PD-AAN-631

TRIP REPORT

NUTRITION CRSP

CAIRO, EGYPT

September 2 - October 1, 1983

Nell

Avanelle Kirksey
Principal Investigator, Purdue University

October 28, 1983

This is a report of the activities and accomplishments for Nutrition CRSP resulting from a trip to Cairo, Egypt, September 2 to October 1, 1983. The itinerary is included as Attachment I. The purposes of the trip were:

1) to work with Egyptian colleagues to finalize plans for Phase II protocols for Reproduction/Lactation and Biological Fluids;
2) to visit field site in the village of Kalama for continued pilot testing of data collection procedures for Reproduction/Lactation and Biological Fluids;
3) to establish reliability of field measurements and laboratory quality control procedures;
4) to work with collaborating Egyptian scientists and project field coordinator with regard to systems for data collection, management and transfer; and
5) to attend the first Egypt Nutrition CRSP Workshop.

I. Refinement of Protocols for Reproduction/Lactation and Biological Fluids

A. Early Information About Newborn Infants

Most families in the village wait until survival outcome of the newborn is known before registration is initiated. Therefore, a system has been developed for the project to obtain early information about new births from the three dayas (mid-wives) who live in the village. The dayas deliver most of the infants in the village and during the pilot study became daily informants to Nutrition CRSP personnel of new births in the village. This method of monitoring births among villagers participating in Nutrition CRSP is important in order that the information about newborns becomes available immediately to project workers. Dr. Nargis Bassily and two assistants, Soheir Shohdy and Nadia Zaki, from the Nutrition Institute appear to have established good rapport with the dayas for obtaining this information. However, incentives for the dayas will be important to the continued success of this system.

B. Data Collection Forms for Reproduction/Lactation

Dr. Amin Said and I collaborated in the development of an additional Reproduction/Lactation form concerned with the identification of congenital abnormalities of infants during the neonatal period (Attachment II). Dr. Cheryl Ritenbaugh, Dr. Farouk Shaheen and I discussed the data collection and recording forms for Reproduction/Lactation in relation to those developed for morbidity assessment. The pre-pregnancy monthly visit form used to ascertain date of last menses was incorporated into the morbidity questionnaire which is administered weekly. This should help to more precisely pinpoint the date of last menses. Also, some revisions were made for maternal anthropometry in the Pregnancy Monthly Visit form (Attachment III) and for infant anthropometry in the Pregnancy Outcome form (Attachment IV). All forms were discussed with Bev Beaton, data manager, University of Kansas and approved for data collection.

C. Protein, Glucose and Ketone Measurements in Urine

Protocols for the urine measurements were developed at Purdue University by Dr. Marlene Borschel and I (Attachment V). These procedures were discussed with Dr. Ahmad Dakroury, Head of the Department of Nutritional Biochemistry at the Nutrition Institute. Also, I conducted a training session at the Nutrition Institute for the physicians who will administer the screening tests in the village of Kalama.

D. Hemoglobin, Hematocrit and Ferritin Measurements

Several discussions were held with Dr. Ahmad Dakroury and with Dr. Mohamad El-Ghorab who is responsible for the hematology measurements. Dr. El-Ghorab was trained in the U.S. at Columbia University under the direction of Dr. Barbara Underwood. He is a very capable scientist and undoubtedly is an asset to this part of Nutrition CRSP. Two different ferritin kits (ELISA, New England Immunology Associates, Cambridge, MA and Ramco, Ramco Laboratories, Houston, TX) were brought to Egypt for Dr. El-Ghorab to continue the experimentation which was begun at the Nutrition Laboratory, Purdue University by Dr. Marlene Borschel and me (Attachment VI). Protocols for hemoglobin, hematocrit and ferritin measurements were developed at Purdue University following discussions with Dr. Ahmad Dakroury during my visit to Egypt in March 1983 (Attachments VII, VIII and IX). Dr. El-Ghorab conducted ferritin assays with the use of each of the kits (ELISA and Ramco) and has continued this work during the past month of October. Dr. El-Ghorab is careful and meticulous and the results of the assays which he has performed attest to this. Presently the laboratory which will be used for hematology measurements in the Nutrition Institute is being rennovated.

II. Field-Testing Procedures in Village of Kalama

Nine visits were made to the village of Kalama for the purpose of field testing certain procedures. Each visit, the three dayas in Kalama were contacted to determine whether any births had occurred during the past 24 hours in the village. Weight data were collected for two newborns. The battery operated K-Tron digital scale (Model DS-I) accurate to 1 g was used to weigh the infants both with and without their clothing. Also, the infant's clothing was weighed (Table 1). These data attest to the collection of accurate birth weight information within 24 to 48 hours of the delivery. When one of the infants was 7 days of age, we were invited to a ceremony in the home where the daya performed a ritual and scattered grains and salt symbolic of 7 days of life.

In March 1983, samples of breast milk were obtained from 22 lactating women in Kalama and were analyzed for several nutrients in the nutrition laboratory at Purdue University (Attachments X and XI). Eight of twenty two mothers whose milk was analyzed for vitamin B-6 were found to have low values for the vitamin and two of the mothers had extremely low values (Attachment X). In September 1983 these two mothers and their infants were re-visited. One of the infants at nine months of age did not sit alone and showed gross neurological problems. The other infant appeared to be normal in development. Additional samples of milk were collected from seven of the eight women who were found previously to have low concentrations of vitamin B-6 in their milk. Vitamin B-6 appears to require further monitoring in breast milk of villagers in Kalama.

III. Reliability of Field Measurements and Laboratory Quality Control Procedures

The close agreement of birth weights of infants measured (a) naked and (b) clothed minus the weight of clothing attests to the accuracy of the weighing procedure for newborns (Table 1).

Table 1

Data Collected from Two Mother/Newborn Pairs in Kalama

Block No. 1 Household No. 161 Date: 22 September 1983

Mother: 29 years of age
5 previous pregnancies
3 living children
2 dead children

Infant: 24 hours of age

Infant + clothing, kg	3.470
Clothing, kg	0.414
	3.056
Naked infant, kg	3.056

Infant: 48 hours of age

Infant + clothing, kg	3.540
Clothing, kg	.493
	3.047
Naked infant, kg	3.047
Physiological weight loss, kg (24 to 48 hours of age)	0.011

Block No. 11 Household No. 43 Date: 24 September 1983
Infant born of normal delivery at 5:00 a.m.
Visit made at 10:30 a.m.
Age of infant 5 hours

Mother: 30 years of age
9 previous pregnancies
3 living children
4 dead children
2 abortions

Infant: 5 hours of age

Infant + clothing, kg	3.710
Clothing, kg	0.566
	3.144
Naked infant, kg	3.138

Infant: 29 hours of age

Infant + clothing, kg	3.476
Clothing, kg	0.438
	3.038
Naked infant, kg	3.026
Physiological weight loss, kg (5 to 29 hours of age)	0.112

Good agreement between expected values for ferritin controls and the values actually observed attests to the accuracy of the procedures used as shown in Table 2 below.

Table 2
Expected and Observed Values for Ferritin Concentrations in Control Samples (ELISA[1] and Ramco[2])

Controls for Ferritin Measurements	ELISA[1] Method		Ramco[2] Method	
	Expected Value	Observed Value	Expected Value	Observed Value
ng/ml	ng/ml		ng/ml	
Low	33±5	40	11±4	18
Medium	95±15	125	58±9	72
High	223±25	230	213±37	260

[1]New England Immunology Assoc., Cambridge, MA 02138

[2]Ramco Laboratories, Inc., Houston, TX 77098

These values represent the first experimental results of Dr. El-Ghorab. He has continued to experiment with the ferritin assay and now reports much closer agreement, especially with the Ramco procedure, between expected and observed values. During the beginning stages of data collection in Kalama, duplicate serum samples will be sent to the Nutrition Laboratory at Purdue University for ferritin analyses. Also, periodically duplicate serum samples will be sent to the Purdue Laboratory for cross-laboratory comparisons.

A project meeting was held to encourage Egyptian/American scientists involved in various areas of the project to discuss procedures for establishing reliability of the methodologies. The unanimous opinion was that the month of October should be spent in checking reliability of the data collection procedures. One week will be spent testing the Reproduction/Lactation questionnaires. However, biological fluid collections will be delayed until the data collection begins in early November.

IV. Data Collection Routines

Egyptian/American scientists, who were in Egypt to attend the Egypt Nutrition CRSP Workshop, met for the purpose of discussing the data collection routines. To facilitate the work in the collection of biological fluids, one person will be assigned to the village Health Center. The data collection routines proposed for Reproduction/Lactation and for Biological Fluid measurements are shown in Table 3. This information was submitted to Bev Beaton, University of Kansas. She will coordinate these activities with those of other areas for a field logistics schedule of all measurements of households and target individuals.

V. Egypt Nutrition CRSP Workshop and Other Project Activities

A. Workshop

The first Egypt Nutrition CRSP Workshop was held on September 6-8, 1983.

Table 3
Data Collection Routine for Pregnancy/Lactation
and for Biological Fluids and Related Data

	Initial	Pregnancy months					Delivery	days		Lactation months					
		5	6	7	8	9		1-3	8	1	2	3	4	5	6
Anthropometry (pregnant lead female)	☐	☐	☐	☐	☐	☐				☐	☐	☐	☐	☐	☐
Physical Exam	☐	☐			☐		☐								☐
Laboratory															
Blood, finger stick (Hb, Ht, ferritin)	☐	☐			☐		☐			☐					☐
Blood, venous (immunology)		☐			☐					☐					☐
Urine specimen (glucose, albumin ketone)	☐	☐	☐	☐	☐	☐				☐					☐
(Schisto screen)															
Stool, ova and parasites	☐	☐			☐					☐					☐
Saliva (lysozyme, secretory IgA, IgC)	☐	☐			☐										
Breast milk sample								☐		☐		☐			☐
Questionnaire: Reproduction Form I	☐														
Morbidity Data Base	☐														
Questionnaire: Pregnancy Monthly Visits Form II		☐	☐	☐	☐	☐									
Questionnaire: Pregnancy Outcome Form III							☐								
Dubowitz Score								☐							
Anthropometry of infant									☐	☐	☐	☐	☐	☐	☐
Questionnaire: Congenital Abnormalities of Infants Form IV										☐					
Questionnaire: Lactation/Infant Feeding Form V									☐	☐	☐	☐	☐	☐	☐
Questionnaire: Supplementary Foods Form VI										☐	☐	☐	☐	☐	☐

All Egyptian/American senior scientists involved in the project, excepting two American scientists, participated in the workshop. The workshop provided an opportunity for all of the scientists to review accomplishments of Phase I and to meet in work sessions to finalize plans for the next phase of data collection. The minutes of the workshop are included as Attachment XII.

B. Visit to Ministry of Health

Following the workshop and at the time Dr. B. J. Liska, Dean of Agriculture, Purdue University was visiting the Nutrition Institute, Drs. Galal, Gabr, Jerome, Harrison, Liska, Wolgemuth and I paid a visit to Dr. Sabri Zaki, Minister of Health, at the Ministry of Health. Dr. Gabr informed him of past accomplishments and future plans for the Nutrition CRSP project in Egypt. During our visit, Dr. Zaki telephoned the Minister of Agriculture to brief him about the project and its potential for providing findings useful to policy-making in Egypt.

C. Project Building

Two trips were made to the village of Kalama to examine a building which is to be up-graded for project use in cognitive testing, resting metabolic measurements and possibly lactation studies, as well as for use by the field workers and observers as a lounge and to review data collection forms.

D. Trip to NAMRU-3

Dr. June Wolgemuth, field coordinator, and I made a trip to NAMRU-3 to investigate the possibility of using some of their discarded laboratory equipment in the Nutrition Institute. Some of the leads for follow-up were presented to Dr. Galal. Dr. Moustafa Mansour, Head of Biochemistry at NAMRU-3 hosted a tour of the new building and facilities.

E. Human Subjects Document

The human subjects document which insures the protection of the rights of human subjects who participate in the Nutrition CRSP project in Egypt was discussed at a meeting of the Executive Committee for the project. Egyptian senior scientists and American scientists who were in Cairo to attend the workshop were invited to the meeting of the Executive Committee. The Committee reviewed the results of Phase I and the protocols for data collection in Phase II and each member of the Committee signed a statement indicating that the rights of the subjects and the community were adequately protected (Attachment XIII).

F. Publications Agreement

Dr. Gabr, Chairman of the Executive Committee, led a discussion at the meeting described previously on the publications agreement. He suggested that (a) the project representative principal investigator clear the permission to publish with representatives from the other countries; (b) there be no overlapping of graduate students in the project and that the number of graduate students per discipline be minimized; (c) Egyptian and American scientists agree to publish in particular areas; and (d) no CRSP data be published until finalization of the project but that project reports, as distinguished from publications of findings, should be disseminated.

VI. Summary of Accomplishments

A. Protocols and training procedures for Reproduction/Lactation and Biological Fluids were refined with input from Egyptian scientists for implementation in the next phase of data collection.

B. Several trips were made to the village of Kalama for the purpose of field testing methodologies and, in particular, to locate and weigh newborn infants.

C. Methodologies for use in measurements of biological fluids were discussed with Egyptian scientists and plans for laboratory quality control were developed.

D. Final plans were made with Egyptian colleagues for the supplies, equipment and staff needed for the implementation of data collection for Reproduction/ Lactation and for Biological Fluids.

E. Plans were discussed among the principal investigators and other scientists for rennovating and furnishing a building for project use in the village of Kalama.

F. Participation in a workshop in which accomplishments of the first phase of the project were presented and discussed by Egyptian/American scientists.

G. Publications agreements and human subject documents were discussed in some detail with American/Egyptian colleagues.

Professional Contacts Made in Egypt (September 2-October 1, 1983)

Contacts were made with several individuals who are involved with Nutrition CRSP activities in Egypt. These individuals include:

Dr. Hekmat El-Sayed Aly, Retired Director of Nutrition Institute and Member of Executive Committee for Nutrition CRSP

Dr. Zeinab Bishry, Professor Child Psychiatry, Department of Psychiatry, Ein-Shams University

Dr. Ahmad Dakroury, Head, Department of Nutritional Biochemistry and Metabolism, Nutrition Institute

Dr. Esmat Ekladious, Department of Microbiology, University of Cairo

Dr. Mohamad El-Ghorab, Department of Nutritional Biochemistry and Metabolism, Nutrition Institute

Dr. A. M. El-Naggar, Work Physiologist, Helwan University, Cairo

Dr. Daisey Fleita, Biochemist, American University, Cairo

Dr. Mamdouh Gabr, Professor Pediatrics, University of Cairo

Dr. Osman Galal, Director, Nutrition Institute and PI Egypt Nutrition CRSP

Dr. Mohammed Hussein Khalil, Chairman, Department of Statistics, High Institute of Public Health, Alexandria, Egypt

Dr. Moustafa Mansour, Head, Biochemistry Department, U.S. Navy Medical Research Unit

Dr. Wafaa Moussa, Head, Department of Surveys, Surveillance and Programs, Nutrition Institute

Dr. Amin Said, Head, Department Clinical Nutrition, Nutrition Institute

Dr. Farouk Shaheen, Dept. of Surveys, Surveillance and Programs, Nutrition Institute

Dr. Zeinab Shaheen, National Center for Social and Criminological Research

Mr. John Wiles, Nutrition Officer, Agency for International Development

Dr. June Wolgemuth, Field Coordinator, Nutrition Institute

Dr. Feisal A. Yunis, Department Psychology, Faculty of Arts, Cairo University

Dr. Sabri Zaki, Minister of Health, Arab Republic of Egypt

ATTACHMENTS

I. Itinerary

II. Congenital Abnormalities of Infant

III. Pregnancy Monthly Visits

IV. Pregnancy Outcome

V. Urinalysis for Protein, Glucose and Ketones

VI. Comparison of Two Procedures for Determination of Ferritin in Plasma and Serum of Capillary and Venous Blood

VII. Hemoglobin (Hgb)

VIII. Hematocrit (Hct) Measurement

IX. Plasma/Serum Ferritin Measurement

X. Protein, Lipid and Vitamin B-6 Concentrations in Milk Samples Obtained from Women in the Village of Kalama, Egypt

XI. Comparison of Total Lipid Content in Pooled Human Milk Analyzed by Creamatocrit and Modified Folch Methods After Freezing and Thawing of Milk and After Different Lengths of Storage

XII. Minutes of Egypt Nutrition CRSP Workshop

XIII. Human Subjects Document

ATTACHMENT I

Itinerary

September 2-3		Travel from West Lafayette to Cairo
4	Sunday	Nutrition Institute
5	Monday	Nutrition Institute
6	Tuesday	Nutrition CRSP Workshop I, Nutrition Institute
7	Wednesday	Nutrition CRSP Workshop I, Nutrition Institute
8	Thursday	Nutrition CRSP Workshop I, Nutrition Institute
10	Saturday	Ministry of Health, Nutrition Institute, Village of Kalama
11	Sunday	Village of Kalama
12	Monday	AID (Cairo), Nutrition Institute
13	Tuesday	Nutrition Institute, American University, Cairo
14	Wednesday	Nutrition Institute
15	Thursday	Nutrition Institute
16-20		High feast celebration
21	Wednesday	Village of Kalama, Nutrition Institute
22	Thursday	Village of Kalama, Nutrition Institute
24	Saturday	Village of Kalama, Nutrition Institute
25	Sunday	Village of Kalama, Nutrition Institute
26	Monday	Village of Kalama, Nutrition Institute
27	Tuesday	Village of Kalama, Nutrition Institute
28	Wednesday	Village of Kalama, Nutrition Institute
29	Thursday	Nutrition Institute, NAMRU Unit 3
30-October 1		Travel from Cairo to West Lafayette

CONGENITAL ABNORMALITIES OF INFANT

(Observation During The First Month of Life)

Block No. ☐☐ Household No. ☐☐☐☐ Date ☐☐ (D) ☐☐ (M) ☐☐ (Y) Examiner ☐☐

Person giving information: Name of Infant _____ Sex ☐M ☐F

Lead Female _____ ☐☐☐ Birth date of Infant ☐☐ (D) ☐☐ (M) ☐☐ (Y)

I.D. No. of Infant ☐☐☐

Code 1 if yes; 2 if no.

- Hydrocephalus ☐
- Microcephalus ☐
- Blindness ☐
- Deafness ☐
- Hare-lip ☐
- Cleft palate ☐
- Abnormalities in Fingers ☐
- Abnormalities in Toes ☐
- Esophageal Stenosis ☐
- Pyloric Stenosis ☐
- Imperforate Prepuce ☐
- Imperforate Anus ☐
- Talepes (club foot) unilateral ☐
  bilateral ☐
- Cyanosis ☐
- Heart Abnormalities ☐
- Other _____ ☐☐ (Office use)

NUTRITION INSTITUTE

ATTACHMENT III

EGYPT NUTRITION CRSP
Reproduction Form II
(Page 1 of 3)

PREGNANCY MONTHLY VISITS

Block No. ☐☐ Household No. ☐☐☐☐ Date ☐☐ D ☐☐ M ☐☐ Y

Weeks of gestation ☐.☐ Date of last menses ☐☐ D ☐☐ M ☐☐ Y

Name of Lead Female _____ I.D. No. ☐☐☐

Anthropometry Recording Form

Observer A _____

Observer B _____

Parameters to be measured	First pair of measurements				Second pair of measurements		
	Observer A	Observer B	Difference of A & B	Acceptable?	Observer A	Observer B	Comments
Weight (kg)	__ __ __.__	__ __ __.__			__ __ __.__	__ __ __.__	
Midarm circumference (cm)	__ __ __.__	__ __ __.__			__ __ __.__	__ __ __.__	
Triceps skinfold (mm)	__ __ __.__	__ __ __.__			__ __ __.__	__ __ __.__	
Biceps skinfold (mm)	__ __ __.__	__ __ __.__			__ __ __.__	__ __ __.__	
Subscapular skinfold (mm)	__ __ __.__	__ __ __.__			__ __ __.__	__ __ __.__	

Pre-set limits: Weight, 0.1 kg; Midarm circumference, 0.2 cm; Triceps skinfold, 3 mm; Biceps skinfold, 3 mm; Subscapular skinfold, 3 mm.

Code 1 if yes; 2 if no; 3 if no answer.

Examiner ☐☐

Nipple enlarged?* ☐

Colored areola?* ☐

Lower limb edema?* ☐

Are you presently breast feeding? ☐

Blood pressure ☐☐☐ S ☐☐☐ D

Fundal height ☐☐.☐ cm

Fetal heart sounds ☐

Fetal movements ☐

*By examination

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MORBIDITY FORM ATTACHMENT

Schedule for Pregnancy

Block No. ☐☐ Household No. ☐☐☐☐ Date ☐☐ D ☐☐ M ☐☐ Y Interviewer ☐☐

Target Individual: Lead Female _____ ☐☐☐

Person Giving Information: Lead Female _____ ☐☐☐

Code 1 if yes; 2 if no; 3 if no answer.

In the past week:

Did you feel quickening/fetal movement? ☐

Did you feel heaviness/tingling/tenderness in your breast? ☐

Did you have bleeding? ☐

If yes, how many days? ☐

Did you have discharge? ☐

Did you feel contractions? ☐

Did you feel morning sickness? ☐

Did you feel craving for special foods? ☐

If yes, list: 1. ☐☐

2. ☐☐

3. ☐☐

4. ☐☐

5. Food in general ☐

Did you feel aversion to particular foods? ☐

If yes, list: 1. ☐☐

2. ☐☐

3. ☐☐

4. ☐☐

5. All foods ☐

Did you eat different than usual? ☐

(If yes, notify supervisor)

Did you feel more or less tired than usual? 1 = less 3 = more ☐

2 = no change

Did you change your activity pattern? ☐

(If yes, attach schedule J)

Are you nursing? ☐

If yes, has your milk changed in quantity? ☐

quality? ☐

Other ☐

If yes, describe _____ ☐☐

(Office use)

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Blood, finger-prick withdrawn (22 and 36 weeks only) Sample obtained at ☐☐☐☐AM/PM

Hemoglobin ☐☐.☐g/dl

Hematocrit ☐☐%

Ferritin ☐☐.☐ng/ml

Blood smear for differential while blood cell count

Neutrophils ☐☐

Basophils ☐☐

Eosinophils ☐☐

Monocytes ☐☐

Lymphocytes ☐☐

Urine (monthly) Sample obtained at ☐☐☐☐AM/PM

Albumin, mg/dl ☐ Neg ☐ Tr ☐ 30 ☐ 100 ☐ 300 ☐ >2000

Glucose, mg/dl ☐ Neg ☐ 100 ☐ 250 ☐ 500 ☐ 1000 ☐ >2000

Ketones, mg/dl ☐ Neg ☐ 5 ☐ 15 ☐ 40 ☐ >80

PREGNANCY OUTCOME

Block No. ☐☐ Household No. ☐☐☐☐ Date ☐☐ (D) ☐☐ (M) ☐☐ (Y) Examiner ☐☐

Name of Lead Female _____ I.D. No. ☐☐☐

Date of Pregnancy Outcome ☐☐ (D) ☐☐ (M) ☐☐ (Y)

Pregnancy outcome
- ☐ Abortion or miscarriage (<28 weeks)
- ☐ Stillbirth (>28 weeks)
- ☐ Live birth

If live born
- ☐ Normal delivery
- ☐ Complicated
  - ☐ Hemorrhage
  - ☐ Forceps
  - ☐ Caesarean

Pleurality ☐ Single ☐ Twins ☐ Triplets

Attended by ☐ Self/Family ☐ "Daya" ☐ Paramedical ☐ Physician/Hospital

NEWBORN:

Birth weight ☐☐.☐ kg at age ☐☐ hours

GESTATIONAL AGE:

☐☐ weeks by menstrual history

☐☐ weeks by date of quickening

☐☐ weeks by fundal height

☐☐ weeks by Dubowitz score at age ☐☐ hours

Continue to next page for anthropometry recording form for 8-day-old-infant.

INFANT (8-day-old):

Anthropometry Recording Form

Observer A _____

Observer B _____

Parameters to be measured	First pair of measurements				Second pair of measurements		
	Observer A	Observer B	Difference of A & B	Acceptable?	Observer A	Observer B	Comments
Length (cm)	__ __.__	__ __.__			__ __.__	__ __.__	
Weight (kg)	__ __.__	__ __.__			__ __.__	__ __.__	
Midarm circumference (cm)	__ __.__	__ __.__			__ __.__	__ __.__	
Triceps skinfold (mm)	__ __.__	__ __.__			__ __.__	__ __.__	
Biceps skinfold (mm)	__ __.__	__ __.__			__ __.__	__ __.__	
Subscapular skinfold (mm)	__ __.__	__ __.__			__ __.__	__ __.__	
Head circumference (cm)	__ __.__	__ __.__			__ __.__	__ __.__	

Pre-set limits: Length, 1 cm; Weight, .1 kg; Midarm circumference, 0.2 cm; Triceps skinfold, 1.5 mm; Biceps skinfold, 1.5 mm; Subscapular skinfold, 1.5 mm; Head circumference, .2 cm.

ATTACHMENT V

URINALYSIS FOR PROTEIN, GLUCOSE AND KETONES

Uristix (Protein and Glucose)
Ketostix (Ketone-Acetoacetic Acid)

Ames Division
Miles Laboratories, Inc.
P.O. Box 70
Elkhart, IN 46515

Supplies

Collection containers for urine sample
Uristix
Ketostix
Stop watch or watch with second hand

Storage of Sticks

Store Ketostix and Uristix in original container at temperatures under 30° C (86°F). Do not store in a refrigerator. Use within 4 months after opening. Do not use after the expiration date.

Specimen Collection

1. Collect urine immediately before analysis in a clean, dry container. The urine specimen must be fresh to avoid false readings.

Procedure

1. The test should be performed in a well-lighted area. Remove one Ketostix from the bottle and replace the cap. Completely immerse the reagent area of the strip in FRESH urine and remove immediately to avoid dissolving out the reagents.

2. While removing, draw the edge of the strip against the rim of the urine container to remove excess urine. Hold the strip in a horizontal position to prevent soiling of the hands with urine.

3. Compare test area to color chart at exactly 15 seconds after wetting the Ketostix. HOLD STRIP CLOSE TO THE COLOR BLOCKS ON THE CHART AND MATCH CAREFULLY.

4. Record results.

5. Repeat procedures 1-4 using Uristix except results for protein measurement may be read immediately and for glucose exactly 30 seconds after wetting the Uristix.

NOTE: For further information refer to the brochure which the Company (Ames, Division, Miles Laboratories, Inc.) supplies with Uristix and Ketostix.

ATTACHMENT VI

Comparison of Two Procedures for Determination of Ferritin in Plasma and Serum of Capillary and Venous Blood

	ELISA[1]		Ramco[2]	
Venous Blood				
Plasma	28.5±3.3[3,4]	(n=5)	23.8[5]	(n=4)
Serum	26.7±1.4	(n=5)	22.5	(n=4)
Capillary Blood				
Plasma	20.5±2.9	(n=4)	21.0	(n=4)
Serum	24.3±7.4	(n=4)	21.8	(n=4)

[1]New England Immunology Assoc., Cambridge, MA 02138

[2]Ramco Laboratories, Inc., Houston, TX 77098

[3]Mean ± SD

[4]50 μl sample for ELISA method

[5]10 μl sample for Ramco method

ATTACHMENT VII

HEMOGLOBIN (Hgb)

Supplies

Blood Lancet
Alcohol Swabs
Bandages
Ice Thermos
Labelling tape
Marking pens
Chemical wipes

UNOPETTE Microcollection System (Becton-Dickinson capillary pipette and diluent reservoir)
Spectrophotometer (540 nm)
Cuvettes
Linear graph paper
Commercial Cyanomethemoglobin HgB Standard (Hycel)
Commercial Hgb Controls (Hycel)

Principle

UNOPETTE test (Becton-Dickinson #5857/5858) measures the following hemoglobins: cyanmethemoglobin, oxyhemoglobin, carboxyhemoglobin and methemoglobin. Whole blood is added to the UNO-HEME Reagent. The ferrous form of hemoglobin is oxidized by potassium ferricyanide to the ferric state to form methemoglobin. This, in turn, combines with potassium cyanide to produce the stable pigment cyanmethemoglobin. These two reactions are rapid and stoichiometric.

The absorbance of cyanmethemoglobin is measured at 540 nm in a spectrophotometer. The absorbance value is used to calculate the grams per deciliter of hemoglobin in the sample, according to a previously constructed hemoglobin standard curve.

WARNING

This product (UNOPETTE test) is for *in vitro* diagnostic use only. Avoid contact of reagents in the reservoir with skin and clothes. If contact occurs, flush area copiously with water. Salts and solutions of cyanide are poisonous and are not to be ingested nor their fumes inhaled. Do not discard diluent into a sink containing acid; pour diluent directly into drain while water is flowing from the tap.

CAUTION: If reservoir is squeezed too hard, the diluted specimen may be expelled through the top of the overflow chamber, resulting in contamination of the fingers.

Dilution Ratio

Sample to Total Volume 1:250

Reagent Stability

The reagent system is stable when stored below 25° C, protected from light and used before the expiration date and under the stated conditions of the test. Diluent normally appears light yellow in color. Do not use if diluent appears to have lost color.

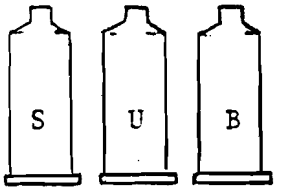
Specimen

Free-flowing capillary blood or venous blood thoroughly mixed with an anti-coagulant. EDTA is the anti-coagulant of choice. The diluted specimen is stable at either room temperature (<25° C) or 4° C for 24 hours.

Storage Instructions

If hemoglobin quantitation cannot be performed immediately, store diluted specimen at either room temperature (<25° C) or 4° C for as long as 24 hours.

PROCEDURE



1. Assemble Components.

   a. Label three UNOPETTE reservoirs containing UNO-HEME reagent as follows: S for standard; U for unknown; and B for blank.

2. Puncture Diaphragm.

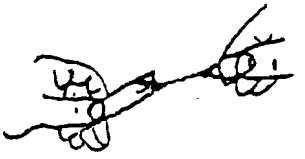
   a. Place reservoir on a flat surface. Grasping reservoir in one hand, take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.

   b. Remove shield from pipette assembly with a twist.

3. Add Sample.

Fill capillary with 20 µl of hemoglobin standard and add to reservoir S as follows:

   a. Holding pipette almost horizontally to ensure complete filling, touch tip of pipette to standard solution. Pipette will fill by capillary action. Filling is complete and will stop automatically when solution reaches end of capillary bore in neck of pipette.



   b. Wipe excess standard from outside of capillary pipette. NOTE: Capillary pipette should be placed over chemical wipe and drawn away to avoid loss of the sample onto the wipe. If sample is lost, refill and rewipe the outside of capillary pipette.

   c. Squeeze reservoir slightly to force out some air. Do not expel any liquid. Maintain pressure on reservoir.

   d. Cover opening of overflow chamber of pipette with index finger and seat pipette securely in reservoir neck.

   e. Release pressure on reservoir. Then remove finger from pipette opening. Negative pressure will draw solution into diluent.

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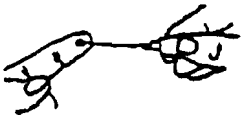
f. Squeeze reservoir gently two or three times to rinse capillary bore, forcing diluent up into, but not out of, overflow chamber, releasing pressure each time to return mixture to reservoir. CAUTION: See previous WARNING section.

g. Place index finger over upper opening and gently invert several times to thoroughly mix standard with diluent.

Fill a second capillary with 20 µl of whole blood. Add specimen to labeled reservoir following steps a to g above.

Insert an empty pipette into labeled reservoir.



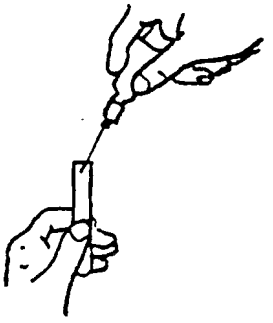
4. Allow to Stand

Let reservoirs stand at room temperature for ten minutes. Diluted specimen is stable for as long as 24 hrs at either room temperature or 4° C.

5. Transfer Contents

Transfer thoroughly mixed contents of each reservoir to appropriately labeled test tubes or corresponding cuvettes as follows:

a. Convert reservoir to dropper assembly by withdrawing pipette and reseating securely in reverse position as shown.



b. Place capillary tip into appropriately labeled test tube or cuvette which will accommodate 5.0 ml of reagent and squeeze reservoir to expel entire contents.

6. Set wavelength on a spectrophotometer at 540 nm.

7. Read Absorbance

a. Wipe outside of test tubes or cuvettes before placing in instrument.

b. Check zero stability of instrument using distilled water.

c. Place test tube or cuvette labeled B (blank) in instrument and set instrument at zero absorbance.

d. Measure absorbance of Standard and Unknown against Blank set at zero absorbance and record.

8. Construct a Calibration Curve

To establish hemoglobin values, a calibration curve must be constructed based on a solution of known hemoglobin content. Commercial preparations of

cyanmethemoglobin are available for this purpose. Reservoirs containing UNO-HEME diluent should be used to make the appropriate dilution of the standard (Hycel) which should be made to obtain at least 3 or 4 points on the calibration curve. All calibration dilutions are read at 540 nm. The curve should closely follow Beer's Law.

a. Procedure

1. Note the dilution factor of technique and select proper standard set for the instrument.

2. Select four clean, dry, well matched cuvettes. Label one cuvette blank and the other three according to the concentration of standards on the vial labels.

3. Fill cuvette labeled blank with cyanmethemoglobin reagent. Fill the other cuvettes with standards matching their concentration, and using the same volume for determination of unknown samples.

4. Allow the standard samples to warm to room temperature.

5. Set the wavelength of the spectrophotometer at 540 nm. Adjust the instrument to zero absorbance using the reagent blank, and then determine the absorbance of the standards.

6. Prepare a calibration curve by plotting the absorbance of the standards against their concentrations using arithmetical graph paper.

7. A new standard curve should be prepared and standardization done at scheduled frequencies.

9. Calculate

a. Determine the absorbance of sample unknowns against a reagent blank in accordance with the test procedure used.

b. Transfer the reading to the standard curve and obtain the hemoglobin concentration in g/dl.

<u>OR</u>

c. Enter points on a calculator (simple linear regression program) and obtain exact concentrations.

For further information consult the UNOPETTE instruction booklet (Becton-Dickinson, Rutherford, New Jersey 07070, Telephone number 1-800-631-0174).

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ATTACHMENT VIII

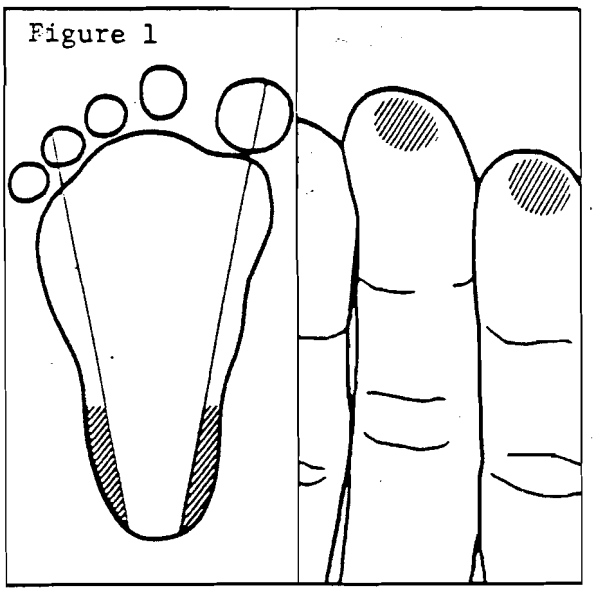
HEMATOCRIT (HCT) MEASUREMENT

Supplies

Ice Thermos
75 mm heparinized micro-hematocrit tubes
Capillary tube sealer
Chemical wipes
Prepared storage tubes
Labelling tape
Marking pen
Plasma ferritin tubes
Diamond point pencil
Lancet
Alcohol swabs
Bandages

Specimen Collection

1. The preferred site for finger prick blood collection is on the index or middle finger on the non-dominant hand in the area illustrated (Figure 1). The preferred site for infant blood collection is the heel in the illustrated shaded areas (Figure 1). Clean the finger/heel area with alcohol and puncture the skin with a disposable blood lancet, or equivalent, to produce a free flow of blood.



2. Grasp the end of a 75 mm heparinized capillary tube nearest the colored ring between the thumb and forefinger.

3. Insert the other end of the capillary tube into the drop of finger blood. THE FINGER SHOULD NEVER BE SQUEEZED TIGHTLY TO INCREASE BLOOD FLOW. Tight squeezing tends to dilute the blood specimen with tissue fluid, causing a false low hematocrit.

4. Fill the capillary tube between 1/2 and 3/4 full as shown in Figure 2. NOTE: The rate of filling may be accelerated by tilting the capillary tube (Figure 3).

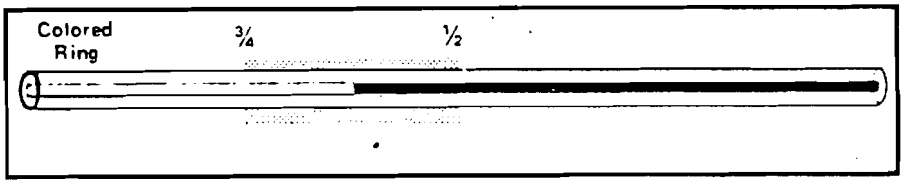


Figure 2 Capillary Tube, Showing Fill Level Region.

5. When properly filled, remove the tube from the finger, holding the tube level so that blood cannot flow out of the end. NOTE: In the event the tube has been over-filled, remove the excess blood by gently dabbing the wet tip with a chemical wipe.

6. TILT THE CAPILLARY TUBE UNTIL BLOOD FLOWS ABOUT 3 MM or 1/8" IN FROM THE BLOOD COLLECTING TIP (Figure 4). Return the capillary tube to a horizontal position so that no blood is lost.

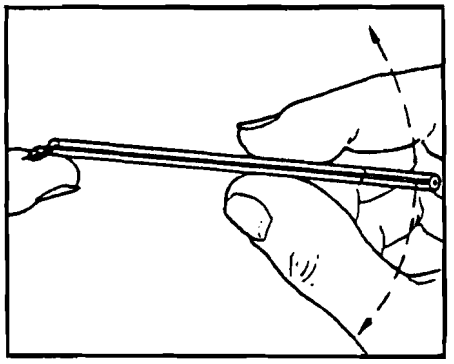


Figure 3 Tilting Capillary Tube to Regulate Blood Flow.

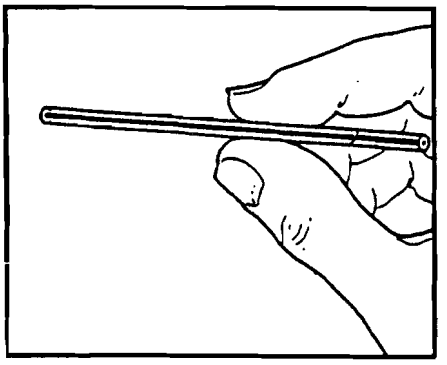


Figure 4 Tilting Tube to Remove Blood From Tip.

7. Wipe any droplets of blood from the outside surface of the capillary tube using a chemical wipe.

8. SEAL THE CAPILLARY TUBE AS FOLLOWS:

   a. Invert the capillary tube so that the dry end, not used for blood collecting, points down.

   b. IMMEDIATELY INSERT THE BLOOD-FREE, DRY END INTO SEALER, as shown in Figure 5. Push the tube fully down into the clay <u>before</u> blood flows into the dry end. THE END OF THE TUBE MUST BE DRY FOR THE CLAY TO SEAL.

      NOTE: If blood reaches the end of the tube before the sealer is forced in, some blood may be lost and the clay will not seal. In this case a new sample should be drawn. The tube is now ready for centrifugation.

9. Place the HCT tube in a test tube with a cotton plug at the bottom and cork tube. HCT tube must be inserted with the sealed end first to avoid loss of the sample. Label the test tube with name, date, household #, etc.

10. Samples should be kept cool and centrifuged within 90 minutes of collection.

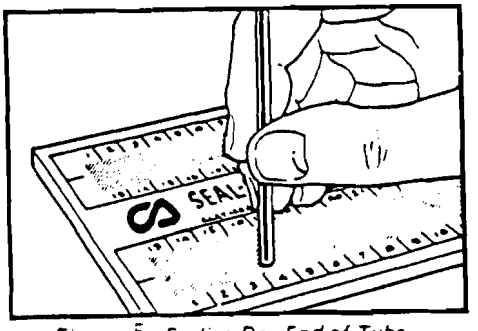


Figure 5 Sealing Dry End of Tube

Centrifugation (Clay-Adams Autocrit II Hematocrit Centrifuge and Reader)

a. Place the loaded carrying tray over the scale plate.

b. Spin the head by hand to check for proper seating and position of the tubes.

c. Place the head cover over the carrying tray so that the spindle fits into the threaded hole in the center of the head cover. Make sure that the index pin on the carrying tray hub (see Figure 6) fits into one of the index holes on the underside of the head cover. A good method to insure proper indexing while tightening the head cover is to hold the cover knob firmly with one hand and spin the entire head assembly counterclockwise with the other hand.

d. Tighten the head cover securely by hand. Do not tighten with the spanner wrench.

e. Close the centrifuge lid and press down until the latch handle is fully retracted. The centrifuge will not operate unless the lid is closed.

f. Turn the TIMER dial clockwise to the desired setting.

g. Press the PUSH TO START Button in the center of the TIMER. The centrifuge head will immediately accelerate and the ON lamp will go on and remain on until the centrifugation is complete and the lid lock is disengaged.
NOTE: Once the TIMER is set, it does not require resetting before additional centrifugations calling for the same spinning time.

h. After the ON lamp goes off and the head slows, pull the latch handle forward to open the lid. Remove the head cover by holding the knurled knob with one hand and rotating the head assembly clockwise with the other hand. Use the spanner wrench if the cover is too tight to be loosened by hand.

i. Read hematocrits of the capillary tubes following the procedures in Paragraph 3, below.

j. For additional centrifugations at the same time setting, repeat Steps a-e, above, and start the centrifuge by depressing the PUSH TO START Button.

Reading Hematocrits

Use the magnifier supplied with the AUTOCRIT II Centrifuge for accurate hematocrit readings. To read hematocrits:

a. Swing the magnifier over the spiral hematocrit scale.

b. Rotate the carrying tray until the tube to be read is at the front of the centrifuge, directly under the center of the magnifier.

c. Slide the capillary tube along its axis until the interface between the bottom of the packed red cell column and the sealing material coincides with the "0" line at the periphery of the spiral scale (see Figure 6).

d. Hold the aluminum rim of the carrying tray steady with one hand. Use the other hand to rotate the scale plate until the top of the plasma column coincides with the "100%" line on the spiral scale (see Figure 6).

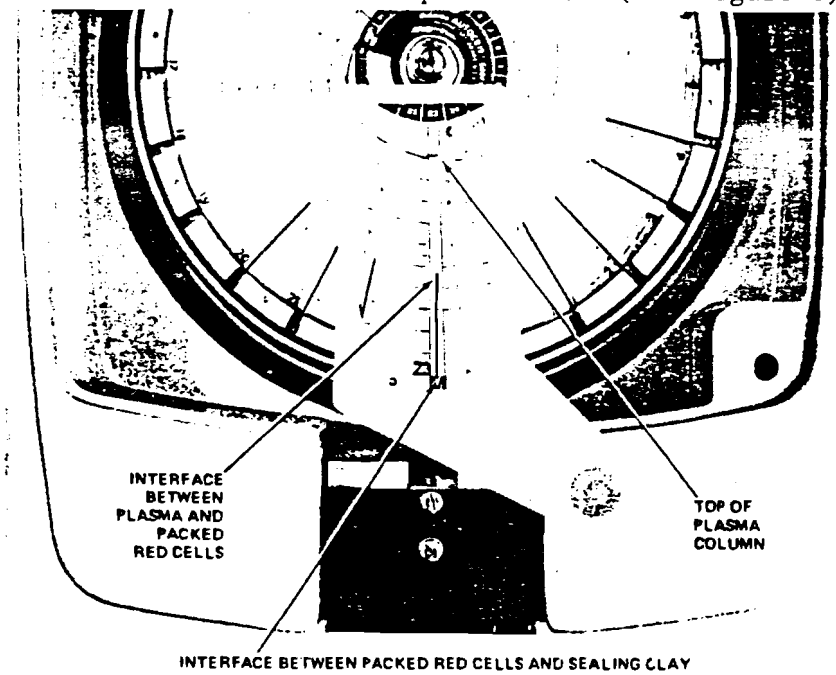


Figure 6 Direct Hematocrit Reading from Scale Plate.

e. The hematocrit value may now be read from the spiral scale at the interface between the packed red cell column and the clear plasma column.

f. After reading the HCT tubes of subjects whose plasma ferritin concentrations are to be analyzed should be handled as follows:

1. One by one each tube should be removed from the tray by carefully sliding each tube out of its hub slot and then lifting it off the tray.

2. The tube should be placed flat on a board and a line etched in the tube just above the packed cell layer with a diamond tip pencil. While maintaining capillary tube in a horizontal position, carefully snap tube at the etched line.

3. Place the open end of the HCT tube in a clear plastic micro-centrifuge tube and tilt to dispense the plasma into the tube.

4. Cap the tube and label. Freeze as soon as possible.

NOTE: THE ACCURACY OF THE MICROHEMATOCRIT METHOD (WHEN REPLICATES ARE READ BY THE SAME OBSERVER) IS $\pm$ 0.21%. THERE IS, HOWEVER, A SIGNIFICANT OBSERVATION ERROR, ABOUT 2% VARIATION, WHEN DIFFERENT TECHNOLOGISTS READ THE SAME MICROHEMATOCRIT PREPARATION (JB Miale, Laboratory Medicine Hematology, Mosby, 1982).

ATTACHMENT IX

PLASMA/SERUM FERRITIN MEASUREMENT

(Enzyme immunoassay)

Spectroferritin
Ramco Laboratories, Inc.
3801 Kirby, Suite 170
Houston, Texas 77098

Test Principle

The enzyme immunoassay for the quantitative determination of serum ferritin is basically a three stage reaction.

Stage 1: The binding of human serum ferritin to a solid phase anti-human ferritin.

Stage 2: The binding of purified anti-human ferritin conjugated with alkaline phosphatase to the insoluble Stage 1 reaction complex.

Stage 3: Incubation of the Stage 2 reaction complex in a substrate solution of p-nitrophenyl phosphate disodium to develop color which is the measure of the amount of bound anti-human ferritin alkaline phosphatase conjugate. The reaction is stopped with 0.1 N NaOH and the color is measured in a spectrophotometer set at 405 nm. The amount of color formed in each sample is directly proportional to the concentration of serum ferritin in the specimen. A standard curve is prepared by plotting a series of ferritin standards supplied with the kit versus absorbance. Samples and controls, run simultaneously with the standards, are then assigned values determined from the curve.

Reagents	Storage
Prediluted Ferritin Standard Solutions 6 vials: Ferritin concentrations- 6,20,60,200,600 and 2000 ng/ml	2-8° C
Solid Phase Anti-human Ferritin	2-8° C
Sample Diluting Buffer	2-8° C
Ferritin Standard Diluting Buffer	2-8° C
Conjugated Anti-human Ferritin	2-8° C
Substrate Buffer	2-8° C
Substrate	less than 0° C
Sodium Hydroxide	room temperature

Precautions: The human ferritin in the standard solutions has been tested and found to be non-reactive for Hepatitis B Surface Antigen by RIA. The standard solutions as well as the samples and control sera should be considered potentially hazardous and handled in accordance with approved laboratory procedures and regulations. In addition, sodium azide has been added to solutions at a concentration of 0.003 M. Although this is a low concentration, it is recommended that copious amounts of water be used to flush the drain pipeline after disposal of these reagents in the plumbing system. Sodium azide is a potential explosive

hazard when it reacts with copper, lead, brass or solder in plumbing systems.

Please Note:

1. Important: Upon arrival, take the bottle of substrate tablets out of the box and store in freezer (below 0° C), at all times except when in use.
2. Do not pipet
3. Avoid contact of Sodium Hydroxide solution with skin.
4. Do not mix reagents from different lots.
5. Do not use reagents beyond the expiration date.

Sample Collection and Storage

Plasma samples, obtained after hematocrit determinations, will be used for the assay. Immediately after the hematocrit determination the capillary tube should be etched at the plasma/red blood cell interface and the tube is then broken at the interface. Plasma is then transferred to a small plastic vial and frozen until assayed for ferritin. Moderate hemolysis will not interfere with the ferritin assay.

Materials

Materials provided: All vials of each reagent (see reagents), 12 reaction trays and 16 bead holder assemblies for 128 beads, log-log graph paper.

Materials required but not provided:

- Precision pipettes to deliver 10 µl, 20 µl, 200 µl (or microliter syringe)
- Pipettes that deliver accurately 1.0 ml
- Spectrophotometer with the following specifications at 405 nm:
  - Bandwidth: 7.0 nm or less
  - Absorbance range: 0.001 to 2.0 OD
  - Repeatability: Less than 0.005 OD at 0 OD
  - Linearity or accuracy: Less than 0.005 OD at 0-1 OD
  - Required Readout Type: Digital preferred
- Ramco Shaker Table or horizontal rotation table (not necessary if refrigerated incubation procedures are used)

Serum Ferritin Assay Procedures

Use of the reaction trays:

Remove the required number of trays and bead holders needed for the assay. Number the trays and bead holders. Example: If three trays are needed for the assay, number the trays 1, 2, 3 and the bead holders 1 through 12. This will keep all the beads in the proper sequence during washing, etc. Duplicate samples should be dispensed side by side in each row. Example: A1, A2 are duplicates of the same sample. Follow this scheme until the first tray is completed. Start again with A1 of the second tray and continue in this fashion until all samples have been dispensed. NOTE: Three sets of trays will be required for the assay, one set for phase ONE, a second set for Phase THREE and a third for Phase FOUR.

If necessary, trays and bead holders may be reused once. These should be thoroughly washed immediately after use.

A flexible time assay procedure will be described since it employs only 10 µl of plasma. Procedures for the 2 hour assay are described in detail in the Spectroferritin instruction booklet.

II. FLEXIBLE TIME ASSAY

PHASE ONE: DISPENSE STANDARDS AND SUBJECT SAMPLES.
Allow reagents to reach room temperature before use.

FERRITIN STANDARDS

TRAY NO. 1	SAMPLE	SAMPLE VOLUME	DILUTING BUFFER	BUFFER VOLUME
A1, A2	0 ng/ml	*	STANDARD	200 µl
A3, A4	6 ng/ml	10 µl	STANDARD	200 µl
A5, A6	20 ng/ml	10 µl	STANDARD	200 µl
A7, A8	60 ng/ml	10 µl	STANDARD	200 µl
B1, B2	200 ng/ml	10 µl	STANDARD	200 µl
B3, B4	600 ng/ml	10 µl	STANDARD	200 µl
B5, B6	2000 ng/ml	10 µl	STANDARD	200 µl

*The 0 ng/ml standard consists of Standard Diluting Buffer only and is used to measure non-specific binding (NSB).
Add the sample or standard to the cuvette before the buffer.
For the most accurate delivery of the 10 µl volume, touch the bottom of the cuvette with the pipette tip when dispensing the standard or subject samples.

SAMPLES AND CONTROLS

TRAY NO. 1	SAMPLE	SAMPLE VOLUME	DILUTING BUFFER	BUFFER VOLUME
B7, B8	LOW CONTROL	10 µl	SAMPLE	200 µl
C1, C2	MED CONTROL	10 µl	SAMPLE	200 µl
C3, C4	HIGH CONTROL	10 µl	SAMPLE	200 µl
C5, C6	SUBJECT PLASMA	10 µl	SAMPLE	200 µl
C7, C8	SUBJECT PLASMA	10 µl	SAMPLE	200 µl

NOTE: Any convenient 1:21 dilution procedure may be used if large sample volumes are desired. EXAMPLE: 25 µl of sample in 500 µl of appropriate buffer, 200 µl of this diluted material must be assayed in each duplicate cuvette. If preliminary dilutions are made of standards these dilutions may be stored and used for the shelf life of the kit.

PHASE TWO: ADD BEADS AND START STAGE 1 INCUBATION.
Place one Solid Phase Anti-Human Ferritin bead in each test well used.
Place the numbered bead holders into the reaction cuvettes.
Place bead holder #1 in row A of tray #1, bead holder #2 in row B, etc.
Place bead holder #5 in row A of tray #2 and continue in this sequence until all bead holders are in place. Press down firmly on each bead holder to assure it grips the beads.
Incubation may be performed using the most convenient of the following two methods. NOTE: Stage I and Stage II methods may be mixed without affecting the results of the test.

METHOD 1: Place the reaction trays on a Ramco Shaker Table or a horizontal rotation table and shake for two hours at moderate speed at room temperature.

METHOD 2: Leave at room temperature for 6 hours or cover and refrigerate at 4°C for 16 hours.

NOTE: Just before proceeding the PHASE THREE, dispense 200 µl of the alkaline phosphatase conjugated anti-human ferritin into each cuvette of the second set of trays that will be used for the Stage 2 incubation. It is necessary to have these trays ready to receive the beads after washing, in order that they do not become dry before being placed into the conjugated antisera.

PHASE THREE: WASH BEADS AND START STAGE 2 INCUBATION.
Stop the Stage 1 reaction by removing the bead holders from the trays and passing the beads under a stream of distilled or deionized water. Gently shake the assembly to remove any excess water and place the beads in the alkaline phosphatase conjugated anti-human ferritin.

This incubation may be performed using the most convenient of the following two methods. NOTE: Stage 1 and Stage 2 methods may be mixed without affecting the results of the test.

METHOD 1: Place the reaction trays on a Ramco Shaker Table or a horizontal rotation table and shake for two hours at moderate speed at room temperature.

METHOD 2: Leave at room temperature for 6 hours or cover and refrigerate at 4°C for 16 hours.

NOTE: About 15 minutes prior to starting PHASE FOUR of the assay, place the appropriate number of Substrate tablets in a separate container and dissolve them in the required amount of Substrate Buffer. Use 2.0 ml of Substrate Buffer for each tablet. Vortexing will make the tablets dissolve faster if necessary. Just before proceeding with PHASE FOUR, dispense 200 µl of the Substrate into each cuvette required in the third set of trays used for the Stage 3 incubation.

PHASE FOUR: WASH AND START STAGE 3 INCUBATION.
Stop the Stage 2 reaction by removing the bead holders from the trays and wash the beads in the same manner as previously described. Place the beads in the trays containing the Substrate and incubate at room temperature for 30 minutes. It is not necessary to use the shaker table for this incubation.

PHASE FIVE:
STOP STAGE 3 INCUBATION WITH SODIUM HYDROXIDE.
Leave the bead holders in the trays and accurately dispense 1.0 ml of 0.1N NaOH into each test well. Mix the contents of the test wells by plunging the bead holders up and down gently several times. After sufficiently mixing the contents discard the bead holders and beads.

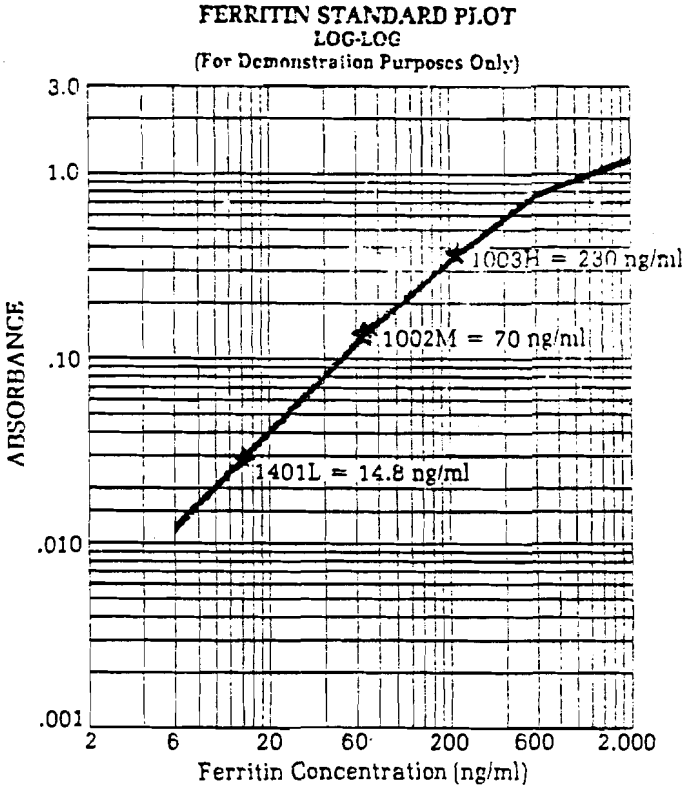
CALCULATIONS

Construct a standard curve by plotting the mean absorbance value obtained for each ferritin standard on the vertical axis (Y) and the corresponding ferritin standard concentration in ng/ml on the horizontal axis (X) of the log-log graph paper supplied with the kit. Draw a curve using a line drawn from point to point. Determine the concentration of each unknown and control directly from the graph of the standard curve.

EXAMPLE DATA
(For Demonstration Purposes Only)

ng/ml	O.D.	MEAN	-NSB	Conc. ng/ml
0 (NSB)[1]	.000 .001	.0005		
6	.011 .012	.0115	.011	
20	.039 .039	.039	.0385	
60	.114 .113	.1135	.113	
200	.346 .333	.3395	.339	
600	.791 .756	.7735	.773	
2000	1.174 1.231	1.2025	1.202	
Sample 1 (1003H)	.388 .355	.3715	.371	230
Sample 2 (1002M)	.126 .122	.124	.1235	70
Sample 3 (1401L)	.028 .029	.0285	.028	14.8

[1]Non-specific binding



Set the spectrophotometer to 405 nm. Zero the instrument using a blank made of 200 µl of substrate solution and 1.0 ml of 0.1N NaOH. Read and record the absorbance of each standard and sample. Readings should be made on a spectrophotometer within one hour of completion of assay.

Absorbance values for the 2000 ng/ml standard should range from 1.0-1.5 O.D. Lower readings may occur in cooler room temperatures of approximately 23°C while higher absorbances may result when room temperatures approximate 27°C.

DILUTION OF SERA OF FERRITIN CONCENTRATION IN EXCESS OF 1000 ng/ml.

1) Dilute 1:10 with Sample Diluting Buffer.
2) If further dilutions are required, these additional dilutions are made with the Ferritin Standard Diluting Buffer.
3) For assay of these diluted sera:
   a) Pipette 10 µl of diluted sera into appropriate cuvette.
   b) Pipette 200 µl of the Ferritin Standard Diluting Buffer into cuvettes which contain the diluted sera.
   c) Proceed with PHASE TWO of the assay procedure.
   d) After determining the concentrations, multiply the value obtained by the dilution factor.

LIMITATIONS OF THE PROCEDURE

Moderate hemolysis has no effect upon the reproducibility or accuracy of the procedure. No drugs or other administered substances have been found to produce any effect on tests. Anticoagulants have not been shown to influence the test so long as they do not result in dilution of the plasma. Strict adherence to precise laboratory procedure is essential for maximum accuracy of the final results. Do not allow beads to dry after removing from Vial.

For further information regarding the assay, consult the Spectroferritin assay instruction booklet (Ramco).

ATTACHMENT X

Protein, Lipid and Vitamin B-6 Concentrations in Milk Samples Obtained from Women in the Village of Kalama, Egypt

Sample	Stage of Lactation	Breast[1]	Total Protein	Total Lipid	Total Vitamin B-6[2]
	mo		g/dl	g/dl	μg/l
1	5 day	RF	2.65	1.0*	12*
2		LF	2.64	0.9*	16*
3	0.5	RF	2.27	3.2	60*
4		LF	2.23	7.1	56*
5	0.75	RF	2.57	3.9	100
6		LF	2.45	2.6	86
7	0.75	RH	2.60	5.8	30*
8		LH	3.32	4.6	29*
9	1	RF	2.94	4.3	69*
10		LF	----	4.7	59*
11	2	H	1.53	4.9	86
12	3	RF	1.85	5.2	143
13	3.5	RH	1.86	3.6	74*
14	3.5	RH	1.45	2.6*	177
15	3.5	RH	1.19	3.7	115
16		LH	1.47	5.9	157
17	4	RF	1.34	5.0	110
18		LF	1.20	4.0	118
19	5	RF	1.45	4.1	88
20		LF	1.43	3.7	101
21	5	LF	1.36	3.3	150
22		RF	1.41	5.6	175
23	7	RF	1.38	1.3*	12*
24		LH	1.67	1.7*	14*
25	7.5	RF	2.15	2.5*	69*
26	9	LF	1.64	0.5*	161
27		RH	1.42	0.4*	138
28	11	RH	1.57	2.3*	172
29		LH	1.20	6.2	126
30	13	RH	1.11	4.7	115
31		lh	1.16	5.5	114
32	14	LH	1.16	4.2	165
33	15	F	1.94	4.4	135
34	17	RH	1.41	5.8	224
35		LH	1.29	7.0	180
36	28	F	2.41	6.1	67*

[1]R-Right breast, L-Left breast, F-Fore milk, H-Hind milk

[2]Convulsive seizures have been observed in breast-fed infants of mothers whose values were <85 μg/l (Pediat. 20:33, 1957; J.A.M.A. 154:406, 1954; Fed. Proc. 40:863, 1981).

*Low values

Comparison of Total Lipid Content in Pooled Human Milk Analyzed by Creamatocrit and Modified Folch Methods After Freezing and Thawing of Milk and After Different Lengths of Storage

Frozen Storage Prior to Analysis	Samples Thawed and Re-Frozen	Method of Analysis	
		Creamatocrit	Modified Folch (Bligh/Dyer)
Days	Times Thawed	Total Lipid, g/dl	
0 (3 hr refrigerated)	0	3.84 (n=4)[1]	4.13 (n=1)
1	1	3.64 (n=4)	3.89 (n=1)
6	2	3.74 (n=4)	3.85 (n=1)
15	3	3.62 (n=4)	3.78 (n=1)
Mean		3.71±0.16[2] (n=16)	3.91±0.15 (n=4)

[1]Number of replicates analyzed from pooled human milk

[2]Mean ± SD

ATTACHMENT XII

MINUTES OF EGYPT NUTRITION CRSP WORKSHOP

SEPTEMBER 6 - 8, 1983

The opening session of the Egypt Nutrition CRSP Workshop was held at 9:00 AM on the 6th of September, 1983 at the Nutrition Institute, Cairo. The Workshop was attended by representatives of four Egyptian Universities (American, Cairo, Ein-Shams and Helwan), three U.S. Universities (Arizona, Kansas and Purdue), the Nutrition Institute, the Ministry of Health, the National Research Center and the NAMRUE-3 Unit.

The opening address was delivered by Dr. M. Gabr, Head of Pediatrics Department, Cairo University and Chr. Executive Committee, Nutrition CRSP in Egypt. He welcomed the participants of the workshop and especially the U.S. scientists who came to participate in the activities of the workshop. He emphasized the efforts of many individuals since 1978 in preparation for the ambitious Nutrition CRSP project which is now undertaken in three countries: Mexico, Kenya and Egypt. He pointed out that it is now one year since the field work and training was started in Egypt in the village of Kalama. Participation of both U.S. and Egyptian scientists during the past year was acknowledged. He stated that it is now time for the U.S. and Egyptian scientists to sit together to review Phase I activities and to discuss how to proceed with Phase II in light of such experience. He pointed out that he is pleased that the revised research design for Phase II was approved by the External Evaluation Panel of Nutrition CRSP and USAID and that permission has been given by the Management Entity, Univ. of California, Berkeley to go ahead with data collection.

Dr. Osman Galal, Principal Investigator in Egypt and Director of the Nutrition Institute, presented an overview of the workshop objectives. He summarized the manpower used and the work accomplished during Phase I of Nutrition CRSP. He reviewed the direct benefits of the research to the village of Kalama and some of the indirect benefits to Egypt. The latter include experience gained in nutrition research, widespread adaptation of the methodologies used, application of the results on a national scale, and potential effects on nutrition policy in Egypt. He stated that more than 100 individuals from 8

institutions in Egypt will contribute to the Egypt Nutrition CRSP project during the next 2 years.

Dr. Gail Harrison, Principal Investigator, University of Arizona and Project Representative from the U.S. responded to the overview presented by Dr. Gabr by extrapolating on the phases through which the Nutrition CRSP has passed since 1975 until the present stage of development. She summarized the challenges ahead in conducting research in a small community for a two-year period. She emphasized the importance of the findings of the research to nutrition policy formulation in terms of the real costs to society of not preventing mild/moderate malnutrition.

COFFEE BREAK

Dr. Wafaa Moussa, Senior Scientists for Food Intake, the Nutrition Institute, reviewed the various methodologies attempted during Phase I to obtain food intake information. These included:

- 24-hour dietary recalls for 3 days on target individuals in 60 households of differing socio-economic status.
- a pilot study to determine food intake protocol and methodology to be implemented during Ramadan
- feasibility study of household food inventory

She pointed out that the results showed clearly that:

- the inventory procedure is not a suitable one because families do not like to give information about the food in their stores.
- the recall and weighing methods are satisfactory
- the intake of more expensive foods is increased during Ramadan and increased more during the feast.

She recommended that it is satisfactory to collect food intake data for only 2 consective days each month by recall and weighing.

Dr. Cheryl Ritenbaugh, Senior Scientist for Anthropometry, University of Arizona, presented the anthropometric data collected in Phase I on all children and lead females in 350 households. Stunting and wasting , defined in reference to WHO standards, become highly prevalent after the sixth month of life. Wasting was uncommon after 3 years of age, but stunting continued through the tenth year with little evidence of catch-up growth. It was recommended that a subsample of children be followed through adolescence to determine the final outcome of this pattern in terms of maturation and adult height. The nutritional status picture is one of mild/moderate undernutrition, similar to the rest of Egypt, and thus satisfies the CRSP requirements for the project village. Data presented on the relationship of socioeconomic status to growth suggest that the growth measurements parallel food intake results.

Dr. Zeinab Shaheen, Consultant for Ethnography, American University, summarized the various ethnographic information which she collected during Phase I in the village of Kalama. She conducted 20 "mini-ethnographies" or limited case studies on specific areas including child care, food exchange, illness, tea drinking and flow of household work. She described Kalama as follows:

- 1,450 Moslem households
- 11 Christian households
- 6 Mosques
- S-shaped canal for drinking water, washing dishes and clothes, swimming, drinking water for animals.
- 156 familes with indoor water and some families share water pumps
- Mud/brick houses of 1-2 stories and 2-4 rooms per story.
- Most children attend school; girls leave school earlier than boys.

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Dr. Amin Abdou, Agricultural Economist from National Research Center, presented results of the sociodemographic and institutional surveys. He recommeded a revision for Phase II of the SES indicators of the families in village by giving scores to various parameters such as lead male occupation, education, structure and material of the house, durable equipment and conveniences in the house...etc.

He emphasised the importance of the demographic survey-currently undertaken-as providing a proper frame for samples selection required for other studies within the program.

Dr. Norge Jerome, Principal investigator, University of Kansas indicated some of the implications from Phase I data for Phase II. She re-emphasized the fact that the pilot studies were intended to improve the research design, data capture instruments and field approaches for Phase II. The results of the study were not intended as results per se to be published in a journal or to be filed.

Dr. Zeinab Bishry, Senior Scientist for cognitive development, Cairo University reported on work in the area of psychological measurements. She stated that training on various cognitive tests was given to 20 dietitians and 5 psychologists and that further training is required during the next 3-4 months to establish reliability. She indicated that observations of children will be for 30 minutes twice per month. Actometers will be used at 6 months of age and for toddlers but not for 7-10 years old.

LUNCH

Dr. Amin Kamel Said, Senior Scientist for Reproduction/Location, Nutrition Institute, reviewed the methods that have been developed to collect information from lead females before pregnancy, during gestation and for 6 months postpertuma as well as the outcome of pregnancy. He presented preliminary data collected during Phase I in Kalama for birth weights. All infants, except two of the 38 measured, were within the normal ranges. A method for the estimation of volume of breast milk intake of infants was tested and evaluated during Phase I. Further work will be done before Phase II in order to estimate volume of milk intake by infants during the night. More training is needed and will be given before Phase II for the estimation of gestational age by use of the Dubowitz scoring system. Also prior to Phase II all data collection forms will be tested for reliability.

Dr. A.H. Ismail, Senior Scientist for work performance, Purdue University, presented the data collected of 33 subjects used in the pilot study. The sample consisted of 19 children (6-16 years), 8 adults (22-48 years) and 6 senior citizens (50 years and above). The results suggested probable effects of mixed diets high in carbohydrates on resting metabolie rate (RMR) and repiratory quotient (RQ). when comparing the adult males who were smokers with the adult females who were not smokers, the negative effect of smoking was reflected on both the RMR and R.Q.

Dr. Ahmed Dakroury, Senior Scientist for Biological Fluids, the Nutrition Institute, pointed out that the biological materials to be examined are blood, urine, milk, saliva, and hair. Hematological measurements include hematocrit, hemoglobin and serum ferritin. These methods are being tested by the technicians in the Nutrition Institute and at Purdue University prior to Phase II data collection. Two kits for serum ferritin measurements have been tested and the results compared. Procedures for protein and creamatocrit measurements of milk have been tested. In Phase II, biological fluids will be used for immunological as well as for hematological and biochemcial measurements. Biochemical from Phase I measurements of blood and milk were presented.

TEA AND COFFEE BREAK

Dr. Farouk Shaheen, Senior Scientists for Morbidity, the Nutrition Institute presented data about the clinical examinations of target individuals in 63 households as compared to morbidity history data for the same households collected weekly over 14 consecutive weeks. Morbidity history did not represent the real disease picture of the community. This was discussed. However morbidity history was thought to be important.

Dr. Esmat Ekladious, Senior Scientist for Immunology, Cairo University, indicated that about 6 ml of blood are required for testing humoral as well as cell mediated immunity. She presented the core and non-core proposals in the area of immunology.

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EGYPT NUTRITION CRSP WORKSHOP - 7th to 8th September, 1983
SENIOR SCIENTISTS WORKING AND DISCUSSION GROUPS

PLANS FOR PHASE II

The plans for Phase II core research were presented by Dr. Gail Harrison. Non-core research should be postponed until Core Research has been successfully implemented (approximately 3 months). Also before Phase II begins the documents for Use of Human Subjects in Research and for the Nutrition CRSP Publications Agreement should be revised.

SAMPLING DESIGN

Dr. Wafaa Moussa has almost completed the household eligibility study in Phase I. She determined that 38 households in Kalama will be eliminated from the sample. Presently 230 eligible households in Kalama can be used to select 100 infants, toddlers and school age children.

DATA MANAGEMENT

To ensure efficient data transmission, the Nutrition Institute will continue to send raw data to the University of Kansas for the first three months of Phase II data collection. Dr. June Wolgemuth suggested that the cost of producing and mailing duplicate copies of new data to Kansas should be compared to the cost of transferring data from the Nutrition Institute to Alexandria to Kansas

FIELD LOGISTICS AND COORDINATION OF RESEARCH ACTIVITIES

Dr. June Wolgemuth reported 23 available seats for transportation of fieldworkers to and from Kalama. Dr. Norge Jerome suggested that investigators map out the field logistics for data collection in their disipline for a three-month period. Investigators should include daily workload for each fieldworker, data-collection shifts and number of families to be visited in the three months period. Dr. June Wolgemuth, the Field Coordinator, will be responsible for designing the final field schedule for Phase II activities. Morbidity investigators must report on the feasibility of afternoon data collection.

CLOSING COMMENTS

Dr. Osman Galal, opened the closing session and introduced Dr. B. Liska, Dean of Agriculture, Purdue University. Dr. Liska stopped in Cairo for a Nutrition CRSP site visit while on a return trip to Purdue University from a trip to Upper Volta. Dr. Liska expressed his interest and support in the activities of Egypt Nutrition CRSP and indicated his desire to visit the field site and to meet with scientists during his brief visit.

Dr. Avanelle Kirksey, Principal Investigator, Purude University and newly appointed Project Representative (to December 1984) presented a brief description of the role of Project Representative as the communication link between Management Entity, University of California, and the Universities of Arizona and Kansas and the Nutrition Institute in Egypt. She pointed out that the workshop has:

- provided a holistic/panaramic picture of the nature of the problem which is being addressed by Nutrition CRSP research.
- provided an apportunity for scientists to present the accomplishments of Phase I, and to exchange information among various parts of the project.
- increased awareness that Nutrition CRSP is an operationally complex research project and of the need for making the communications system as near perfect as possible.
- renewed interest and generated enthusiasm to move ahead towards the next important phase of the project which is data collection.
- provided for informal interaction among American and Egyptian scientists including the opportunity to taste Egyptian Food through the hospitality of many Egyptians.
- challenged the participants to successfully complete the research activities of Egypt Nutrition CRSP not only for what it means to Egypt but also as a scientific model which can be useful for future nutrition research.

Dr. Osman Galal officially closed the Egypt Nutrition CRSP Workshop at 4:00 pm on September 8, 1983.

ATTACHMENT XIII

COUNCIL OF INSTITUTE OF NUTRITION
16, KASR EL-AINY ST.
P.O, KASR EL-AINY
CAIRO - EGYPT

12 September 1983.

To whom it may concern,

The responsibility for insuring the protection of the rights of human subjects in the Nutrition CRSP Project in Egypt, continues to reside in this Executive Committee, which has been designated to assume this responsibility by the Ministry of Health. This committee will continue to insure that subjects will not be coerced into the study, that any potential risks are minimized and that both individual subjects and the community will benefit from the project.

We have reviewed the results of Phase I and the agreed-upon protocol for Phase II data collection. We are satisfied that the subjects and community are adequately protected in these matters.

Dr. Mamdouh Gabr
Head of Pediatrics Department
Cairo University

Dr. Ramses Gomma
Under Secretary
Ministry of Health
Dr. R. A. Gomma

Dr. Mahmoud Abdel Kader
Head of Nutrition Institute
Council
M. M. Abdel Kader

Dr. Hekmat El Sayed
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