Memorandum

To: David Burch
From: Daniel J. Fink
Date: July 2, 1998
Re: Trip Report for June 1 - 10, 1998 to Kiev and Minsk

Attached is my Trip Report for the June trip to Kiev and Minsk. I have concentrated only on the laboratory aspects of this visit.

Trip Report
Kiev
June 2 to June 5, 1998

The laboratory aspects of this trip to Kiev focused on the following issues:

1. Blood collection procedures
2. Proper collection and measurement of Ionized Calcium
3. Testing required by the project
4. Progress and schedule for testing
5. Quality Control Procedures

These issues were discussed during the plenary sessions, a visit to the screening center at the institute, a visit to a district hospital where a mobile team was screening patients, and two visits to Dr. Epstein's laboratory (one with his technical staff and one with him).

Blood Collection Procedures

Nurses trained and certified by the laboratory under Dr. Epstein's direction performed phlebotomy. I observed blood drawing both at the institute screening center and at the district hospital. Blood collection was done professionally and correctly. However, at both sites, the blood was collected in a syringe and transferred to containers. This was due to a lack of heparinized vacutainers although serum separator vacutainers were available. I discussed this with Dr. Epstein; he said the heparinized vacutainers are now available in sufficient numbers and would be used beginning the week after our visit.

In general, patients bring urine for Iodine measurements to the screening center rather than collecting it during screening. I did not check to see what Instructions were given to the patients, but instructions ensure uniformity of collection, especially if a first morning urine is the desired specimen.

Proper Collection and Measurement of Ionized Calcium

Another issue that was discussed was the requirement that Ionized Calcium be collected only if the patient is fasting. Up to this point, blood has not been collected for Ionized Calcium if the patient was not fasting. This procedure was questioned by the mobile team and during the plenary session.

While it is true that the most accurate values of Ionized Calcium are obtained from a fasting specimen, there is variation from factors other than diet. Also, patients often misstate whether they have fasted or not.

After discussion, we agreed that Ionized Calcium data from non-fasting specimens was better than no data at all. Therefore, the screening process will be modified to begin

collecting blood for Ionized Calcium even if the patient is not fasting. If possible, data forms should be modified to indicate whether the patient is fasting or not.

Testing Required by the Project

The issue of what tests should be performed on every patient was discussed at the plenary session and in my meeting with Dr. Epstein. Dr. Markov had asked at the plenary session whether TG and PTH should be measured on every patient; anti-TPO and anti-TG were also mentioned.

Dr. Epstein and I reviewed the testing requirements during our meeting and agree on the following:

- Ionized Calcium and TSH should be measure on every patient
- PTH should be measured only on patients with Calcium abnormalities
- TG is not a good marker for cancer and should not be measured
- Anti-TPO and anti-TG are not required except in special cases.

I believe that TG, PTH, and the antibody tests were excluded from the routine testing called for in the study protocol in order to minimize the cost of performing the study. On a research basis, gathering information on these tests might be interesting. Therefore, it might be worthwhile to review the literature on TG and anti-TPO and to discuss this further with Dr. Robins, Dr. Mincey, and Dr. McConnell.

Progress and Schedule for Testing

I made two visits to Dr. Epstein's laboratory during this trip. During the first visit, I met with his laboratory technicians; the second visit was with Dr. Epstein.

It should be noted that no hormone testing has been performed yet for study patients. The endocrinologists expressed some unhappiness with this state of affairs and are unable to write final clinical summaries for these patients until they receive these results.

During the visit with the laboratory technicians, I discussed the routine operation of the laboratory. Dr. Epstein's laboratory measures over 30 different hormones and proteins including T_4, Free T_4, TSH, T_3, T Uptake, and anti-TG. Although the laboratory has a chemiluminescence instrument, it is not used for routine testing because reagents are too expensive. The laboratory performs mainly RIA tests using manual pipetting with Eppendorf precision pipettes, separation, and counting on a Beckman gamma counter connected to a computer for data reduction. This methodology is still used in many US laboratories but it is older technology that is disappearing in the US.

Common assays such as TSH or T_4 are run every other day in batches of 60 – 70 patients. Standards are run in duplicate but patient specimens are run only once. Results are generated by the computer and transferred manually to reports. Control material from kits or frozen patient specimens are run for quality assurance purposes

but there is no systematic monitoring of the results. Normal values have been assigned to the hormones measured in the routine laboratory by running approximately 80 normal patients. This used to be easy using military recruits but is more difficult recently.

In terms of the study, the plan is to use chemiluminescence assays. The manufacturer of the chemiluminescence analyzer is coming soon to service the instrument in the laboratory. No testing has been performed yet for hormones or antibodies because of reagent issues. The current plan is to begin testing in September. One issue is that Immulite reagents for 1000 assays have been delivered but are not being used. Dr. Epstein may be planning to discard these reagents since they are no longer being manufactured. Arrangements have been made to purchase Brahams reagents that will start coming in September.

Ionized Calcium and urinary Iodine testing has been performed on the specimens received to date. Of note is that the laboratory now thinks that the ionized calcium measurements should be done in the main lab because there is only one analyzer and it is felt that the analyzer does not travel well.

The computer program to enter data has not been tested yet.

Quality Control Procedures

There is no systematic program for monitoring the accuracy and precision of testing. Frozen patient specimens are run with each batch of routine specimens and the results reviewed. If the variation from the previous value is too large, (?10 percent), the results are reviewed. However, no records are kept of this process and no data is collected on accuracy and precision. For any given assay, this cut-off may be either too narrow or too wide to be of much use, and instead, should be set for each assay specifically.

I brought three papers on laboratory Quality Control for Dr. Epstein (they need to be translated) and reviewed with him the basic concepts. We discussed the use of Levy-Jennings plots of QC results to identify results that might represent analytical failure and require intervention. This identification is based upon a rule set of quality control rules. We went over the simplest 3 SD rule but also discussed the more complicated Shewart rules. Dr. Epstein seemed quite interested in this approach and said he would set this up as soon as feasible. I suggested that he Email or fax me if he had any additional questions or if questions arose during implementation.

Finally, we discussed exchanging unknown specimens. He would be eager to receive unknown specimens from either a laboratory or a proficiency testing program. We discussed whether there would be customs problems and he said he would investigate. We agreed I would try to send him specimens before my next visit.

Minsk
June 8 – June 10, 1998

During our visit to Minsk, the following questions were raised and discussed either in plenary sessions or in meetings with the laboratory staff:

1. Test results to date
2. Reagent purchasing issues
3. Need for TG, anti-TG, anti-TPO, and PTH
4. Continuation of Iodine measurements after first screening
5. ICA storage conditions
6. Quality Control
7. Broken analyzer
8. Move of laboratory

During our visit, I met with Dr. Petrenko in two long and one brief session to discuss laboratory issues. These issues also came up in various group and plenary sessions.

Test Results to Date

The examination of blood and urine specimens is going slowly. Blood and urine has been collected on about 1,550 different patients in 2,692 screening visits since 1996. To date, urinary Iodine determinations have been done on most of these specimens but Ionized Calcium and TSH have been performed only on the patients screened in 1998, approximately 600 patients. Antibody studies have not been performed on any patients. There are over 2,000 frozen specimens awaiting analysis. I suggested that a milestone be added to catch up on the testing over the next 1 or 2 quarters. Also, data entry programs have just become available and have not been tested.

Dr. Petrenko reviewed the results of urinary Iodine testing with me. The average values varied considerably from Oblast to Oblast with the highest values seen in Minsk and Gomel Oblasts, presumably because of better diets. Of some concern is that values on repeat patients varied considerably from year to year, and the average values for the Oblasts also varied greatly (+64% to – 25%) from year to year. There are three possible explanations for this. First, in a small series of experiments, first morning urinary Iodine varied considerably from day to day based on diet. Second, there was a calibration problem early in the study that may have altered the results of patients studied early on. Third, patients were bringing the urine to the screening so the conditions under which it was collected may not have been uniform; perhaps a 24-hour urine would give a more accurate result.

Reagent Purchasing Issues

Dr. Petrenko complained of slow arrival of reagents. However, a review of orders with Dr. Masnyk showed that all requested reagents had been ordered and scheduled for delivery. To date, 600 TSH tests from Abbott have been received and run. A total of 1800 more TSH tests will be received by October. In addition, Brahams reagents for anti-TG (1000), anti-TPO (1000) and PTH (100) have been ordered and scheduled for delivery in the next 6 weeks. These are radioimmunoassay tests with a shelf life of 2 months. How to use these reagents and the impact of the impending move on these reagents will be discussed below.

Need for TG, anti-TG, anti-TPO, and PTH

The need for additional testing was discussed. There have been repeated suggestions by individuals that these assays be done more frequently than was contemplated. At first glance, it would seem that these tests are adjuncts and the expense of trying to do them for a significant number of patients would be too high. It has been suggested that they be run based on clinical or laboratory indications or that they be run on a fixed percentage of patients. Since we are not sure of the clinical usefulness of these tests, targeted testing might be worthwhile. Patients with the 5 % highest and 5 % lowest TSH values would be tested for these analytes to see if there are any correlations between disease and these parameters. In addition, all patients with nodules and or carcinomas should be tested. These matters will be reviewed when we return home and a final recommendation made to the advisory committee.

Continuation of Iodine Measurements after First Screening

It was envisioned that Iodine testing would be stopped after an initial screening period of 1 or 2 years. However, the variability in the results to date suggests that this be re-examined. We need to determine if the variability is due to the mode of collection or some other factor such as the calibration problem noted early on in the study. I would suggest we await more results, review the literature on this testing, and discuss it further on our next visit in order to make a recommendation to the advisory committee.

iCa Storage Conditions

Given that there is only one iCa analyzer, it must remain at the institute. Furthermore, the Ukrainians have found that it does not travel well. However, Dr. Petrenko feels that iCa is not stable, even when frozen, based on a small series of experiments that he has done. Furthermore, he is concerned that the long trip back from the field to the laboratory will make it impossible to maintain frozen conditions. His findings are at variance with my impressions and I will review this issue in the literature before the next visit.

Quality Control

Although reference urine is run with urinary Iodine tests and Abbott controls are run with TSH tests, Quality Control is not approached in a systematic fashion and accuracy and precision are not tracked. In the case of urinary Iodine testing, the technicians run

a reference serum with each batch. However, each technician maintains his/her own reference pool and there is not a protocol for monitoring or reacting to outliers. In at least one case, an outlier was generated but no action was taken.

This finding led to a discussion of the Quality Control literature I had brought for Dr. Petrenko. We discussed the general approach to Quality Control and I showed him with graphical illustrations the basic ideas behind this approach. The key concepts discussed were Levy Jennings charts and the Shewart rules for detecting outliers. These concepts are described in the procedure manual developed for the laboratory by Dr. Mincey. However, the concepts and their implementation had not been discussed with Dr. Petrenko so he had never attempted to implement them. He said that our discussion and the documentation I gave him give him a basis for using these techniques; he stated that he intends to implement these techniques into his routine testing procedures.

Finally, we discussed the issue of exchanging unknown specimens. He would be eager to receive unknown specimens from either a laboratory or a proficiency testing program. We agreed I would try to send specimens to him before my next visit.

Broken Analyzers

On the last day of our visit, we learned that the Abbott TDx analyzer had broken the previous afternoon. Dr. Petrenko and I looked at the instrument. The stepper screw that controls the motion of the reagent probe is broken. No testing can be done until this is repaired. Abbott has a repairman for Minsk but a mechanism of payment must be established. If the instrument was acquired as part of a reagent rental contract, maintenance is covered at no additional charge. However, the exact method of acquisition was not known at the time of the visit and would have to be determined.

There has also been a broken component on the gamma counter for 2 years and repair has not been performed. The project must establish a general approach to maintenance issues.

Move of the Laboratory

The move of the dispensary will have a negative impact on laboratory testing. The laboratory will have to move twice because the renovations in the new dispensary will not be completed before the laboratory has to move out of the old dispensary. Furthermore, the interim space is not good laboratory space and may not be suitable for testing, much less RIA testing.

Dr. Petrenko will have to suspend testing while these issues are resolved and a new RIA permit issued by the government. Furthermore, the recently ordered RIA test kits have a shelf life of 2-3 months and if testing is suspended, these kits may expire unused. A delay in shipment is probably required.

Finally, all the frozen specimens must be moved without thawing or other damage.