, range of normal, and detection limits. In order to determine these, a few basic and essential statistical calculations have to be done. These include the mean, variance, standard deviation, and coefficient of variation. Some definitions and points to consider when deciding on which anemia detection test to use are described below. This is followed by the formula for calculating hemoglobin at high altitudes.

Mean: The mean, or average, is a value which is typical or representative of a set of data. It takes account of all observations and is determined by calculating the sum of all the observations and dividing by the number of observations. The following formula is used to calculate the mean:

$\bar{x} = (x_1 + x_2 + x_3 ... + x_n)/n$

$\bar{x}$ is the arithmetic mean

x_1, x_2, x_3, and x_n represent the value of each individual observation

n is the number of observations

For example, if we wish to determine the average hemoglobin level from 8 different hemoglobin measurements, 12.5, 12.5, 12.6, 8.0, 12.5, 12.3, 12.6, 12.5

$\bar{x} = (12.5 + 12.5 + 12.6 + 8.0 + 12.5 + 12.3 + 12.6 + 12.5)/8$

$= 11.9$

Two other calculations, the variance and the standard deviation, are used to determine how widely the values scatter about the mean.

Variance: Variance is a measure of the dispersion of results around the mean. It is the average of the sum of the squares, i.e. the average of the squared deviations where the mean is subtracted from each value. Because it is a squared quantity, the value of the variance will not be assigned the same units of measure as the individual observations (e.g. g/dl of hemoglobin) thus its use is limited.
Variance (V) is calculated:

$$V = \frac{\sum (x - \bar{x})^2}{n-1} = \frac{\left(x_1 - \bar{x}\right)^2 + \left(x_2 - \bar{x}\right)^2 \ldots + \left(x_n - \bar{x}\right)}{n-1}$$

Using the data from the first example:

$$V = \frac{(12.5-11.9)^2+(12.5-11.9)^2+(12.6-11.9)^2+(8.0-11.9)^2+(12.5-11.9)^2+(12.3-11.9)^2+(12.6-11.9)^2+(12.5-11.9)^2}{8-1}$$

$= 2.54$

Standard Deviation: Standard deviation is also a measure of dispersion about the mean and is the square root of variance. The standard deviation is the most common measure of dispersion because it is expressed in the same units as the original observations. It is determined by taking the positive square root of the variance;

$$SD = \sqrt{V} = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

The standard deviation will be small when the group of observations are bunched closely about the mean and large if they are scattered.

Using the data from the example above:

$$SD = \sqrt{V} = \sqrt{2.54} = 1.6 \text{ g/dl Hb}$$

The Coefficient of Variation: The coefficient of variation is a measure of relative precision that is determined by dividing the standard deviation by the mean and is expressed as a percentage. The coefficient of variation is normally used for determining the precision of several measurements of the same sample. Generally, for tests to be acceptable, the coefficient of variation (CV) should be less than 5%.

$$CV = \frac{S\ D}{\bar{x}} \times 100$$

Sensitivity: Sensitivity is the ability to accurately detect a condition and is expressed as a percentage. "Positiveness of condition" is defined as the proportion of test results that are positive (true positives) among all those with the condition (true positives and false negatives). The higher the sensitivity of a test, the greater the ability to minimize false negatives.

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives plus false negatives}} \times 100$$

Specificity: Specificity is the ability to correctly determine the absence of a condition and can be thought of as "negativeness of condition." Specificity is defined as the proportion of true negatives among the healthy (true negatives and false positives). The higher the specificity of a test, the greater the ability to minimize false positives.

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives plus false positives}} \times 100$$

Factors that will influence the sensitivity and specificity of anemia diagnostics tests include:

How the blood is collected. Capillary blood differs from venous blood. 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.

The type of test used and the population studied. If a test only detects severe anemia but the prevalence of mild to moderate, rather than severe anemia, is high, then the actual number of correctly identified anemic cases may be lower than if a test with a broader detection ability is used. Hemoglobin values are more accurately measured when they fall in certain ranges for some methods. For example, the copper sulfate method is more sensitive for hemoglobin levels below 9.0 g/dl.

Laboratory versus field conditions. In general, laboratory settings have a more controlled environment than field settings. Taking duplicate measurements, controlling ambient conditions such as temperature, and following the proper test procedures influence the sensitivity and specificity of a test.

Reference Method: The reference method serves as a comparison against which new tests are measured. In determining hemoglobin values, the hemiglobincyanide method is considered the reference method.

Accuracy: Accuracy is a measure of closeness between an observed result and the true value. An accurate test is free from both random and systematic errors.

Precision or Reproducibility: Precision is the degree of agreement between repeated measurements of the same test. Good precision, or reproducibility, does not ensure the accuracy of the test; it is a measure of variability of the test when repeated under the same conditions. Factors that affect reproducibility include patient and laboratory conditions.

Range of Normal: The range of normal values will vary from population to population—men, women, pregnant women, children, and those living at high altitudes will differ from one another in their normal range of hemoglobin values. There may be overlap from group to group, but it is important to acknowledge the varying cutoff levels when diagnosing anemia.

Detection Limits: The detection limits, or sensitivity, of each method vary and is an important factor when determining which method is most appropriate. The hemiglobincyanide colorimetric method can accurately measure hemoglobin to within +/- 0.5 g/dl, whereas the visual colorimetric methods detect ranges of hemoglobin to within +/- l.0 g/dl and are only useful for qualitative screening. The noninvasive test based on clinical signs detects the presence of severe anemia, but does not indicate actual hemoglobin levels. It is important to acknowledge the varying detection limits of the different anemia detection methods so that they are used in appropriate situations.

In cases where severe anemia is suspected, it is important to accurately measure the level of hemoglobin in order to avoid unnecessary blood transfusions. Blanket iron supplementation programs require baseline hemoglobin data as well as follow-up data to capture changes in hemoglobin levels in order to determine whether treatment was effective; thus a sensitive method is needed.

When hemoglobin ranges are in the upper end of normal, as much as one-fifth of the blood volume may be lost before anemia is detected. Using tests with more sensitive detection limits can identify these types of conditions. Both case management and monitoring community-based interventions require sensitive methods in order for the results to be valid. The visual comparison methods are unsuitable for this purpose as the limits of detection are too wide to give meaningful results.

Determining Hemoglobin Levels at High Altitude: Living at a high altitude results in increased red blood cell production to compensate for the reduced level of oxygen in the air; thus, the level for normal hemoglobin is greater at higher elevations than at sea level. It is important to determine whether anemia exists among populations living at high altitudes. An equation has been developed to adjust for the altitude effect on hemoglobin, where the altitude is known:

Hb adjusted = Hb observed - Hb adjustment for Altitude

To determine the Hb adjustment for Altitude:

Hb adjustment for Altitude = 0.032 x Altitude + 0.022 x (Altitude x Altitude)

Therefore:

Hb adjusted = Hb observed - 0.032 x Altitude + 0.022 x (Altitude x Altitude)

Altitude = Altitude in 1,000 feet units or in 1,000 meter units x 3.3;
e.g. 4,000 feet = 4 x 1,000 feet unit

For example: A man living at an elevation of 6,000 feet and with an observed hemoglobin of 18.5 g/dl, will have an adjusted hemoglobin of 17.5 g/dl using the above formula:

Hb adjusted = 18.5 g/dl - ((0.032 x 6) + (0.022 x (6 x 6)))
= 18.5 g/dl - (0.192 + (0.022 x 36))
= 18.5 g/dl - (0.192 + 0.792)
= 18.5 g/dl - (0.984)
= 17.5 g/dl

Where the exact altitude is not known, the Centers for Disease Control recommend that the following altitude Hb adjustments be made by subtracting the value listed for each altitude value from the hemoglobin value obtained from the patient:

Altitude (feet)	**Hb** (g/dl)	**Altitude** (meters)	**Hb** (g/dl)
<3,000	0	<1,000	0
3,000 - 3,999	+ 0.1	1,000 - 1,499	+ 0.1
4,000 - 4,999	+ 0.2	1,500 - 1,999	+ 0.4
5,000 - 5,999	+ 0.4	2,000 - 2,499	+ 0.7
6,000 - 6,999	+ 0.6	2,500 - 2,999	+ 1.2
7,000 - 7,999	+ 1.0	3,000 - 3,499	+ 1.8
8,000 - 8,999	+ 1.2	3,500 - 3,999	+ 2.5
9,000 - 9,999	+ 1.5	4,000 - 4,499	+ 3.4
10,000 - 10,999	+ 1.9	4,500 - 4,999	+ 4.3
11,000 - 11,999	+ 2.3	5,000 - 5,999	+ 5.4
12,000 - 12,999	+ 2.8	>6,000	+ 6.6
13,000 - 13,999	+ 3.3		
14,000 - 14,999	+ 3.9		
15,000 - 15,999	+ 4.5		
16,000 - 16,999	+ 5.1		
17,000 - 17,999	+ 5.8		

Glossary

Absorbance	A measure of the amount of light absorbed by a substance.
Accuracy	The extent to which the measured value agrees with the true value.
Acid hematin	The compound that results when hydrochloric acid reacts with hemoglobin in blood. It is used in the Sahli method.
Blank	A method of establishing a baseline or zeroing an instrument so that subsequent measurements are of the substance.
Buffy coat	The layer of white blood cells which separates out when a blood sample is centrifuged, as in the hematocrit method.
Colorimetry	A procedure to measure the absorbance of specific wavelengths of light passed through a solution by means of a colored filter. The amount of light absorbed is used to determine the concentration of a substance in a solution. Also called filter photometry.
Drabkin's diluting fluid	A fluid consisting of potassium ferricyanide, potassium cyanide, and sodium bicarbonate that is used to convert hemoglobin to hemiglobincyanide. A modified Drabkin's diluting fluid can be prepared by using a phosphate buffer and surfactant, which results in a more rapid conversion of hemoglobin to hemiglobincyanide.
EDTA	Ethylenediamine tetra acetic acid. The dipotassium or tripotassium forms used in blood collection tubes prevent blood from clotting.
Hematocrit	A measure of anemia, in which the volume of red blood cells is expressed as a percentage of the total blood volume.
Hemiglobincyanide	The compound that results when hemoglobin is added to Drabkin's diluting fluid. When measured photometrically at 540nm, and calibrated with WHO/ International Committee for Standardization in Haematology, International Reference Preparation, the method is considered the "reference method" for hemoglobin measurement.
Hemoglobin	The pigment that gives color in the red blood cells. Hemoglobin consists of protein chains and heme molecules containing ferrous iron. Hemoglobin carries oxygen from the lungs to the tissues and carbon dioxide from tissues to lungs.
Normal reference	A substance or device that conforms to national or international reference standards.
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 or method to reproduce a measured value.
Quality assurance	A system of activities that assures that tests are performed within defined standards to assure quality.
Reference standard	A substance with a known concentration of substrate that is used to check the precision of a test procedure in routine practice.
Saponin	A detergent that disrupts the red blood cell wall and releases hemoglobin into the plasma.
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 with the density of water. It is important in the copper sulfate method.
Spectrophotometry	A procedure that uses a given wavelength of light from a continuous spectrum to measure the concentration of a substance in a solution by the amount of light absorbed.
Surfactant	A detergent used to decrease the surface tension of water.

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