TRIP REPORT: INDONESIA

Gastric Delivery System (GDS) Project
Surabaya, Indonesia

July 9 - 20, 1993

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Report Prepared for
The Agency for International Development
Contract # DPE-5966-Z-00-8083-00

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ACKNOWLEDGEMENTS

I would like to express my sincerest thanks for the gracious assistance of the following persons, without whose help this trip would not have been the successful venture that it was: Dr. Samuel G Kahn, Dr. James D. Cook, Dr. Poedji Rochjati, Dr. Marsianto, Dr. Mary Hansell, Dr. Kenneth Farr and Ratna Kurniawata.

I. EXECUTIVE SUMMARY

The first shipment of samples from the gastric delivery system (GDS) project in Surabaya arrived at the University of Kansas Medical Center in poor condition. Therefore, Dr. Samuel Kahn of the Office of Nutrition at the US Agency for International Development (USAID), Washington, DC, and Dr. James Cook of the University of Kansas Medical Center, requested that Carol Flowers travel to Indonesia to observe, first-hand, the collection and processing of samples for storage and also the preparation of these samples for shipment to the U.S.A. Dr. Kenneth Farr of the USAID Office in Jakarta, Indonesia gave authorization for this visit.

The goal of this consultancy was to visit the GDS project, meet with the principal investigator and the project coordinator and discuss the collection and preparation of the samples for storage and for shipment. In addition, aims were to visit the clinics to observe the methods of collections, processing and storage of samples. In the event this process could be improved this would be discussed with the principal investigator and a list of recommendations left in country.

In addition, the consultant would provide instruction and assistance in the preparation and packaging of the remaining samples and duplicates of samples from the original failed shipment, for transport to the University of Kansas.

A. Key Findings

1. The first shipment of samples to Kansas arrived thawed with no dry ice evident. The labels were not suitable for freezing and had come loose on

many tubes. Marking of the tubes had been done with non-permanent ink. When the labels became wet, on thawing, the ink ran, making many of the numbers unreadable. Values for the transferrin receptor measurements were lower than expected in samples from iron-deficient women, indicating there had been some deterioration of the samples.

2. Blood samples were not collected into fresh, clean and sterile vacutainer tubes. The tubes used for processing were not adequately washed to prevent possible cross-contamination or bacterial contamination of the blood if they were at ambient temperature for any length of time.
3. The samples were stored and shipped in glass tubes which risk breakage from repeated freezing and thawing or rough handling during shipment.
4. The freezer in which the samples were stored malfunctioned over one weekend. The samples thawed before they could be transferred to another freezer.
5. Finger-stick methods employed during the study often necessitated milking of the finger to obtain blood for hemoglobin (Hgb) readings, introducing the possibility of artificially low values.

B. Key Recommendations

1. Shipments of samples should be made more often during the term of the project with enough dry ice to make the entire journey. Adequate instructions should be given for proper preparation and packing to persons involved in this process.

2. Adequate supplies should be provided for proper collection, processing and storing of samples.
3. Incorporation of laboratory or technical personnel into the planning stage of the project would be crucial.
4. Adequate supervision at the outset of the project would catch potential difficulties before they become major problems.

II. PURPOSE OF VISIT

The University of Kansas is providing laboratory support for the Indonesian GDS project. Unfortunately, the first shipment of samples arrived in very poor condition and consequently may not be fully used. Because there appears to be a problem in the correct preparation and shipment of the samples, both R&D/H/Office of Nutrition and the University of Kansas felt the latter should send one of their staff to oversee the process of sample collection and shipment preparations in order to ensure arrival of viable samples.

Under this scope of work, Carol Flowers would meet with Dr. Poedji, the principal investigator, to discuss the process for collection and preparation of the samples for shipment. Methods would include first-hand observation and evaluation of the practices used by persons involved in the GDS project for the collection, processing, and storage of blood samples, visits to the clinics and health center where the actual blood collections were done, discussions with key personnel to assess any potential areas where problems may have occurred, instruction and assistance with preparation and packaging of the remaining samples and

duplicates of the first failed shipment, and return with viable samples to the University of Kansas Medical Center for laboratory analysis.

III. BACKGROUND

It is presently estimated that over a billion people in the world are anemic and that the major cause of this anemia is a deficiency in the supply of dietary iron relative to the iron needs of the population. The population segment most vulnerable to this nutritional iron deficiency is pregnant women. The global prevalence of nutritional anemia during pregnancy exceeds 50% and although this figure is much higher in Third World countries, no region is exempt. Recent studies have highlighted the significance of iron deficiency anemia during pregnancy. Mild anemia is associated with an increase in the rate of premature delivery and low birth weight, whereas more severe anemia is estimated to account for up to 20% of maternal deaths during parturition. In the light of the dismal success in reducing the global prevalence of anemia in this highly vulnerable segment of the population, there is a need to develop novel methods to improve the iron status of pregnant women.

For the past several decades the provision of oral iron supplements to pregnant women has been the primary method to decrease the prevalence of anemia. A commonly cited reason for the ineffectiveness of iron supplementation in the developing countries is the reluctance of women to adhere to a regimen that is often associated with gastrointestinal side effects. One way of circumventing this problem is to supply iron in a slow release form while retaining it in the stomach,

proximal to the iron absorbing region of the small intestine, a formulation referred to as GDS. Recent studies indicate that this preparation not only eliminates the side effects of oral iron but also results in a more efficient assimilation of the nutrient.

There has not been sufficient research to document the etiology of the problem of anemia in Indonesia; however, poor absorption of dietary iron from rice-based diets appears to be a contributing factor. Recently, the new delivery system GDS has been tested in field studies in Jamaica and it appears to significantly lower the adverse side effects while still reducing anemia. This would then decrease problems with compliance. Therefore the present study was undertaken in two separate trials in Surabaya, Indonesia. The first trial would assess the ability of the GDS formulation to reduce anemia in pregnant women while reducing side effects in comparison with the standard ferrous sulfate tablet. The second trial would assess compliance in groups of anemic non-pregnant non-lactating women.

These studies would be done at the Dr. Soetomo Hospital by the research group there and the laboratory analysis of samples collected during the study would be done at the University of Kansas Medical Center.

It was expected that, because of the low Hgb values, the measured transferrin receptor levels would range from normal levels to those elevated due to increased erythropoiesis in iron deficiency anemia. However, study samples which arrived in Kansas for evaluation had unusually low receptor levels indicating deterioration of the samples. This was most likely due to increased temperature during shipment as they had arrived with no dry ice and completely thawed. Because of the poor condition of the samples when they arrived it was suggested that an attempt be

made to determine the cause and salvage the rest of the study by transporting, by hand, the remaining samples and the duplicates of those already sent.

IV. TRIP ACTIVITIES

On Friday July 9, I left Kansas City for Indonesia carrying a large cooler with which to transport frozen plasma samples to KU from Surabaya. Phone calls to airline agents and officials had assured us there would be no problem transporting the samples on board the airplanes, provided the proper letters of verification of the non-hazardous nature of the shipment were in hand. These letters were supplied by the Department of Health and Human Services, Centers of Disease Control in Atlanta, Georgia and the USAID office in Jakarta, Indonesia.

On Sunday, July 11, I arrived in Jakarta, Indonesia and on Monday met with Mission officials at USAID in Jakarta for briefing. I met with Mary Jo Hansell and Ratna Kurniawata to discuss the condition of the samples as they had arrived in Kansas City. We discussed my scope of work and the concerns that the project was salvageable. I listed for them the things I needed to accomplish in Surabaya to make it so. We also discussed Dr. Kahn's hopes to obtain individual Hgb values for each blood sample collected, and Dr. Cook's desire to obtain a copy of the revised protocol as it was finally administered. Ratna gave me the letter for Customs Officials to aid in return of the samples to the USA. I met Dr. Kenneth Farr when he delivered this letter to Ratna.

On Tuesday, July 13, I flew to Surabaya where I was met by Dr. Marsianto. We discussed the layout of the Dr. Soetomo Hospital and the logistics of sample

collection, processing, and finally transportation for freezing and storage. He described the distances from clinics and health centers to the central storage area and the time taken for each day's activities. Dr. Marsianto indicated that samples collected from the two pregnancy clinics at the hospital were frozen immediately after collection and separation of the plasma, however because the power supply to the clinic area was unreliable they were transferred and stored in a freezer located next to the operating room (OR). This electricity was more dependable, however, the freezer did malfunction over one weekend and the thawed samples had to be transferred to another on Monday. The samples from the Mojo Health Center were kept at the center until the day's collection was complete then carried back to the hospital where they were processed in the delivery room, which is one of the few areas open 24 hours a day. The rest of the hospital closes at 1:00pm. Ambient temperature during these collections was 25-30° C.

On Wednesday, I met with Dr. Poedji to discuss the problems with the original shipment and my schedule of activities while at Dr. Soetomo hospital. She gave some background information of the medical situation in Indonesia. There are four types of hospitals in the country, types A,B,C and D. There are only two type A hospitals in Indonesia, one in Jakarta and Dr. Soetomo in Surabaya. These have all areas of specialization and sub-specialization. The Dr. Soetomo hospital has 1500 beds in the inpatient area and each of the outpatient clinics sees between 50 and 200 patients a day. The hospital closes at 1:00pm Monday - Thursday and Saturday, but closes at 11:00 am on Friday as most of the staff are Moslem and this is their time of worship. Type B hospitals employ only doctors in five or six

areas of specialization and type C only three or four. Type D are the Health Centers located in each provincy.

I was introduced to, and chatted briefly with, the hospital director, Professor Karjadi Wirjoatmodjo. We then walked to the Obstetrics and Gynecology Department where I met with the head of the department, Professor R. Prajitno Prabowd. From here we visited the Pregnancy Clinic I where the first part of Trial I took place. Here I watched while the nurse screened a patient for Hgb with a finger-stick and HemoCue reading. The Hgb was in the target range of 8.0-10.9, therefore 3 cc of blood was drawn via venous puncture using a sterile syringe with needle. The blood was transferred to a vial containing powdered EDTA and mixed. It was then transferred to another tube for centrifugation and separation of the plasma from red cells. The plasma was pipetted to a small vacutainer tube using a glass transfer pipet, sealed with a stopper and immediately frozen in a small freezer located in the clinic. At the end of the day, these tubes were carried to the central freezer located in the OR. After each sample was drawn the vial, tube, and pipet were washed with soap and water or just water and inverted to dry for the next day's collections.

Then we visited the Pregnancy Clinic II where the second half of Trial I was conducted. The women seen in this clinic were from families of government workers, with a higher standard of living than those in Clinic I. The process was essentially identical to that in Clinic I except the distance to the central freezer was longer.

The methods employed at both clinics for obtaining blood from finger-sticks encourages milking of the fingertip and can sometimes give artificially low Hgb

measurements. I was concerned that the vials, tubes and pipets were reused again and again without proper washing procedures. This can introduce cross-contamination or bacterial contamination if the blood is at ambient temperatures for extended periods of time.

Dr. Marsianto indicated that there is an inherent problem, in Indonesia, with the study design. There is a prevailing fear or reluctance to have blood drawn from venipuncture. Many subjects were lost because of the number of blood draws involved in completion of the study.

On Thursday, I met the midwives involved in Trial II with non-pregnant anemic women at the Mojo Health Center. I met with the director of the health center briefly and,because there were no patients there,we went to do home visits for the follow-up blood collection on some of the study subjects. We only found one of these subjects at home. The blood was drawn from finger-stick for Hgb and then from venipuncture with a 3 cc syringe. The blood was transferred, as before, to the vial containing EDTA. However,after mixing, it was carried in the vial during the rest of the trip until we reached the hospital. We walked to the area's community center where blood was drawn from one of the nurses for an additional fresh sample. From here we walked back to the Delivery Room, where the centrifuge is located, for separation of the plasma. The processing was the same from this point as I have described for the other clinics. However, if there were more patients in one day than available centrifuge tubes, those already used were rinsed, shaken dry, and reused for subsequent samples. Residual water in these tubes would slightly dilute subsequent samples. The Delivery Room staff were in the midst of several deliveries while we were working there.

In the afternoon I met the laboratory personnel who would be assisting in the preparation and packaging of samples for transport to the USA. A schedule of activities was set up as special arrangements had to be made since we would be working in the afternoons, after regular work schedules had finished. We spent the rest of the day sorting and remarking samples with permanent markers.

Thursday and Friday were spent at the OR freezer sorting and remarking samples. This would not have been necessary if the markings had been in permanent ink and proper storage racks had been supplied initially so that duplicate samples could have been separated at the time of collection.

On Saturday, the tubes were packed 10-15 per group, wrapped with a rubber band and packed into individual Ziploc bags, each bag was secured with an additional rubber band. This method of packing was used to ensure adequate cushioning for the tubes in the least possible space. The samples were returned to the freezer as soon as each package was complete. On Monday morning, all samples would be packed in the cooler with 30 lbs of dry ice.

I met with Dr. Poedji and discussed my findings and recommendations with her, returned to the hotel where I drafted these recommendations, and had them typed for her.

Monday, after final packing, we travelled to the airport where we encountered difficulties with the Singapore Air agents. They insisted that an excess baggage ticket be purchased if the cooler was to travel with me on the plane. I had no difficulties at the Singapore Airport or on the NorthWest Airlines flights. However, I had problems again with the agents for American Airlines in Chicago. It is my belief that a phone call from an embassy official to the airport personnel for each

of the airlines would have completely eliminated all of the difficulties that encountered. Apparently telephone calls to the airlines alone were not sufficient.

V. RESULTS/CONCLUSIONS

The trip to Indonesia allowed me to make observations of the actual blood collections and processing at the various clinic sites. I was able to view actual procedures done by the personnel involved in the two research trials. This enabled me to determine several potential problem areas. There may be several factors that have acted in concert to affect the measured protein values. These would have a cumulative effect on the plasma samples.

1. The most significant factor may be the actual shipment of plasma to Kansas. These arrived thawed with no dry ice evident. It is not known how long they were in this condition. There was either not enough dry ice to make the entire journey or it was not replenished in Singapore as had been previously arranged.
2. During the actual collection of samples, the heat of the Surabaya climate seems only a possible factor in those samples collected at the Mojo Health Center. These would have been at ambient temperature for 2-4 hours, either at the center or on home visits, until they could be processed at Soetomo hospital. The plasma may have warmed while being prepared for shipment, however, either of these conditions would only contribute to small losses.
3. Some of the earlier samples from the studies thawed when the OR freezer malfunctioned over a weekend. These were transferred to another freezer on

Monday, however it is not known how long they were thawed or how warm they got.

4. A factor in the low receptor values may also be the lack of adequate supplies for the blood collection, processing and storage. The blood was collected into vials containing EDTA prepared by the laboratory personnel. It was mixed and transferred to centrifuge tubes, centrifuged and then transferred to clean 3 ml glass tubes with a glass transfer pipet. This glassware was either rinsed or washed with soap or detergent and allowed to air dry. Blood proteins stick tenaciously to glass surfaces and with inadequate washing procedures these would carry over to the next sample. Any residual soap would also carry over. Bacterial contamination is a possibility if the blood samples are kept at ambient temperatures for extended periods of time. Any of these factor could affect measured proteins values.
5. If there were more samples in a day's collections than there were available centrifuge tubes, these were rinsed, shaken dry and reused for subsequent samples. Any residual water left clinging to the surfaces would dilute the next sample.
6. Adequate supplies were not provided for proper storage of the samples.
7. The finger-stick methods employed by the nurses introduced the possibility of artificially low Hgb values. These were only measured on the first and last blood draws.
8. Mike Linnan is the only person with the keys to the capsule codes as administered in the final revised protocol.

9. Analysis of a representative sampling of duplicate tubes of plasma returned with me to KU indicates the transferrin receptor values average 30% higher than those measured on the same samples from the May shipment. This would point to the shipping process as the major factor in the deterioration of the samples.

I believe that inadequate attention was given to technical details for completion of this project. A few more dollars spent on permanent markers and proper storage containers would have eliminated many problems. More careful attention to the process of packaging and shipment of samples plus an adequate supply of dry ice would have prevented deterioration of samples.

VI. RECOMMENDATIONS

It is my feeling that if a future project hopes for the best possible results there are several points that need careful consideration.

1. More frequent shipments should be made to the USA with careful attention given to proper packaging procedures and an adequate supply of dry ice inside the foam container to make the entire journey to the US without replenishment.
2. Care should be given that samples are handled the shortest amount of time and the least number of times.
   a. Individual blood collection tubes containing EDTA should be provided.
   b. Storage tubes should be provided that are appropriate for freezing and shipping. These should be polypropylene microtubes with snap-caps or

screw-caps. These should have frosted "write-on" surfaces. Humidity in Indonesia makes adhesive labels unusable.

c. Storage racks or containers which take little space would facilitate separation of duplicates as they are collected rather than trying to accomplish this when preparing samples for shipping.

3. Markers that have permanent ink are imperative.
4. Disposable transfer pipets for transferring plasma to the freezing tubes would be an ideal solution, however, lacking this, a detailed protocol of stringent glassware washing procedures would be essential.
5. Input from laboratory personnel at the planning stages of the project would be extremely helpful.
6. Close supervision by USAID/John Snow, Inc. (JSI) personnel at the outset of the project might help spot potentials problems early.
7. Contact of an embassy official with airport personnel of authoritative position would avert difficulty in transportation of samples to the USA.
8. If samples are carried by USAID or JSI personnel, I would recommend carrying bungee cords for tying down the container on board the aircraft.
9. More frequent shipments of samples would eliminate the potential for losses of major portions of any study.

NOTE: Federal Express does have service out of Surabaya. This would probably make more frequent shipments easier.