

Manual M-2, Clinical Affairs. Part I, General

**Chapter 22, Sickle Cell Screening and Counseling Program
(Sections I and II, including Paragraphs 22.01 through 22.13;
Appendix 22A through Appendix 22G)**

This document includes:

Memorandum, dated **July 23, 1985**

Contents page for M-2, dated **June 1989**

Title page and title page verso for M-2, Part I, dated **February 9, 1990**

Contents page and Rescissions pages for M-2, Part I, dated **April 7, 1995**

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Text for Chapter 22, dated **September 24, 1974** (Change 56)

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Change 56, dated **September 24, 1974**



Veterans
Administration

Memorandum

Date:

From: Actg. ACMD for Clinical Affairs (11)

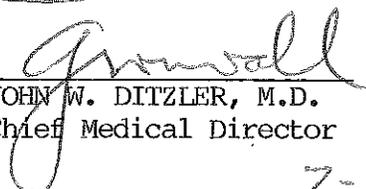
Subj: Redesignation of Manual M-2

To: Director, Regulations and Publications (10A1B)

VA Department of Medicine and Surgery Manual M-2, "Professional Services," has been redesignated as VA Department of Medicine and Surgery Manual M-2, "Clinical Affairs."


HOWARD D. COHN, M.D.

APPROVED/DISAPPROVED:


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Chief Medical Director

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M-2 MANUALS

M-2

Part I General

Part II Chaplain Service

Part III Dietetic Service

Part IV Medical Service

Part IV Nuclear Medicine Service

Part V Nursing Service

Part VI Pathology & Allied Sciences Service

Part VI Drug Dependency Treatment Program

Part VII Pharmacy Service

Part VIII Physical Medicine & Rehabilitation Service

Part IX Prosthetic & Sensory Aids Service

Part X Psychiatry, Neurology & Psychology Service

Part XI Radiology Service

Part XII Social Work Service

Part XIII Medical & General Reference Library Staff - *Rees (see M-8, Pt III 8/14/87)*

Part XIV Surgical Service

Part XV Resc. by M-2, Part IV, Chg. 6(11-62) Pulmonary Disease (TB) Service

Part XVI Resc. by M-2, Part X (4-65) Vocational Counseling Service

Part XVII Voluntary Service - *M-1, Pt I, Ch 3*

Part XVIII Audiology & Speech Pathology (II 10-66-20, 6-8-66)

Part XIX ~~Extended Care Service (Domiciliary)~~ *Replaced by M-5* ~~Spinal Cord Injury~~

XX Nuclear Medicine

XXI Vocational Counseling Service

XXII Prosthetic & Sensory Aids Service

XXIII *Blind Rehabilitation Service*

XXIV *Spinal Cord Injury*

**DEPARTMENT OF
VETERANS AFFAIRS**

PROGRAMS

**CLINICAL AFFAIRS
GENERAL**

**M-2, Part I
February 9, 1990**

**Veterans Health Services and
Research Administration
Washington, DC**

Department of Veterans Affairs
Veterans Health Services and
Research Administration
Washington, DC

February 9, 1990

Department of Veterans Affairs, Veteran Health Services and Research Administration
Manual M-2, "Clinical Affairs," Part I, "General," is published for the compliance of all
concerned.

John A. Gronwall, M.D.
JOHN A. GRONWALL, M.D.
Chief Medical Director

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RESCISSIONS

The following material is rescinded:

1. COMPLETE RESCISSIONS

a. Manuals

Par. 112f, M10-3.

Pars. 129f and 169, M10-6.

M-2, Part I, changes 2 through 5 through 9, 11, 12, 13, 14, 16, 18 through 21, 25, 30, 32 through 40, 41, 44, 45, 49, 50, 51, 52, 55, 57, 60.

VHA Supplement MP-1, Part I, Chapter 2, Section A and Appendices D and E, change 43, dated October 27, 1987 (Effective October 1, 1992).

VHA Supplement MP-1, Part I, Chapter 2, Section A, change 44, dated July 26, 1991 (Effective October 1, 1992).

M-2, Part I, Chapter 35, dated August 7, 1992 and Supplements 1 and 2.

b. Interim Issues

II 10-156

II 10-161

II 10-184

II 10-188

II 10-270

II 10-292, pars. I, II, III, App. A

II 10-300

II 10-381

II 10-68-31

II 10-71-33

II 10-71-26 by M-2, part I, chg. 67

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c. Circulars/Directives

261, 1946, Sec.1

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10-91-059

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- 10-93-004
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- Par. 2, TB 10A-191
- Pars. 1b, 2 through 5, 6a and 9c, TB 10A-246
- TB 10A-256
- TB 10A-295 (except sec. XXI)
- TB 10A-359
- TB 10A-324 (This completes the rescission of TB 10A-324.)

f. AB Station Letters and Other Communications

<u>Date</u>	<u>Subject</u>
December 5, 1949	Officer of the Day Reports
March 3, 1952	Furnishing of Meals to Officers of the Day
April 8 1952	Domiciliary Care for Paraplegics
April 16 1952	Transfer of Quadriplegic Patients
April 17, 1952	Accomplishment of Recheck Examinations and Treatment of current Conditions Involving Paraplegics at VA Hospitals Other Than Paraplegia Centers.
June 23, 1952	Monthly Report of Service-Connected Blinded Veterans and Blinded Military Personnel
August 18, 1952	Proposals for Membership, American College of Physicians
September 19, 1952	Establishment of Paraplegia Organizational Segment
January 4, 1954	Certificate of Medical Feasibility, VA Form 4555b

g. Instructions (pertaining to Public Law 702, 80th Congress, as amended)

- Pars. 2d and 2e, Inst. 1-B
- Inst. 1C
- Inst. 1-D

2. LIMITED RESCISSIONS

The following material is rescinded insofar as it pertains to this manual.

a. Manuals

- M10-3, par. 115h

a. Manuals - Continued

M10-6, pars. 9b, 42e, 70c, 86, and 132h
M10-11, pars. 22b, 92e, 96d, 133b, and 172

b. Circulars

10-65-57, pars.2 and 3

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CHAPTER 22. SICKLE CELL SCREENING AND COUNSELING PROGRAM

SECTION I. GENERAL

22.01 SCOPE

This chapter defines and establishes procedures for implementing the Sickle Cell Screening and Counseling program. The provisions of this chapter apply only to VA hospitals listed in appendix 22A.

22.02 POLICY

a. Title 38, United States Code, sections 651-654, authorizes a Sickle Cell Anemia program within the VA. The specific provisions of this authority are:

- (1) Screening, counseling and medical treatment of eligible sickle cell anemia patients. (See par. 22.07.)
- (2) Research and research training in diagnosis, treatment and control of sickle cell anemia—based on the screening examinations and treatment.
- (3) Participation by the patient will be voluntary.
- (4) Confidentiality of information and records of the patient will be maintained.
- (5) Annual report of the administration of this program, including recommendations for additional legislation which may be deemed necessary.

b. The Chief Hematologist at the hospital having a VA centrally authorized Sickle Cell Screening and Counseling program has overall responsibility for administration of the program.

22.03 OBJECTIVES OF THE PROGRAM

a. To provide a voluntary screening program for all patients who are recognizable as risks for hemoglobin (Hb) S and/or glucose-6-phosphate dehydrogenase (G-6-PD) deficiency who are admitted to the hospital or who are eligible for treatment in the outpatient clinic.

b. To provide a voluntary educational program on the basic medical and genetic aspects of the hemoglobinopathies and enzyme deficiency to all such patients and their spouses.

c. To educate physicians, nurses and other VA personnel about sickle cell disorders and G-6-PD deficiency and to provide testing and education to such individuals at their requests.

22.04 HISTORY

The rationale for a VA Sickle Cell program is:

a. In the United States, sickle hemoglobin is found in 8-10 percent of the black population. Hemoglobin C, another abnormal hemoglobin, is present in 2-4 percent of blacks. About 1 in 400-500 black newborns will have sickle cell anemia and the great majority of such children are destined to live a shortened life span during which they will suffer from frequent painful crises and anemia. In addition to the hemoglobinopathies, the red blood cells of about 10 percent of black persons show deficiency of an enzyme, G-6-PD, which may produce a variable degree of hemolytic anemia under certain circumstances. Data are lacking on the incidence or severity of sickle cell trait (AS) + G-6-PD deficiency, hemoglobin SC + G-6-PD deficiency, and other possible combinations. Caucasians of Mediterranean ancestry may also show red blood cell sickling. Only recently has public comprehension of the magnitude of the problem of sickle cell anemia and related disorders become evident.

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b. The VA is uniquely equipped to undertake such a program. There are approximately 4.93 million black veterans in the United States and about 17,000 black patients have been recorded on a daily basis in VA hospitals.

c. Based on the information gained from a pilot program of screening and counseling carried out in six VA hospitals since July 1972, the procedures in section II will be used for the satisfactory conduct of a Sickle Cell Screening and Counseling program.

22.05-22.06 (Reserved.)

SECTION II. PROCEDURES

22.07 PATIENT SELECTION

Persons otherwise eligible for bed care or outpatient treatment and VA personnel requesting approved medical services as an employee benefit are potential candidates for participation in the Sickle Cell program. Where applicable and medically indicated, spouses of patients may participate in the program but actual treatment is limited to eligible veterans. Preliminary screening of persons for identification of potential candidates will be performed in the admissions office. A patient's racial background or ethnic origin will be ascertained and placed in the lower right corner of item 1 of VA Form 10-10m, Medical Certificate and History. For uniformity of data analysis, race or ethnic origin and codes for race contained in coding instructions in MP-6, part XVI, Supplement No. 1.1, for the Patient Treatment File program will be used. A rubber stamp or other suitable means similar to the following will be used for designating (✓) the appropriate race of the applicant:

Race or Ethnic Origin of Applicant	
<input type="checkbox"/> (1) Spanish American-White	<input type="checkbox"/> (4) Black
<input type="checkbox"/> (2) Spanish American-Black	<input type="checkbox"/> (5) Asian
<input type="checkbox"/> (3) American Indian (Includes Aleutians and Eskimos)	<input type="checkbox"/> (6) White
	<input type="checkbox"/> (7) Other

22.08 PATIENT CONSENT

Except for preliminary screening in the admissions office, participation in the Sickle Cell program by the applicant will be wholly voluntary on the part of the applicant and will not be a prerequisite for any other VA benefit. An SF 522 will be used to obtain the applicant's written consent. Item A1 of the form will include the statement "Participation in Sickle Cell Screening and Counseling Program." Item B1 of the form will include the statement "Necessary laboratory examination(s) for hemoglobinopathies." All other applicable items on the form will be completed. Patients who are hospitalized and who have given written consent for participation and testing will be appropriately identified on the daily "Gains and Losses" sheet.

22.09 DRAWING BLOOD SAMPLES

The sickle cell counselor or hematologist will use a copy of the "Gains and Losses" sheet to determine which patients will have blood samples drawn. Samples should be obtained as soon as possible after admission. If possible, tests for hemoglobin identification and G-6-PD determination should be completed on the same day the blood samples are obtained. Results of tests will be recorded on the appropriate standard forms.

22.10 LABORATORY METHODOLOGY

a. **Hemoglobin Electrophoresis.** All blood samples will be examined by electrophoresis. A suggested method is found in appendix 22B. It is suggested that cellulose acetate be used as the supporting medium. Utilization of

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whole blood has proved to be satisfactory. Samples showing abnormal or suspicious patterns are repeated utilizing hemolysates. (See app. 22C.) The electrophoretic diagnosis of Hb S is verified by performance of the dithionite solubility test. (See app. 22D.) Both techniques are necessary because 2 percent of all blacks have other hemoglobin abnormalities (e.g., Hb C, Hb D, beta-thalassemia, etc.) which must be separated from the normal group. Reference laboratories at VA hospitals—Chicago (West Side) and San Francisco are available for questionable results.

b. **Glucose-6-Phosphate Dehydrogenase.** Two tests are currently in use in the VA Sickle Cell Anemia program. The "Brewer methemoglobin reductase test" and "Beutler fluorescent spot test" are being performed (apps. 22E and 22F), since there appear to be occasional unexplained discrepancies. Until one test has proven to be superior to the other, both relatively easy procedures are being followed. Appropriate positive and negative controls are run simultaneously. Reference laboratories at VA hospitals—Chicago (West Side) and San Francisco are available for questionable results.

22.11 REPORTING RESULTS

The results of Hb electrophoresis and G-6-PD examinations are recorded on the standard laboratory form and sent to the appropriate ward to be included in the patient's chart. The sickle cell counselor maintains a daily log book identifying each patient tested by name, hospital number, ward and results of the tests. At the end of each month, results of findings for that period are tabulated (app. 22G) and retained at the station for future analysis and annual report. A monthly listing of patients and spouses attending educational sessions will be maintained. All persons tested will be given an ID card, VA Form 10-1450, indicating results of Hb and G-6-PD examinations.

22.12 EDUCATIONAL SESSIONS

a. The hematologist will determine the best technique for the method of conducting educational programs for patients, their spouses and hospital personnel. One of the effective methods is to hold daily or triweekly sessions of all patients (screened or otherwise) conducted by the counselor, utilizing such modalities as brochures, film strips, movies and lantern slides with appropriate narration. A question and answer period can be instituted following the formal session. A patient with a hemoglobin disorder or G-6-PD deficiency should be given the opportunity to attend a private counseling session, preferably with spouse (who may be tested voluntarily) so that genetic as well as medical aspects of the findings may be discussed. With the patient's written permission, tape recordings of these sessions may be made and the discussions later reviewed by the counselor with the hematologist for suggestions and/or recommendations about counseling techniques and medical information.

b. If possible, educational exhibits describing sickle cell disorders may be constructed and placed in the lobby of the hospital, with educational brochures, to make this information available and act as a reminder for personnel and visitors. Availability of nearby sickle cell centers for non-VA beneficiaries can be disseminated through such exhibits.

22.13 STAFFING

Previous experience from the VA's pilot study suggests that the following complement of personnel can effectively handle the needs of a program having 600-1,000 beds in a hospital.

a. **Medical Technician.** One medical technician is needed to provide the necessary laboratory support.

b. **Counselor**

(1) This position can be adequately filled by a person (GS-7) who will be responsible for the education and counseling of veterans and spouses found to have hemoglobin disorders and/or G-6-PD deficiency. The counselor will function under the technical and administrative supervision of the Chief, Hematology Section.

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(2) The position of counselor requires a practical understanding of the methods and techniques used in interviewing and counseling. The counselor will have the ability to communicate effectively with black veterans and their spouses and with persons from other population groups who may be tested with respect to the highly sensitive area of sickle cell disorders. Also required is the ability to develop sufficient knowledge of the medical and genetic aspects of sickle cell disorders and G-6-PD deficiency in order to convey and reinforce a practical understanding of the significance and implications of these problems. Imagination and skill in adapting and creating teaching aids and information materials are requisite. A prototype job description for a GS-7 counselor is available from the Central Office Personnel Service. A 1-week course or apprenticeship in counseling techniques is desirable before initiating the program. There are numerous centers and considerable resource material available for training in counseling and introduction of educational concepts. For further information contact VA Central Office.

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APPENDIX 22A

**HOSPITALS AUTHORIZED TO PARTICIPATE IN THE
SICKLE CELL SCREENING AND COUNSELING PROGRAM**

Region 1

Brooklyn, New York
Buffalo, New York
East Orange, New Jersey

Region 2

Washington, D.C.

Region 3

Birmingham, Alabama
Jackson, Mississippi
Memphis, Tennessee
Tuskegee, Alabama

Region 4

Allen Park, Michigan
Chicago (W.S.), Illinois
Cleveland, Ohio
Hines, Illinois

Region 5

Dallas, Texas
Little Rock, Arkansas

Region 6

St. Louis, Missouri

Region 7

San Francisco, California

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APPENDIX 22B

**HEMOGLOBIN ELECTROPHORESIS SCREENING METHOD
USING MICROZONE CELL**

1. Fill microzone cell with barbital buffer (pH 8.6).
2. Soak cellulose-acetate strip in TRIS-EDTA Borate buffer (TEB)—blot—place on bridge and equilibrate for 2 minutes.
3. Apply well-mixed *whole blood* sample, using special microzone applicator.
4. Run at 250 V and 2-3 MA for 1 hour.
5. Known AS and AC blood samples are applied with each run.
6. Air dry strip after run. No staining is necessary.
7. If a hemoglobin band is found in position other than that of hemoglobin A, electrophoresis is carried out on the hemolysate, in addition to the solubility test for hemoglobin S.
8. Any other suitable zone electrophoresis apparatus may be used if available. Directions of manufacturer should be followed. Known AC and AS should be run to determine optimal voltage, amperage and duration for buffer and equipment used that will produce the best (clean) separations.

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APPENDIX 22C

PREPARATION OF HEMOLYSATE

1. Venous blood is collected in any suitable anticoagulant (EDTA or heparin) and red cells packed by centrifugation at 3000 rpm for 10-15 minutes.
2. After plasma and buffy coat are removed, red cells are washed 3 times with 0.9 percent sodium chloride solution. They are then recentrifuged and supernatant discarded.
3. To each volume of red blood cells are added 1.25 to 1.50 volumes distilled water and 0.5 volume toluene. The mixture is placed in a shaker for 20 minutes, or alternatively, may be allowed to stand overnight at 4°C.
4. The hemoglobin, water, toluene mixture is centrifuged at 3000 rpm for 15 minutes. The toluene layer and precipitated material at the toluene interface are removed by careful aspiration. The hemoglobin solution is filtered through a small Whatman #1 filter paper in a short stem funnel. This filtrate is now ready for use for electrophoresis and alkali denaturation. If the solution is not crystal clear it should be refiltered.

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APPENDIX 22D

**SOLUBILITY TEST
FOR Hb S**

Yakulis & Heller
Blood 24, 198, '64

A. REAGENTS

1. **Stock Buffer.** 217 gm. K_2HPO_4 and 169 gm. KH_2PO_4 are dissolved and made up to total volume of 1 liter with distilled water.

2. **Working Buffer.** Add 100 mg. of sodium dithionite (sodium hydrosulfite) to 50 ml. of stock buffer and dissolve for use. Working buffer can be stored for several days in a tightly capped container.

B. PROCEDURES

1. To 2 ml. aliquots of working buffer add 1-2 drops of clear hemolysate used for electrophoresis. Mix and allow to stand for 3-5 minutes.

2. The presence of Hb S is indicated by a persistent turbidity. A clear solution indicates absence of Hb S.

3. The exact amount of hemolysate required depends on concentration of Hb in hemolysate and varies from 1-2 drops. Usually 1 drop will be adequate.

**G-6-PD DEFICIENCY SCREENING
MET Hb REDUCTION TEST**

BREWER JAMA p. 386, 1962

MODIFICATION

A. REAGENTS

1. Deionized water and glass tubes should be used in preparation of reagents and performance of G-6-PD tests.
2. Methylene blue (MeB1) 0.015 percent—Weigh out 0.15 gm. of MeB1 and bring to 1000 ml. in a volumetric flask.
3. Dye solution—Weigh out 0.5 gm. of dextrose and bring to 100 ml. with .015 percent MeB1 solution. (See step 1, above.)
4. Sodium nitrite—Weigh out 250 mg. in each of a series of screw-capped test tubes. Store tightly capped tubes in dry place at room temperature. To use, add 20 ml. of water to one tube, mix and use. Store sodium nitrite solution at 4°C and use for 1 week only, then discard.
5. To prepare unknown tubes, mix 5 ml. of dye solution and 5 ml. of sodium nitrite. Add 0.2 ml. of this mixture to sufficient tubes for day's work. Discard remainder of mixture after day's screening is complete.
6. Positive control tube (1 per day) is prepared by adding 0.1 ml. sodium nitrite to a single test tube.
7. Negative control consists of a clear dry glass test tube.
8. With each day's run, include a known abnormal blood from a previously determined G-6-PD deficiency.

B. PROCEDURE

1. Venous blood is collected in any suitable anticoagulant (heparin or ACD).
2. Perform microhematocrit determination on test sample. Concentrate packed red cells to 40 volume percent if necessary.
3. Add 2 ml. of well-mixed blood to 0.2 ml. of dye and sodium nitrite mixture previously pipetted. (See step 5, sec. A, above.) Mix and incubate for 3 hours at 37°C in a stoppered glass test tube.
4. With each run, include a positive and negative control, by adding 2 ml. of any blood sample into a positive reference tube which contains 0.1 ml. sodium nitrite (step 6, sec. A, above) and a negative tube which is empty (step 7, sec. A, above). Incubate as in step 3, above.
5. At the end of the incubation, remove tubes, mix and transfer 0.05 ml. of each sample into 5 ml. of distilled water. Mix and compare with 2 reference tubes within 2-10 minutes after dilution.

C. COLOR INTERPRETATION

1. Normal—clear red in color, like negative reference tube. (See step 7, sec. A, above.)
2. G-6-PD deficient—brown, like positive reference tube. (See step 6, sec. A, above.)

BRIEF REPORT**Special Modifications of the Fluorescent Screening Method
for Glucose-6-Phosphate Dehydrogenase Deficiency**

By ERNEST BEUTLER AND MARGARET MITCHELL

WE HAVE RECENTLY DESCRIBED a rapid, simple screening method for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency.¹ This method depends upon the fluorescence of reduced triphosphopyridine nucleotide (TPNH) as an indicator of G-6-PD activity. Ten μ l. of whole blood are added to 100 μ l. of a screening solution containing glucose-6-phosphate, TPN, buffer, and saponin. A spot is made on ordinary filter paper after 5 minutes incubation at room temperature. The spot is permitted to dry, and is then examined for fluorescence under long-wave UV light.

We now introduce modifications of the procedure which may enhance its usefulness under certain circumstances. It has been found that when the test reagent originally described is used the fluorescence on filter paper fades relatively rapidly. It is difficult to differentiate normal from enzyme-deficient samples after more than 24 hours. While this does not pose any problem for the ordinary clinical laboratory, it may limit the usefulness of the technic for field surveys. In such studies it may be desirable to examine the results of tests under ultraviolet light in a distant central laboratory, possibly after several days' delay. It has been found that the stability, and indeed the initial intensity, of fluorescence can be greatly enhanced by substituting an appropriate tris-hydrochloride buffer for phosphate buffer.

A second modification makes it possible to eliminate almost entirely the slight fluorescence which sometimes appears when blood from individuals with the mildest forms of glucose-6-phosphate dehydrogenase deficiency are examined, especially using a reaction mixture buffered with tris. This fluorescence can be eliminated by adding oxidized glutathione (GSSG) to the reaction mixture. When GSSG is present, TPNH is oxidized through the action of glutathione reductase (GSSG-R). But the activity of glutathione reductase is normally only approximately one-quarter of that of the activity of the glucose-6-phosphate dehydrogenase-phosphogluconic dehydrogenase sys-

From the Department of Hematology, City of Hope Medical Center, Duarte, California.

This investigation was supported, in part, by U. S. Public Health Service Grant No. HE 07449 from the National Heart Institute, National Institutes of Health.

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ERNEST BEUTLER, M.D.: Chairman, Division of Medicine, and Director, Department of Hematology, City of Hope Medical Center, Duarte, California, Clinical Professor of Medicine, University of Southern California Los Angeles, California. MARGARET MITCHELL: Research Technician, Department of Hematology, City of Hope Medical Center, Duarte California.

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FLUORESCENT SCREENING METHOD

		0'	5'	10'	15'	DAY
<u>REGULAR</u>	NORMAL					0
	DEFICIENT					
<u>TRIS</u>	NORMAL					
	DEFICIENT					
<u>TRIS-GSSG</u>	NORMAL					1
	DEFICIENT					
<u>REGULAR</u>	NORMAL					
	DEFICIENT					
<u>TRIS</u>	NORMAL					3
	DEFICIENT					
<u>TRIS-GSSG</u>	NORMAL					
	DEFICIENT					

Fig. 1.—Spot tests for G-6-PD deficiency using three different reaction mixtures as shown in Table 1. 10 μ l. of whole blood from a normal and G-6-PD deficient subject were incubated with 100 μ l. of reaction mixture at room temperature. Spots were made on Whatman #1 filter paper at the beginning of incubation, after 5 min., 10 min., and 15 min. The spotted filter paper has been photographed under long-wave UV light through a yellow filter on the day of preparation, after one day, and after 3 days.

tem. In effect, the difference between GSSG-R activity and activity of the G-6-PD-6 PGD system is measured. Thus, samples which have only 10 or 20 per cent of the normal glucose-6-phosphate dehydrogenase activity are unable to maintain appreciable concentrations of TPNH in the reaction mixture. In normal samples, however, the effect of glutathione reductase activity is negligible.

Table 1 summarizes the method of preparation of the originally described mixture, a mixture in which Tris is substituted for phosphate, and a mixture in which GSSG has been added. Figure 1 shows the appearance of a piece of filter paper spotted with normal and A- type G-6-PD deficient blood at 0, 5, 10, and 15 minutes incubation at room temperature (22 C). It is apparent that the initial fluorescence is much brighter and its stability greater than when phosphate is used. The presence of GSSG eliminates virtually all fluo-

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Table 1.—*Screening Mixtures for Detecting G-6-PD Deficiency*

Reagent		1 (Regular)	2 (Tris)	3 (Tris-GSSG)
Glucose-6-P	.01 M	0.1	0.1	0.1
TPN	.0075 M	0.1	0.1	0.1
Saponin (Mann)	1%	0.2	0.2	0.2
Potassium Phosphate Buffer, pH 7.4	.25 M	0.3	0.0	0.0
Tris-HCl Buffer, pH 7.8	.75 M	0.0	0.3	0.3
GSSG	.008 M	0.0	0.0	0.1
H ₂ O		0.3	0.3	0.2

rescence when the deficient sample is studied, even in Tris buffer, and even when the incubation time is prolonged to periods much greater than the recommended 5 minutes. We believe that the third mixture would probably be most satisfactory for general screening purposes, although an additional potential variable has been introduced by coupling the system through GSSG-R. It is also of interest that blood collected on filter paper retains sufficient enzyme activity for use not only in the galactosemia screening system, as has been reported previously,^{2,3} but also for G-6-PD screening.^{4,5} This mode of collecting blood has gained widespread use in the screening of newborn infants for metabolic defects such as phenylketonuria and galactosemia, and the same samples could be used to detect G-6-PD deficiency.

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The reagent is prepared in accordance with the formula (table 1, column 3) given in this appendix. The only modification is the addition of 0.1 percent sodium azide as a preservative.

STORAGE AND STABILITY: The prepared reagent is extremely stable and can be stored frozen with little deterioration. Once thawed, *and thoroughly mixed*, the reagent must be kept in the refrigerator with minimal exposure at room temperature.

PERFORMANCE OF THE TEST: One-tenth ml. of reagent is pipetted into a small test tube. 0.01 ml. of blood is added to the reagent, the blood and reagent are mixed and are allowed to stand at room temperature for not less than 5 or more than 10 minutes. The exact volume of screening solution and of blood sample used is of no importance. Rapid, approximate pipettings are quite acceptable. It is not necessary to adjust the hematocrit of the blood sample nor is it necessary for the blood sample to be fresh. A droplet of the mixture is then placed on a piece of Whatman #1 filter paper and the spot is permitted to dry completely. This may require 15 or 20 minutes, and is quite important. After the spot is completely dry, it is examined in a darkened place with longwave ultra violet light. Any longwave ultra violet hand lamp found in various scientific equipment catalogues, or an ordinary Wood's light is satisfactory.

QUALITY CONTROL: It is desirable to test a normal and a G-6-PD deficient sample with each group of blood samples being screened for G-6-PD deficiency. The normal sample should fluoresce brightly while the G-6-PD deficient sample should be virtually devoid of fluorescence. Intermediate results are generally observed only when the blood of heterozgous women is examined. Intermediate results in men implies the presence of some extraordinary circumstance such as an unusual G-6-PD variant or a very young red cell population in an individual with G-6-PD deficiency. Such a sample may require more detailed biochemical study.

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Part I, "General," VA Department of Medicine and Surgery Manual M-2, "Professional Services," is changed as indicated below:

NOTE: The purpose of this change is to add Chapter 22, "Sickle Cell Screening and Counseling Program."

- ✓ Pages v through viii: Remove these pages and substitute pages v through ix attached.
- ✓ Pages 22-1 through 22G-1: Insert these pages attached. (Ch. 22 added.)



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